Renal Cortical Blood Flow in Glycerol-Induced Acute Renal Failure in the Rat

THEODORE W. KURTZ,* ROY M. MALETZ, M.D.,† AND CHEN H. HSU, M.D.

ABSTRACT Renal hemodynamics and renal function were evaluated in rats at 3, 24, and 48 hours and at 7 days after the induction of acute renal failure (ARF) by glycerol injection. Three hours after induction of ARF, creatinine clearance was 0.04 ml/min/100 g; renal blood flow (RBF), 1.99 ml/min/100 g; and filtration fraction, 3.7%. All were abnormally low. Although the administration of isotonic saline (total dose, 3% of body weight) to rats 3 hours after glycerol injection significantly improved creatinine clearance (0.17 ml/min/100 g), RBF (2.54 ml/min/100 g), and filtration fraction (12.9%), these values still were significantly lower than those of controls (creatinine clearance = 0.50 ml/min/100 g, RBF = 4.92 ml/min/100 g, filtration fraction = 20.0%, all P values <0.001). Serum creatinine concentrations were significantly elevated at 24 hours (3.72 gm/100 ml), 48 hours (4.69 mg/100 ml), and 7 days (0.66 mg/100 ml) after glycerol injection compared to control (0.46 mg/100 ml, all P <0.01). RBF during these phases was not different from normal (4.41 ml/min/100 g), RBF 3 hours after bilateral ureteral obstruction was measured to determine the effects of tubular obstruction on renal hemodynamics. The RBF of rats with ureteral obstruction (4.12 ml/min/100 g) was not significantly different from controls (4.41 ml/min/100 g), suggesting that tubular obstruction in this model of ARF is probably not the cause of decreased RBF. The depressed glomerular filtration, as reflected by the decreased creatinine clearance that occurs during glomerular-induced ARF, is probably related to altered intrarenal vascular resistance or to changes in glomerular capillary permeability, or both.

THE PATHOGENESIS of the oliguria in myoglobinuric acute renal failure has been the subject of investigation ever since the association between myoglobinuria and renal injury was first noted. The preponderance of recent evidence has indicated that depression of glomerular filtration due to sustained cortical ischemia is responsible for the acute renal failure (ARF). These studies, however, have involved the use of gas washout techniques to evaluate renal blood flow (RBF) under circumstances in which tissue necrosis, cell swelling, and edema occur. That such conditions may impugn the reliability and accuracy of hemodynamic data obtained by washout methods was explained by Aukland et al., who thoroughly examined the methodology of hydrogen washout. Development of the radioactive microsphere technique for evaluating renal hemodynamics has obviated the technical problems associated with the gas washout methods. The recent adaptation of the microsphere technique for use in the renal cortex has made it possible to investigate the hemodynamics of the renal cortex in the studies outlined below.

From the Nephrology Division, Department of Internal Medicine, The University of Michigan, Ann Arbor, Michigan. *Trainee of the Undergraduate Cardiovascular Research Program (NIH Grant HL-05682) at The University of Michigan. †Formerly a Renal Fellow at The University of Michigan Medical Center; present address: St. Joseph Hospital, Boston, Massachusetts. Received August 4, 1975; accepted for publication October 1, 1975.
awake rat has provided us with the opportunity to perform a serial study of total RBF and intracortical blood flow distribution (ICBFD) during four critical stages of glycerol-induced myohemoglobinuric ARF. Recently Finn et al. reiterated the potential significance of tubular obstruction in the development of ARF; hence we performed additional studies of RBF and ICBFD 3 hours after ureteral obstruction to determine the relationship between obstruction of tubular flow and renal hemodynamics.

Methods

Male Spraque-Dawley rats weighing 200–300 g were given Purina Lab Chow pellets and tap water ad libitum. ARF was induced by intramuscular injection of 50% glycerol, 1 ml/100 g of body weight, into the hind limbs of animals previously dehydrated for 15 hours. Water was freely available thereafter. We performed measurements of RBF and ICBFD on awake rats at 3, 24, and 48 hours, and at 7 days after glycerol injection, using the radioactive microsphere technique recently adopted by our laboratory for use in the rat. Blood flow measurements were also performed in normal control rats.

