Cardiovascular disease is the leading cause of mortality in patients with systemic lupus erythematosus (SLE), a chronic autoimmune inflammatory disorder that predominantly affects women of reproductive age.1–3 The prevalence of hypertension, a major cardiovascular risk factor, is much higher in women with SLE than age-matched healthy women.4–8 However, the factors that underlie the increased prevalence of hypertension during SLE remain unclear.

Because of the partiality toward women, estrogen is commonly implicated as an important contributor to SLE disease progression. Using an established mouse model of SLE (female NZBWF1), we tested whether estrogen has a causal role in the development of hypertension in adulthood. Thirty-week-old SLE and control mice (NZW/LacJ) underwent either a sham or ovariectomy (OVX) procedure. 17β-Estradiol (E2; 5 μg/mouse, twice/week, subcutaneously) was administered to a subset of OVX mice. Mean arterial pressure (in mmHg) was increased in SLE mice (134±4 versus 119±3 in controls). Contrary to our hypothesis, OVX exacerbated the hypertension in female SLE mice (153±3; P<0.05 versus SLE sham), and repletion of E2 prevented the OVX-induced increase in blood pressure (132±2). The prevalence of albuminuria was increased in SLE mice compared with controls (37% versus 0%). OVX increased the prevalence of SLE mice (70% versus 37% in SLE shams). Repletion of E2 completely prevented albuminuria in OVX SLE mice. Renal cortical tumor necrosis factor α was increased in SLE mice compared with controls and was further increased in OVX SLE. The OVX-induced increase in renal tumor necrosis factor α expression was prevented by repletion of E2. Treatment of OVX SLE mice with the tumor necrosis factor α inhibitor, etanercept, blunted the OVX-induced increase in blood pressure (140±2) and prevalence of albuminuria (22%). These data suggest that 17β-estradiol protects against the progression of hypertension during adulthood in SLE, in part, by reducing tumor necrosis factor α. (Hypertension. 2014;63:616-623.)

Key Words: estrogens ■ lupus erythematosus, systemic ■ pressure

Cardiovascular disease is the leading cause of mortality in patients with systemic lupus erythematosus (SLE), a chronic autoimmune inflammatory disorder that predominantly affects women of reproductive age.1–3 The prevalence of hypertension, a major cardiovascular risk factor, is much higher in women with SLE than age-matched healthy women.4–8 However, the factors that underlie the increased prevalence of hypertension during SLE remain unclear.

Because of the partiality toward women, estrogen is commonly implicated as an important contributor to SLE disease progression. In addition, estrogens can promote humoral (antibody)-mediated immunity9,10 that is essential for the formation of immune complexes, resulting in the tissue injury and inflammation characteristic of SLE. A role for estrogens in SLE is commonly supported by studies in experimental animal models in which removal of estrogens early in life (typically <6–8 weeks of age) delays the production of autoantibodies and the subsequent renal injury and mortality.11,12 Although these early life manipulations of estrogen suggest that they are important for the onset of SLE, the effect of estrogens on SLE in adulthood has not been widely studied, particularly with regard to their role in the development of hypertension.

Understanding the influence of estrogens on cardiovascular risk factors like hypertension in adulthood is important for women with SLE because autoimmunity accounts for up to 50% of premature ovarian failure, and women with SLE have increased risk for early menopause,13 an important risk factor for hypertension and cardiovascular disease. Using an established experimental model of SLE (female NZBWF1 mice), this study was initially designed to test the hypothesis that estrogen, commonly considered an important factor in the development of SLE, has a causal role in the development of hypertension in adulthood. However, contrary to the hypothesis, the data suggest that estrogens may have an anti-inflammatory role that protects against the further development of hypertension.

Methods and Materials

Animals

Adult (30-week-old) female NZBWF1 (SLE) and NZW/LacJ (Ctrl) (Jackson Laboratories, Bar Harbor, ME) mice were used in this study.
Mice were maintained on a 12-hour light/dark cycle in temperature controlled rooms with access to chow and water ad libitum. All studies were performed with the approval of the University of Mississippi Medical Center Institutional Animal Care and Use Committee and in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Ovariectomy
Ovariectomy (OVX) was performed through a dorsal midline incision. The abdominal muscle and peritoneum were incised, and a silk ligature was tied below the ovaries to remove them. To confirm the efficacy of OVX, the uterus was collected and weighed at the conclusion of the study.

