Offspring of Normal and Diabetic Rats Fed Saturated Fat in Pregnancy Demonstrate Vascular Dysfunction

E. Koukkou, MD; P. Ghosh, BSc; C. Lowy, MD, FRCP, MSc; L. Poston, PhD

Background—Disturbances of the in utero environment may “program” for disease in later life. In this study, we determined whether dietary fat supplementation and/or diabetes in pregnancy can adversely affect vascular function in the offspring.

Methods and Results—Female Sprague-Dawley rats were fed a breeding diet or a diet high in saturated fat (30% wt/wt) for 10 days before mating, throughout pregnancy, and postpartum. Endothelium-dependent relaxation to acetylcholine was blunted in isolated femoral arteries of 15-day-old weanling pups from dams fed the 30%-fat diet. Endothelial dysfunction and enhanced constrictor responses to norepinephrine were also observed in an additional study of 60-day-old offspring of dams fed 20% saturated fat. Rats with streptozotocin-induced diabetes were also fed saturated fat during pregnancy. Femoral arteries from their 15-day-old offspring showed impairment of endothelium-dependent dilation and enhanced constrictor responses to norepinephrine and the thromboxane mimetic U46619 compared with young offspring of high-fat-fed normal dams. The 30%-fat diet was also deleterious to vascular function in the maternal diabetic animals when assessed in mesenteric arteries 16 days postpartum.

Conclusions—A high-fat diet in pregnancy led to vascular dysfunction in rat weanlings and young adult offspring. Vascular function further deteriorated in weanlings if the maternal rat was diabetic. (Circulation. 1998;98:2899-2904.)

Cardiovascular disease may have origins in fetal life. Retrospective population-based studies suggest that smaller size at birth or disproportionate growth is associated with increased incidence of hypertension and heart failure. Experimental studies in animals to investigate the proposed fetal “programming” of adulthood cardiovascular disease have therefore concentrated on the sequelae of maternal undernutrition, and little attention has been paid to possible in utero effects of a diet high in saturated fat, despite overwhelming evidence linking saturated fat intake to cardiovascular disease. Others have suggested that maternal diabet es may also have lasting adverse consequences on cardiovascular function of the next generation, particularly because offspring of diabetic pregnant rats demonstrate overt insulin resistance in adulthood.

In this study, we determined the effect of a high-saturated-fat diet in pregnant rats on vascular function of isolated femoral arteries from 15-day-old and young adult offspring. In 15-day-old offspring, we also investigated the effect of maternal diabetes and the potential interaction between a high-saturated-fat diet and maternal diabetes.

Responses to constrictor agonists and to the endothelium-dependent vasodilator acetylcholine (ACh) were assessed in femoral arteries of the offspring by use of a small-vessel myograph. Mesenteric small arteries from the maternal circulation of control and diabetic animals were also investigated 16 days postpartum.

Methods

Animals and Diets

Study 1: Vascular Function in Dams and 15-Day-Old Offspring

Female Sprague-Dawley rats (12 to 14 weeks old) were fed a standard breeding diet (4% fat, 22% protein, 51% carbohydrate, 5% fiber) or a diet high in saturated fat (30% lard, 18% protein, 40% carbohydrate, 3% fiber) for 10 days before mating, throughout pregnancy, and for 16 days postpartum. On day 1 to 2 of pregnancy, diabetes was induced in half the dams by caudal injection of streptozotocin (STZ; 30 mg/kg). Some control and diabetic rats fed the 30%-fat diet experienced difficulty in labor and were humanely killed. Diabetes was confirmed by demonstration of glycosuria (by Glucostix). Urinary glucose and ketones were monitored every 2 days during pregnancy by use of Glucostix and Ketostix, and to partially control diabetes, an insulin implant (one-half tablet; release rate 2 U/24 hours per implant for >40 days) was inserted subcutaneously after detection of ketonuria. Animals were weighed every 2 days until 20 to 21 days’ gestation. At delivery, litter size was reduced to 5 to 6 pups. Vascular function was assessed in 15-day-old pups and in the dams at 16 days postpartum.
Dietary Fat and Offspring Vascular Function

Study 2: Vascular Function in 60-Day-Old Offspring

Study 1 demonstrated abnormalities of vascular function in the weanlings of normal dams fed the 30%-fat diet. A second study was performed to determine whether this defect persisted into young adulthood. Because 30%-fat-fed dams had experienced difficulty in labor and a high neonatal mortality rate, the maternal diet was reduced to 20% (wt/wt) after weaning. Offspring were fed a diet of standard rat chow (3% fat) until 60 days old, when vascular function was assessed.