Briefly, animals were weighed and lightly anesthetized with ether. Cannulas were placed in the femoral artery for blood collection and in the left ventricle via the carotid artery for injection of microspheres. After the animals awoke from anesthesia, they were placed in restraining cages for recovery for 45–60 minutes prior to injection of the microspheres. Approximately 0.1 ml of a concentration (2-3 mg/ml in 10% dextran solution) of vigorously agitated 85Sr-labeled microspheres, 15 ± 5 μm in diameter (3M Co.), was injected within 5–7 seconds into the left ventricle via the carotid catheter. Immediately upon injection of the spheres, the femoral catheter was opened and blood was allowed to flow freely into a preweighed tube for exactly 1 minute. Approximately 0.1–0.2 ml of blood was collected in this fashion. We repeated the procedure 1 hour later in the 24-hour, 48-hour, and 7-day postglycerol groups, using 141Ce-labeled spheres. Arterial blood was then taken for creatinine determinations, the animals were killed, and the appropriate steps for determination of total RBF and ICBFD blood flow were followed as previously reported. Postmortem examination verified left ventricle catheter placement.

The 3-hour postglycerol rats were surgically treated as described. In addition a PE 50 catheter was placed in the bladder through a midline suprapubic incision for urine collection and determination of creatinine clearance. The clearance study was begun 2½ hours after injection of glycerol, and urine was collected for 60 minutes. At the midpoint of the collection period (3 hours after the administration of glycerol) 85Sr- and 141Ce-labeled microspheres were injected (approximately 5 minutes apart) for determination of RBF and ICBFD. To avoid hemodynamic changes due to the bleeding, arterial blood for measurement of serum creatinine and hematocrit was drawn at the end rather than during the middle of the clearance period. Kidneys were removed for determination of RBF and ICBFD. Another group of animals, studied 3 hours after glycerol injection, were similarly treated but, in addition, cannulas were placed in the femoral vein for infusion of isotonic saline (in a dose equal to 3% of body weight) at 0.2 ml/min. For controls, normal rats received saline via intramuscular injection.

The volume expansion with the isotonic saline (3% of body weight) was begun approximately 1½ hours after surgery was completed. Commencement of the infusion was timed so that at approximately 30 minutes after the infusion was completed the clearance period would begin at the 2½-hour point following glycerol injection. We determined urine volumes by weighing the urine and dividing the weight by a specific gravity of 1.000. Creatinine in the blood and urine was determined on a Technicon Autoanalyzer.

RBF and ICBFD were determined in rats subjected to bilateral ureteral obstruction of 3 hours' duration. Animals previously dehydrated for 15 hours were anesthetized with ether, a midline abdominal incision was made, and both ureters were doubly ligated at the midportion. Catheters were placed in carotid and femoral arteries for blood flow measurements, and the animals were placed in restraining cages. We determined RBF and ICBFD 3 hours after the ligation procedure, using 85Sr- and 141Ce-labeled spheres injected approximately 5 minutes apart.

Renal plasma flow (RPF) and filtration fraction (FF) were calculated by these formulas:

\[ \text{RPF} = \frac{\text{RBF}}{1 - \text{hematocrit}} \]

\[ \text{FF} = \frac{\text{creatinine clearance}}{\text{RPF}} \times 100\% \]

The results for 85Sr and 141Ce determinations of RBF and RPF were averaged for each rat. Both RBF and RPF represent the total RBF and RPF of both kidneys combined, and are expressed as ml/min/100 g of body weight. All data are expressed as mean ± SEM. For statistical analyses, we used Student’s t-test.

Results

In 3-Hour Postglycerol Rats

Renal hemodynamic and renal function data for animals studied at 3 hours after glycerol injection, with and without volume expansion equaling 3% of body weight, are presented in Table 1. ARF occurred within 3 hours after glycerol injection, regardless of whether isotonic saline in a total dose equal to 3% of body weight was administered. Creatinine clearance of glycerol-treated animals subjected to volume expansion was significantly lower than that of controls (P < 0.001). Creatinine clearance of ARF animals without volume expansion was extremely low, 0.04 ml/min/100 g of body weight, with total anuria occurring in three of eight rats studied.