17β-Estradiol Repletion
17β-Estradiol 17-valerate (E2: 5 μg/mouse; Sigma, St. Louis, MO, Catalog no. E1631) dissolved in 0.1 mL corn oil14–18 was injected subcutaneously twice per week for 4 weeks. Mice not receiving E2 were injected with 0.1 mL corn oil.

Etanercept Administration
A subset of mice was administered the tumor necrosis factor α (TNF-α) inhibitor etanercept (Wyeth, Thousand Oaks, CA; 0.8 mg/kg) or sterile water by subcutaneous injection once weekly for 4 weeks as we previously published.19

Blood Pressure Measurements
Mean arterial pressure (MAP; in mm Hg) was recorded via indwelling carotid artery catheters in conscious mice as previously described by our laboratory.20

Autoantibody Production
Plasma anti-double-stranded DNA (dsDNA) antibodies, a hallmark of SLE disease, were measured by commercial ELISA (Alpha Diagnostic International, San Antonio, TX) as previously described.21

Albuminuria
Urinary albumin was monitored weekly by dipstick analysis (Albusitx, Tarrytown, NY) of overnight urine samples. Animals were considered to be positive for albuminuria at >100 mg/dL as previously described.21 Urinary albumin was confirmed by ELISA (Alpha Diagnostic International, San Antonio, TX) in urine samples collected at the end of the study as previously published by our laboratory.21

Renal TNF-α
Renal cortical protein expression of TNF-α was determined using standard Western blot technique as previously published by our laboratory.19 A mouse monoclonal anti–TNF-α antibody (1:250; Santa Cruz Biotechnology, Dallas, TX) and rabbit anti–β-actin (1:4000; Abcam Inc, Cambridge, MA) were used. Proteins were visualized using an IR700-conjugated donkey anti-mouse IgG (1:2000) and IR800-conjugated donkey anti-rabbit IgG (1:2000; Rockland Immunologicals, Gilbertsville, PA). Blots were analyzed using the Odyssey Infrared Scanner (LI-COR Biosciences, Lincoln, NE). Data are presented as units of protein, based on band optical density, normalized to β-actin.

Protocol 1
Thirty-week-old SLE and control mice underwent either OVX or sham operation. Both SLE and control mice were assigned to one of the following treatment groups: sham, OVX, or OVX+E2. Sham and OVX mice in the E2 repletion study that were injected with oil were grouped with all other sham or OVX, respectively, because vehicle had no effect on any parameters measured. Body weight was measured weekly. Urine was collected weekly and assessed for the presence of albumin. MAP was measured at 34 weeks of age, and tissues were collected.

Protocol 2
A subset of 30-week-old female OVX SLE and OVX Ctrl mice was administered etanercept via subcutaneous injection once a week for 4 weeks. Mice were followed to 34 weeks of age for measurement of MAP and tissue collection.

Statistical Analysis
Data are presented as mean±SEM. Statistical analyses were performed using GraphPad Prism 6 software. A 1-way ANOVA was used to test for treatment interactions with a Tukey multiple comparison post hoc test. *P<0.05 was considered statistically significant.

Results
To test the hypothesis that estrogen, which is commonly considered an important factor in the development of SLE, has a causal role in the development of hypertension in adulthood, 30-week-old control and NZBWF1 mice were subjected to either OVX or sham operation. Consistent with our previously published work, MAP was increased in SLE shams compared with control shams (134±4 versus 119±3 mm Hg; P<0.05; SLE versus Ctrl; 2-way ANOVA). Contrary to our hypothesis, MAP was significantly increased in OVX SLE mice compared with SLE shams (Figure 1B; 153±3 mm Hg; P<0.05). MAP was not altered by OVX in control mice (Figure 1A; 114±3 mm Hg). In a subset of animals, E2 was administered to OVX SLE and control mice to determine whether the loss of E2, rather than other ovarian hormones such as progesterone, was responsible for the further increase in blood pressure. Repletion of E2 in OVX SLE mice prevented the increase in MAP caused by OVX

![Figure 1](https://example.com/figure1.png)
Repletion of E2 in OVX control mice did not alter MAP (Figure 1A).