Evaluation of Vascular Function

Animals were killed by CO₂ inhalation and cervical dislocation. Blood samples were obtained by cardiac puncture, and plasma was stored at –70°C before analysis. One pup from each litter was studied. Third-order branches of the mesenteric artery from the adult rat and the femoral artery from the offspring were mounted as ring preparations on a small-vessel myograph. Offspring mesenteric arteries were found to be very friable, whereas femoral arteries demonstrated reproducible viability. These were therefore used for the study. Arteries failing to produce tension equivalent to a pressure of 100 mm Hg to 5 mm Hg were rejected (a total of 5). Concentration response curves were constructed to NE for maternal mesenteric arteries (10⁻⁷ to 10⁻² mol/L). In offspring arteries, a concentration response was also performed to the thromboxane mimetic U46619 (10⁻⁶ to 10⁻² mol/L). Arteries were then preconstricted with NE to achieve ~80% maximum response and relaxation to ACh assessed (10⁻⁴ to 10⁻⁵ mol/L). A similar protocol was followed to evaluate relaxation to the NO donor spermine NONOate (10⁻⁹ to 10⁻³ mol/L).

Blood Glucose, Fructosamine, and Lipids

Glucose concentrations were determined with a commercially available assay based on an enzymatic (HK/G6P-DH) UV test. Fructosamine, as an alternative estimate of glucose control, was determined by use of a commercially available assay involving the formation of formazan from nitro blue. Total plasma cholesterol and triglyceride concentrations were determined by assays based on the colorimetric evaluation of enzyme activity [UNIMATE CHOL [cholesterol oxidase] and UNIMATE TRIG [glycerol phosphate dehydrogenase], respectively].

Chemicals

Chemicals were used, and spermine NONOate (Alexis Corp). All chemicals for PSS (in mmol/L: NaCl 119, NaHCO₃ 25, D-glucose 5.5, KH₂PO₄ 1.18, MgSO₄ 7, H₂O 1.17, KCI 4.7, CaCl₂ 2.5, EDTA 0.026) were from BDH. STZ was from Upjohn Co. Glucostix and Ketostix were from Boehringer Mannheim. Insulin implants were from Linkplant. Kits for estimation of glucose, total cholesterol (UNIMATE CHOL), and triglycerides (UNIMATE TRIG) were from Roche Diagnostic Systems. High-saturated-fat and normal breeding (No. 3 breeding diet) diets were from Special Diet Services.

Statistical Analysis

All values are given as mean±SEM. To account for small differences in vessel diameter and in tension development, NE-induced tension is expressed as percent of the maximum contractile response to KPSS. Relaxation to ACh and spermine NONOate was expressed as a percentage of NE-induced precontraction. The negative log of the concentration (mol/L) of a drug required to produce 50% of the maximum response (pEC₅₀) was calculated after data were fit to a sigmoidal curve (GraphPad Software Inc). Two arteries from each animal were studied, and the mean pEC₅₀ was used in analysis. One-way ANOVA and Student’s independent t-test were used for single comparisons, and the Student-Newman-Keuls test was used for multiple comparisons between groups of arteries. The Kruskal-Wallis nonparametric ANOVA test was used for statistical comparisons of total cholesterol and triglyceride levels (Instat; GraphPad). Significance was assumed if P<0.05.

Results

Study 1: Vascular Function in Dams and 15-Day-Old Offspring

Weight and Plasma Analyses

Maternal

Pregnancy-associated weight gain was significantly less in diabetic dams fed a high-fat diet than in nondiabetic dams fed the breeding diet (respective weight at 21-day gestation: 305.6±7.4 g, n=8 versus 386.4±8.9 g, n=10; P<0.001). Other weights during pregnancy were similar. Postpartum weights were similar to nonpregnant values in all groups. Mortality among the diabetic dams fed the 30%-fat diet was significantly greater than that among control dams fed the same diet (P<0.01), and pups (>5) survived from only 4 of the 8 litters delivered. Plasma glucose and fructosamine concentrations in the diabetic dams 16 days postpartum were significantly raised compared with control dams fed the breeding diet (diabetic versus control: glucose 26.4±2.3 mmol/L, n=9 versus 11.8±0.7 mmol/L, n=10, P<0.01; fructosamine 178±12 mmol/L, n=9 versus 125±7 mmol/L, n=10, P<0.01) and fed the 30%-fat diet (diabetic versus control: glucose 26.3±2.5 mmol/L, n=8 versus 12.8±1.3 mmol/L, n=10, P<0.01; fructosamine 193±23 mmol/L, n=8 versus 114±5 mmol/L, n=10, P<0.01). The raised plasma glucose concentrations in the control dams were equivalent to those we have previously reported when animals are killed by CO₂ inhalation. Plasma triglyceride concentrations were significantly increased in the diabetic dams fed the 30%-fat diet (4.58±0.90 mmol/L, n=8 versus 1.27±0.25 mmol/L, n=10 for control dams on breeding diet; P<0.01) but not in any other group, whereas the total cholesterol concentration was significantly different only in the normal dams fed the 30%-fat diet, in which it was reduced (1.54±0.15 mmol/L, n=10 versus 1.90±0.09 mmol/L, n=10 for control dams on breeding diet; P<0.05).