Total RBF of the volume-expanded animals, 3 hours after induction of ARF, was 2.54 ml/min/100 g of body weight. This value was significantly lower than that of controls, 4.92 ml/min/100 g of body weight (P < 0.001). Since microspheres are trapped in glomerular capillary beds, the measurement determines renal glomerular blood flow. Absolute regional cortical flows to the outer two-thirds and inner one-third of the cortex also were significantly lower in this glycerol-treated group. However, total kidney
weight corrected for body weight was significantly higher in the animals with ARF \((P < 0.001)\), therefore regional cortical flows expressed in ml/min/g of kidney weight will tend to underestimate true regional flow in these animals. Nevertheless, as total cortical blood flow expressed in terms of body weight was decreased in rats injected with glycerol, the low values of regional flow therefore reflect a true decrease in outer and inner cortical perfusion. In addition, the fractions of total cortical flow to the outer and inner cortex of volume-expanded animals with ARF were significantly different from those of controls. The percentage of flow to the outer cortex was slightly elevated in the volume-expanded animals injected with glycerol (75%) as compared to that of controls (69%, \(P < 0.05\)). Fractional flow to the inner cortex of ARF animals was proportionately lower. However, since it was not determined whether the increase in kidney weight occurred homogeneously throughout the cortex, caution should be taken when evaluating fractional distribution of flow. A comparison of the renal hemodynamic data of non-volume-expanded rats 3 hours after glycerol injection and the non-volume-expanded control rats (Table 2) revealed that total cortical blood flow, outer cortical blood flow, and inner cortical blood flow were significantly lower in the rats with ARF (all \(P < 0.001\)). The percentage of total cortical flow to the outer cortex was higher in the 3-hour postglycerol group (78.1%) than in the controls (66.8%). Fractional flow to the inner cortex of glycerol-treated animals was proportionately reduced.

The mean filtration fraction of volume-expanded rats with ARF (12.9%) was significantly lower than that of controls (20.0%, \(P < 0.001\)). The effect of the infusion of isotonic saline (3% of body weight) on the renal hemodynamics and function of animals injected with glycerol can be appreciated by comparison of filtration fractions of the two glycerol-treated groups. The mean filtration fraction of non-volume-expanded animals was 37%, significantly lower than that of the volume-expanded rats (12.9%, \(P < 0.001\)). Although both glomerular filtration rate (GFR) and RBF were significantly elevated by volume expansion \((P < 0.025\) and \(P < 0.001\), respectively), this difference in filtration fraction indicates that the volume expansion served to elevate GFR much more than RBF. Absolute outer cortical flow was slightly increased by volume expansion \((P < 0.05)\), whereas inner cortical flow was not significantly affected. Fractional distribution of flow and total kidney weight were not significantly different between the volume-expanded and non-volume-expanded animals. Hematocrit, however, was slightly lower in the volume-expanded group (46.96%) than in the controls (45.0%). Fractional flow to the inner cortex of glycerol-treated animals was proportionately reduced.

Renal hemodynamic data determined after 3 hours of bilateral ureteral obstruction are also presented in Table 1. Total RBF and absolute regional flows were significantly lower in non-volume-expanded rats 3 hours after glycerol injection than in those with ureteral obstruction \((P < 0.001)\). Fractional flow to the outer cortex was slightly higher in the glycerol-treated group, with fractional flow to the inner cortex proportionately lower. Total RBF of rats with ureteral obstruction was not significantly different from that of normal control rats (4.12 vs. 4.41 ml/min/100 g of body weight, respectively).