Because repletion of E2 prevented the OVX-induced increase in MAP, the question of the mechanism by which this protection occurs was examined. Estradiol has known anti-inflammatory effects and reduces proinflammatory cytokines including TNF-α,22–24 previously shown by us to mechanistically contribute to hypertension in female NZBWF1.19 Renal cortical TNF-α expression was significantly increased in SLE sham mice compared with controls (Figure 2; 1.8±0.4 versus 1.0±0.1; *P<0.05). In OVX SLE mice, renal cortical TNF-α expression was increased (3.2±0.8), and repletion of E2 after OVX prevented the OVX-induced increase in renal cortical TNF-α expression (2.2±0.3). Renal TNF-α expression was not altered in controls by OVX or repletion of E2 after OVX.

To determine whether the increased TNF-α after OVX contributes to the OVX-induced increase in blood pressure in adult SLE mice, OVX SLE and control mice were treated with etanercept, an inhibitor of TNF-α biological activity. Treatment with etanercept in OVX SLE mice ameliorated the OVX-induced increase in MAP in female SLE mice (Figure 1B; 140±2 mm Hg), suggesting that the exacerbated hypertension caused by OVX was mediated, in part, by TNF-α. This is consistent with our previously published work related to the effect of etanercept on blood pressure during SLE.19 Treatment with etanercept did not alter MAP in controls.

The efficacy of OVX and E2 repletion was confirmed in these studies by measuring uterine weight at the time of euthanization. As expected after OVX in adult mice, uterine weight was significantly reduced in both control (Figure 3A; Ctrl sham: 0.097±0.012 g versus Ctrl OVX: 0.042±0.006 g; *P<0.05) and SLE mice (Figure 3B; SLE sham: 0.094±0.008 g; SLE OVX: 0.045±0.008 g; *P<0.05). Uterine weight in mice replete with estradiol was significantly greater than corresponding sham controls (Ctrl OVX+E2: 0.25±0.02 g; SLE OVX+E2: 0.2±0.02 g; *P<0.05). Treatment with etanercept after OVX did not alter uterine weight in comparison with OVX mice (Ctrl OVX+E2: 0.03±0.002 g; SLE OVX+E2: 0.039±0.01 g).

SLE is characterized by renal injury including albuminuria, and previous work from our laboratory shows that the prevalence of albuminuria in the adult female SLE mice at 34 weeks of age as assessed by dipstick assay is ≈40%.21,25 Consistent with this, the prevalence of albuminuria in 34-week-old SLE shams in this study reached 37% (Figure 4A). No control mice developed urinary albumin during the course of the study (data not shown). Urinary albumin, as measured by ELISA in samples collected at 34 weeks of age, was increased in SLE mice compared with controls as previously shown in our laboratory (Figure 4B and 4C; 3.20±1.5 versus 0.02±0.0 mg/mL; *P<0.05; SLE versus Ctrl; 2-way ANOVA).19,21,25 The percentage of SLE mice with albuminuria was increased to 70% after OVX,

(Figure 1B; 132±2 mm Hg). Repletion of E2 in OVX control mice did not alter MAP (Figure 1A).

Because repletion of E2 prevented the OVX-induced increase in MAP, the question of the mechanism by which this protection occurs was examined. Estradiol has known anti-inflammatory effects and reduces proinflammatory cytokines including TNF-α,22–24 previously shown by us to mechanistically contribute to hypertension in female NZBWF1.19 Renal cortical TNF-α expression was significantly increased in SLE sham mice compared with controls (Figure 2; 1.8±0.4 versus 1.0±0.1; *P<0.05). In OVX SLE mice, renal cortical TNF-α expression was increased (3.2±0.8), and repletion of E2 after OVX prevented the OVX-induced increase in renal cortical TNF-α expression (2.2±0.3). Renal TNF-α expression was not altered in controls by OVX or repletion of E2 after OVX.