<table>
<thead>
<tr>
<th>Group</th>
<th>Capped saline (6)</th>
<th>1-2 hr post-glycerol (12 rats)</th>
<th>III-IV</th>
<th>I-III</th>
<th>Probability values</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.24 ± 0.19</td>
<td>1.34 ± 0.10</td>
<td>1.99 ± 0.13</td>
<td>1.92 ± 0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II</td>
<td>1.09 ± 0.16</td>
<td>1.07 ± 0.52</td>
<td>1.07 ± 0.22</td>
<td>2.80 ± 0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>III</td>
<td>1.08 ± 0.15</td>
<td>1.06 ± 0.21</td>
<td>1.01 ± 0.19</td>
<td>3.13 ± 0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV</td>
<td>1.32 ± 0.25</td>
<td>1.35 ± 0.22</td>
<td>1.32 ± 0.19</td>
<td>3.32 ± 0.19</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

| Probability values | <0.001 | <0.001 |

**Table 1: Renal Cortical Blood Flow and Creatinine Clearance of Normal and Acute Renal Failure Rats 3 Hours after Glycerol Injection**

<table>
<thead>
<tr>
<th>Group</th>
<th>RBF (ml/min/100 g)</th>
<th>CGF (ml/min/100 g)</th>
<th>CRF (mg/min/100 g)</th>
<th>TRW (g/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.24 ± 0.19</td>
<td>1.34 ± 0.10</td>
<td>1.99 ± 0.13</td>
<td>1.92 ± 0.18</td>
</tr>
<tr>
<td>II</td>
<td>1.09 ± 0.16</td>
<td>1.07 ± 0.52</td>
<td>1.07 ± 0.22</td>
<td>2.80 ± 0.12</td>
</tr>
<tr>
<td>III</td>
<td>1.08 ± 0.15</td>
<td>1.06 ± 0.21</td>
<td>1.01 ± 0.19</td>
<td>3.13 ± 0.19</td>
</tr>
<tr>
<td>IV</td>
<td>1.32 ± 0.25</td>
<td>1.35 ± 0.22</td>
<td>1.32 ± 0.19</td>
<td>3.32 ± 0.19</td>
</tr>
</tbody>
</table>

**Abbreviations:** RBF = renal blood flow, CRF = creatinine clearance, CGF = creatinine clearance, TRW = total renal weight.
In 24-Hour, 48-Hour, and 7-Day Postglycerol Rats

Renal hemodynamic and renal function data of animals studied at 24 hours, 48 hours, and 7 days after glycerol injection are presented in Table 2. Data for normal control rats are included for comparison. ARF clearly persisted 24 hours after injection of glycerol, as the mean serum creatinine of these rats was significantly higher than that of controls, 3.72 and 0.46 mg/100 ml, respectively (P < 0.001). However, mean total cortical blood flow of those animals with ARF was not significantly different from that of the controls, 4.01 and 4.41 ml/min/100 g of body weight, respectively. Although mean outer and inner cortical flows of glycerol-treated animals were slightly lower than those of controls, possibly as a result of increased kidney weight, the differences were not statistically significant. Furthermore, there was no significant difference in the fractional distribution of total cortical flow in the two groups. Mean kidney weight corrected for body weight was significantly higher in the glycerol-treated group compared to that of controls, 0.75 and 1.14 g/100 g of body weight, respectively (P < 0.001). At 48 hours after induction of ARF, mean serum creatinine was significantly higher than in the controls (4.69 vs. 0.46 mg/100 ml, P < 0.001). Mean total RBF of animals studied 48 hours after glycerol injection appeared slightly lower than that of controls (3.92 vs. 4.41 ml/min/100 g of body weight) but the difference was not statistically significant. Absolute outer and inner cortical flows were significantly lower in animals studied 48 hours after glycerol than in controls. Fractional flow to the outer cortex was significantly higher in the 48-hour postglycerol group than in controls, and fractional inner cortical flow was proportionately lower. Again, mean kidney weight corrected for body weight was significantly higher in the glycerol-treated group than in the control group, 1.12 vs. 0.75 g/100 g of body weight (P < 0.001).

As mentioned previously, the increased kidney weight of glycerol-treated animals makes interpretation of absolute and fractional flow values difficult. Furthermore, the swollen nature of the kidneys made delineation of the corticomedullary junction uncertain, thereby contributing additional error to these measurements. Seven days after injection of glycerol, mean serum creatinine, although approaching the normal range, was still significantly higher than control (0.66 vs. 0.46 mg/100 ml, respectively, P < 0.01). Mean total RBF was not significantly different from that of controls. Outer cortical flow was not different from control, whereas inner cortical flow was significantly lower. Fractional outer cortical flow was higher in the 7-day postglycerol group, and fractional inner cortical flow was lower. Mean kidney weight was almost twice that of controls (1.48 vs. 0.75 mg/100 g of body weight, P < 0.001), thereby making interpretation of outer and inner cortical flow data unreliable.

Discussion

The oliguria that occurs in ARF has been attributed to a reduction in glomerular filtration resulting from sustained cortical ischemia. Evidence supporting this pathogenetic concept has come from investigators demonstrating significantly depressed RBF in both man and experimental animals with renal failure of diverse origin. Our present study, while demonstrating reduced cortical blood flow in the initial phase of glycerol-induced ARF, does not show significant reductions in total renal perfusion 24 and 48 hours postglycerol that could account for maintenance of the depressed GFR as reflected by elevation of serum creatinine. This is in direct contrast to the reports of Ayer et al. and Chedru and associates, who found significant decreases in RBF 24 hours after glycerol injection. The reasons for such differences are unclear but probably relate to the fact that the studies of Ayer et al. and Chedru et al. both involved gas washout techniques for determining RBF, whereas we utilized the microsphere method. Since the washout techniques are measurements of flow to volume ratios, changes in renal volume will directly affect the washout values obtained. Enlargement of the kidney was clearly noted at 3, 24, and 48 hours and at 7 days after glycerol injection. Increases in kidney weight anywhere from 50% to 100%, as found in our study, may thereby lead to falsely low estimates of renal perfusion by the gas washout technique. Furthermore, Aukland et al. showed that, in a situation involving tissue necrosis and cell damage, underes-
timation of blood flow by the hydrogen washout technique is a distinct possibility. In addition, the effect of increased tissue water content on the partition coefficient of a gas must also be assessed to determine whether the values utilized provide an accurate index of solubility in all areas of the kidney. 19

Investigators using techniques other than gas washout methods have also reported RBF to be normal in uranyl nitrate13 and ischemic models of ARF.19 Jaenike14 reported that ARF persists despite a return of RBF toward normal levels at 24 hours after injection of hemoglobin.

Our study has demonstrated that at 3 hours after the injection of glycerol renal cortical blood flow and creatinine clearance were markedly reduced. Filtration fraction was low regardless of whether an infusion of saline equal to 3% of body weight was administered. The low filtration fraction indicates that predominant preglomerular vasoconstriction with resultant changes in glomerular capillary pressure and in the protein oncotnic pressure profile may be contributing to the decreased GFR in the initial phase of the lesion. Micropuncture measurements of efferent arteriolar pressure in rats have been lower than normal in the first 6 hours following administration of methemoglobin.14 Such low efferent pressures may be secondary to increased preglomerular resistance and low RBF. Furthermore, silicone rubber cast and morphometric analyses of arterioles have demonstrated the existence of preglomerular vasoconstriction in glycerol-induced ARF.18

Additional factors contributing to the early decrease in GFR probably involve changes in the coefficient of ultrafiltration (Kf). Suzuki and Mostofi14 and Dach and Kurtzman17 have demonstrated the presence of an amorphous particulate material covering the endothelial surface of glomeruli within 3 hours after glycerol injection. It seems likely that such a material may affect the permeability of the glomerular capillary or reduce the effective surface area for filtration, or both. Furthermore, Blantz18 has shown that glomerular permeability is reduced in uranyl nitrate-induced ARF. There is no definitive evidence which indicates that tubular leakage or tubular obstruction are primarily responsible for the development of glycerol-induced ARF.19-21 The increase in filtration fraction from 3.7% to 12.9% following saline expansion might be attributed to an increase in effective glomerular filtration pressure, as a result of decreased glomerular capillary oncotnic pressure, subsequent to dilution of plasma protein.22 Alternatively, decreased preglomerular vasoconstriction or increased glomerular permeability might be involved in the increase of creatinine clearance.