To determine whether the increased TNF-α after OVX contributes to the OVX-induced increase in blood pressure in adult SLE mice, OVX SLE and control mice were treated with etanercept, an inhibitor of TNF-α biological activity. Treatment with etanercept in OVX SLE mice ameliorated the OVX-induced increase in MAP in female SLE mice (Figure 1B; 140±2 mm Hg), suggesting that the exacerbated hypertension caused by OVX was mediated, in part, by TNF-α. This is consistent with our previously published work related to the effect of etanercept on blood pressure during SLE.19 Treatment with etanercept did not alter MAP in controls.

The efficacy of OVX and E2 repletion was confirmed in these studies by measuring uterine weight at the time of euthanization. As expected after OVX in adult mice, uterine weight was significantly reduced in both control (Figure 3A; Ctrl sham: 0.097±0.012 g versus Ctrl OVX: 0.042±0.006 g; *P<0.05) and SLE mice (Figure 3B; SLE sham: 0.094±0.008 g; SLE OVX: 0.045±0.008 g; *P<0.05). Uterine weight in mice replete with estradiol was significantly greater than corresponding sham controls (Ctrl OVX+E2: 0.25±0.02 g; SLE OVX+E2: 0.2±0.02 g; *P<0.05). Treatment with etanercept after OVX did not alter uterine weight in comparison with OVX mice (Ctrl OVX+E2: 0.03±0.002 g; SLE OVX+E2: 0.039±0.01 g).

SLE is characterized by renal injury including albuminuria, and previous work from our laboratory shows that
and urinary albumin increased compared with SLE shams (10.2±3.9 mg/mL). The OVX SLE mice replete with E2 did not develop albuminuria as measured by dipstick, which was confirmed by ELISA (0.01±0.003 mg/mL). The prevalence of albuminuria in OVX SLE mice treated with etanercept was blunted (22%) as was urinary albumin in OVX SLE mice. *P<0.05 vs OVX+E2.

Figure 4. A, Weekly percentage of systemic lupus erythematosus (SLE) mice with positive urinary albumin as measured by dipstick assay (n≥8/group). No control mice developed albuminuria. B, Urine albumin in control mice at 34 weeks of age as measured by ELISA (n≥8). Urine albumin was similar between groups. C, Urine albumin in SLE mice at 34 weeks of age as measured by ELISA (n≥8). Urine albumin was increased after ovariectomy (OVX) in comparison with shams. Repletion of estradiol (E2) prevented the OVX-induced increase in urine albumin. Treatment with etanercept blunted the increase in urine albumin in OVX SLE mice. *P<0.05 vs OVX+E2.

Discussion

The prevalence of hypertension is markedly increased in women with SLE4–8 and represents a major independent risk factor for mortality in this patient population26; however, the mechanisms contributing to the prevalent hypertension have not been widely studied. Using an established mouse model of SLE, we previously investigated some of the underlying factors that promote hypertension. These include impaired renal hemodynamic function,27 peripheral vascular function,28 inflammatory cytokines,19,21 and oxidative stress.25 Based on their presumed role to promote SLE disease progression, the present study sought to test whether estrogens could be contributing to the development of hypertension. The major new findings of this study are that (1) in adulthood, OVX accelerates rather than protects against the development of hypertension and albuminuria during SLE; (2) the exacerbation of hypertension caused by OVX in adulthood during SLE is mediated by a loss of 17β-estradiol and is associated with increased renal TNF-α expression; and (3) the increased renal TNF-α expression mechanistically contributes to the exacerbated hypertension in adult SLE mice because etanercept blunts the effect of OVX. These data provide new insight for...
understanding the complex role that estrogens have in SLE disease progression and the associated cardiovascular risk factors such as hypertension.

Estrogens have been widely implicated in the development of SLE, in part, based on the strong predilection of the disorder for women of reproductive age. A role for estrogens in autoimmunity has been supported by reports that the production of immunoglobulins (IgG and IgM) is increased in human peripheral blood mononuclear cells incubated with 17β-estradiol but not other estrogenic compounds (ie, estrone, estriol). Studies in experimental animal models have also consistently supported an important role for estrogens in SLE disease progression. For example, NZBWF1 estrogen receptor (ER)-α knockout mice exhibited reduced development of anti-dsDNA autoantibodies over time that correlated with increased survival time. O VX NZBWF1 mice, treated with a selective ER-α agonist, had increased levels of anti-dsDNA IgG-specific antibodies. These studies support earlier data in the literature, which show that autoantibody production and mortality are increased by estrogen supplementation in female NZBWF1 mice castrated at 2 weeks of age. However, it is worth noting that the dose of E2 (6–7 mg in silastic tubing) used in this previous work is considerably higher than the dose used in the present study and could have caused nonspecific effects, including enlarged bladder and associated kidney injury from the chronic muscle relaxation. Finally, treatment of NZBWF1 mice with tamoxifen, a selective ER modulator, starting at 6 to 8 weeks of age increased survival but did not affect antibody production.

Based on this work, we anticipated that removal of the ovaries would reduce autoantibody production, decrease albuminuria, and protect against the development of hypertension; however, this was not the case. In fact, the data clearly demonstrate a protective role for estrogens against hypertension and albuminuria, independent of changes in antibody production. The finding that estradiol has a protective role against the further progression of hypertension is important because blood pressure is a critical factor for increasing cardiovascular risk, the leading cause of death in women with SLE. Disease severity was not changed. Disease severity was also dependent on the timing of treatment with E2 in a study of ovariectomized DBA/1 mice with collagen type II arthritis. These studies suggest a high likelihood that estrogen has different temporal effects during inflammatory diseases. One possible explanation for the changing roles of estradiol may be related to alterations in the expression of the ER over time. Roa et al showed that NZBWF1 mice have fewer ERs per mg cytosol protein than nonautoimmune BALB/c mice. ER receptor abundance per mg cytosol protein in the liver was increased in NZBWF1 mice and the parental NZW strain compared with the parental NZB strain, whereas no differences were seen in thymus, spleen, or uterus. Another study noted changes in binding affinity and capacity of the ER in MRL mice, a model of lupus, with aging. Whether NZBWF1 mice exhibit similar changes with aging is not known.

Although studies in both humans and experimental models of SLE have examined and speculated on the role of estrogens, the effect of estrogens on blood pressure during SLE has not been carefully examined until now. In addition to the fact that hypertension is an independent predictor of mortality and that blood pressure control is an important clinical consideration for reducing cardiovascular and renal risks in women with SLE, understanding the role of estrogens in blood pressure control in adulthood during SLE is especially important to this patient population because the risk of premature ovarian failure and early menopause is high. Several studies report that hormone therapy in women with SLE is well tolerated, despite the assumption that estrogens promote SLE disease. In one study assessing the relationship between cardiovascular disease risks and hormone therapy in women with SLE, hormone therapy did not predispose to coronary artery disease. In addition, hormone therapy in the SELENA (Safety of Estrogens in Lupus Erythematosus-National Assessment) study of 351 women with SLE did not lead to cardiovascular events. Similarly, in the LUMINA (Lupus in Minorities: Nature vs Nurture) study, hormone therapy was not associated with vascular arterial events, and Fernández et al even speculated that hormone therapy may be a protective factor against the development of arterial vascular events in women with SLE. Our data are consistent with these studies, suggesting a potential cardioprotective role for estrogens in adulthood during SLE.

To gain insight into the mechanism by which estrogen could protect against hypertension, we focused on the inflammatory cytokine, TNF-α. Our previously published work showed renal TNF-α to be an important factor in the pathogenesis of hypertension in this model. In addition, low levels of estrogen or removal of estrogen promotes a pro-inflammatory response and the production of cytokines. For example, TNF-α is increased in postmenopausal women compared with premenopausal women. In experimental animal studies, estrogen-deficient rats have increased serum levels of TNF-α that are reduced after estrogen repletion, and TNF-α inhibition in estrogen-deficient rats was associated with improved vascular function. Consistent with...
these data, we show that OVX adult female SLE mice have increased renal cortical protein expression of TNF-α and that the enhanced blood pressure response caused by OVX was largely prevented by treatment with etanercept. Although these data suggest that TNF-α is an important mediator of the OVX-induced increase in pressure, it is possible that etanercept prevents the increased pressure caused by OVX, independent of the blood pressure-lowering effects of E2.