The mechanism for the early decrease in RBF following glycerol injection is unknown. Although in numerous studies attempts have been made to implicate the involvement of the renin-angiotensin axis,23-24 direct evidence for its pathogenetic role has never been established. Acute volume contraction induced by glycerol injection27 may contribute to the decreased renal perfusion in the initial stages of ARF. This cannot be the only cause, however, as rats receiving a volume expansion with isotonic saline equal to 3% of body weight still demonstrate significantly reduced RBF. On the basis of renal hemodynamic studies of ureteral ligation and ARF produced by 1 hour of renal artery occlusion, Finn et al.3 have recently suggested that after 24 hours of ureteral obstruction there is a relationship between obstruction to tubular flow and the development of preglomerular vasoconstriction. In the present study, RBF determined 3 hours after ureteral obstruction was not significantly different from normal, whereas RBF in rats 3 hours after glycerol injection was significantly reduced. Others have shown increased RBF after acute elevation of ureteral pressure.28,29 These findings suggest that tubular obstruction, if present, probably is not the cause of decreased RBF in the early stage of glycerol-induced ARF. Furthermore, RBF 24 hours after glycerol injection returns to the normal range, whereas RBF 24 hours after ureteral ligation is low (unpublished observations). Although the alterations in RBF observed following glycerol injection and ureteral obstruction do not rule out a relationship between obstruction to tubular flow and preglomerular vasoconstriction, the serial changes in total RBF following glycerol-induced ARF are probably not related to tubular obstruction.

In order to account hemodynamically for the maintenance of decreased GFR in the presence of relatively intact RBF 24 and 48 hours after glycerol injection, opposite changes in pre- and postglomerular resistances must be postulated. Preglomerular vasoconstriction occurring simultaneously with decreased efferent arteriolar resistance could lower glomerular capillary pressure and GFR without reducing RBF. Another factor that possibly could account for the decreased GFR in the presence of normal RBF is a change in glomerular permeability. As far as alteration in glomerular morphology is concerned, Cox et al.22 and Stein et al.33 described a marked abnormality in the epithelial structure of the glomerulus in a unilateral model of ARF caused by norepinephrine infusion and uranyl nitrate-induced ARF, respectively. However, the electron microscopic studies demonstrating deposits of granular material on the glomerular capillary 3 hours after glycerol injection failed to show any abnormalities 24 hours after induction of ARF.37 Nevertheless, this does not exclude the possibility of decreased capillary hydraulic conductivity. In conclusion, the severe depression of glomerular filtration that occurs in glycerol-induced ARF is most probably due to hemodynamic aberrations or altered glomerular permeability. The maintenance of decreased GFR at 24 and 48 hours post-glycerol, however, does not seem to be dependent on decreased RBF.

Acknowledgment

We gratefully acknowledge the assistance of Denise Lowell in the preparation of this manuscript.

References

Renal Blood Flow and Its Response to Angiotensin II

An Interaction between Oral Contraceptive Agents, Sodium Intake, and the Renin-Angiotensin System in Healthy Young Women

NORMAN K. HOLLENBERG, M.D., PH.D., GORDON H. WILLIAMS, M.D., BRUNO BURGER, M.D., WILLIAM CHERINTZ, M.D., IRAJ HOOSMAND, M.D., AND DOUGLAS F. ADAMS, M.D.

ABSTRACT A variety of estrogen- and progestin-containing oral contraceptive agents reduced renal blood flow (RBF) significantly in 23 healthy, nonhypertensive young women, to a mean of 75 ± 3.3% of the value expected for their age and dietary sodium intake (P < 0.001). There was also significant activation of the renin-angiotensin system: renin substrate was increased approximately 3-fold in association with a striking increase in the circulating renin activity and angiotensin II levels in relation to sodium intake and excretion. Two observations suggest that the RBF reduction was directly mediated by angiotensin II. A striking increase in the circulating renin activity and angiotensin II levels in relation to sodium intake and excretion. Two observations suggest that the RBF reduction was directly mediated by angiotensin II.

RESTRICTION of sodium intake reduces renal perfusion1-2 and renal vascular responsiveness to angiotensin II in man.1-4 It has not been possible, to date, to dissociate a...
Renal cortical blood flow in glycerol-induced acute renal failure in the rat.
T W Kurtz, R M Maletz and C H Hsu

Circ Res. 1976;38:30-35
doi: 10.1161/01.RES.38.1.30
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1976 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/38/1/30

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Circulation Research is online at: http://circres.ahajournals.org/subscriptions/