Separating changes in albuminuria from changes in blood pressure is always difficult. Female NZBW/F1 mice represent a long-established model of SLE with immune complex-mediated glomerulonephritis; therefore, it is unlikely that the development of albuminuria is strictly related to hypertension. In support of this, we recently published work showing that bilateral renal denervation in this model ameliorates albuminuria independent of changes in blood pressure.51 In addition, studies in the NZBW/F1 model show that lowering blood pressure alone is not sufficient to reduce renal injury.52 Nevertheless, the albuminuria caused by OVX seems to track well with the increase in blood pressure. Interestingly, however, albuminuria was ameliorated in the SLE mice replete with 17β-estradiol, although the blood pressure is similar to the sham SLE group. The reason for the marked effect of 17β-estradiol on albuminuria is not clear. The dose of estradiol selected for this study was based on previously published work of others,14-16 and the regimen (twice per week) was chosen to better mimic estrous cycling. However, based on the fact that uterine weights were increased over sham controls in animals replete with estradiol, the circulating levels of estradiol achieved were most likely high. We did not measure serum estradiol in this study because levels in mice are low (<5 pg/mL), which typically falls below the sensitivity of even liquid chromatography-mass spectrometry methods and leads to significant variability in radioimmunoassays, even when comparing OVX with intact animals.53 Irrespective of the absolute circulating levels of estradiol, these data support the idea that the albuminuria in this model is not solely the result of increased arterial pressure. Taken together, the results of these studies showing a protective role of estradiol in adulthood during SLE highlight the need to carefully examine the temporal effects of estrogen on disease activity and may help to explain why there is considerable variability in human data with regard to the role of estrogens.

Perspectives

The role of estrogens in adult women with SLE is much less clear than what is commonly presumed. Therefore, it may be particularly informative to consider more carefully these data in the context of what is known about SLE in women. For example, during the course of a normal menstrual cycle, women with SLE report greater disease activity during menses but not during the normal hormonal surge that is associated with the highest levels of estrogen during the estrus cycle.54 Estrogen-based oral contraceptive use is generally associated with the highest levels of estrogen during the estrus cycle.55,56 There are even reports suggesting that estrogens have a protective role against SLE. For example, disease flares were reduced in pregnant women with SLE during the third trimester when estrogen levels are at their highest,57 and in some studies, lower levels of estrogen are associated with increased SLE disease activity.58 In the present study, we show that removal of estrogen during adulthood in female NZBW/F1 SLE mice exacerbates hypertension and increases renal injury and renal TNF-α, all of which are prevented by repletion of estrogen. These data suggest that estrogen may play a protective role against a major cardiovascular risk factor in adulthood during SLE. In addition, these data suggest that hormone therapy or repletion of estrogen can be cardioprotective during SLE while not altering autoantibody levels or encouraging SLE disease activity.

Sources of Funding

E.L. Gilbert is the recipient of an American Heart Association Greater Southeast Affiliate Predoctoral Fellowship (12PRE12050150). This work was supported by HL085907 and a University of Mississippi Medical Center (UMMC) Intramural Research Support grant to M.J. Ryan and HL051971 to UMMC Physiology.

Disclosures

None.

References


What Is New?

- In adulthood, loss of estrogen accelerates rather than protects against the development of high blood pressure and kidney injury during lupus disease.
- The exacerbation of high blood pressure caused by loss of estrogen in adulthood during systemic lupus erythematosus (SLE) is associated with increased inflammation in the kidney.

What Is Relevant?

- Cardiovascular disease is the leading cause of death during SLE. Furthermore, hypertension, a major cardiovascular risk factor, is highly prevalent in women with SLE for reasons that remain unclear.

Summary

Estrogen plays a protective role during adulthood in SLE at least against the progression of hypertension, a major cardiovascular risk factor. These data provide new insight for understanding the complex role that estrogens have in SLE disease progression and the associated cardiovascular risk factors such as hypertension.
17β-Estradiol Protects Against the Progression of Hypertension During Adulthood in a Mouse Model of Systemic Lupus Erythematosus
Emily L. Gilbert, Keisa W. Mathis and Michael J. Ryan

Hypertension. 2014;63:616-623; originally published online December 23, 2013;
doi: 10.1161/HYPERTENSIONAHA.113.02385
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 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/63/3/616

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/