Offspring (15 Days)

The offspring of the diabetic dams fed the 30%-fat diet were smaller than offspring of normal dams fed the breeding diet (27.9±4.2 g, n=4 versus 39.6±1.0 g, n=10; P<0.001). All other young offspring were of similar weights. Plasma glucose and triglyceride concentrations were similar between the 4 groups, whereas total cholesterol concentrations were significantly lower in offspring of control and diabetic rats fed the 30%-fat diet compared with control offspring of normal dams fed the breeding diet (Table).

Vascular Function

There were no significant differences in internal artery diameter among groups except between the mesenteric arteries of the nondiabetic dams fed the high-fat diet and the control dams fed the breeding diet (301±8 μm versus 337±7 μm, n=10; P<0.05).
Plasma, Glucose, Total Cholesterol, and Triglyceride Concentrations in 15-Day-Old Offspring

<table>
<thead>
<tr>
<th>Dam</th>
<th>Glucose, mmol/L</th>
<th>Total Cholesterol, mmol/L</th>
<th>Triglycerides, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/breeding diet (n=10)</td>
<td>16.0±1.3</td>
<td>4.17±0.10</td>
<td>2.35±0.10</td>
</tr>
<tr>
<td>Diabetic/breeding diet (n=8)</td>
<td>13.6±1.4</td>
<td>3.89±0.31</td>
<td>1.87±0.23</td>
</tr>
<tr>
<td>Control/30% fat diet (n=8)</td>
<td>14.6±0.8</td>
<td>3.30±0.12</td>
<td>2.29±0.24</td>
</tr>
<tr>
<td>Diabetic/30% fat diet (n=4)</td>
<td>12.7±2.6</td>
<td>2.92±0.30</td>
<td>1.62±0.37</td>
</tr>
</tbody>
</table>

*P<0.01 vs control/breeding diet.
Values are mean±SEM.

Constrictor Responses

Maternal (16 Days Postpartum). Maximal response to NE was increased in arteries from the diabetic dams fed the 30%-fat diet compared with controls fed the breeding diet (140.9±18.8% of contraction to KPSS, n=8 versus 99.7±5.3%, n=10; P<0.05). Neither diabetes alone nor the 30%-fat diet alone was associated with an abnormal maximal response to NE (102.6±3.0%, n=9 and 94.2±2.7%, n=10, respectively). Sensitivity to NE was similar among groups (pEC 50: control/breeding diet, 5.84±0.07 [n=10]; diabetic/breeding diet, 5.84±0.08 [n=9]; control/30%-fat diet, 5.74±0.05 [n=10]; diabetic/30%-fat diet, 5.90±0.08 [n=8]; Figure 1A).

Offspring (15 Days). The maximal response to NE was increased in arteries from offspring of diabetic dams fed the 30%-fat diet compared with offspring of the control dams fed the breeding diet (122.1±14.4% of contraction to KPSS, n=4 versus 83.3±3.2%, n=10; P<0.01). Neither maternal diabetes alone nor a maternal high-fat diet alone was associated with an abnormal maximal NE response in the offspring (102.6±3.0%, n=9 and 94.2±2.7%, n=10, respectively, PS vs control/breeding diet). Sensitivity to NE was similar among groups (pEC 50: control/breeding diet, 6.10±0.11 [n=10]; diabetic/breeding diet, 6.46±0.13 [n=8]; control/30%-fat diet, 5.89±0.08 [n=8]; diabetic/30%-fat diet, 6.26±0.14 [n=4]; Figure 1B). The maximal constrictor response to U46619 was also increased in offspring of diabetic dams fed the 30%-fat diet (148.3±23.3% of contraction to KPSS, n=4; P<0.001 compared with offspring of controls fed the breeding diet), but sensitivity was unaffected (data not shown).

Endothelium-Dependent Relaxation

Maternal (16 Days Postpartum). Maximal relaxation to ACh was impaired in the mesenteric arteries from the diabetic dams fed the 30%-fat diet compared with those of control dams fed the breeding diet (79.6±4.9% of NE-induced contraction, n=8 versus 90.8±3.9%, n=10; P<0.01). Maximal ACh relaxation was similar to controls in diabetic dams fed the breeding diet and control dams fed the 30%-fat diet (94.3±1.9%, n=9 and 93.5±1.6%, n=10, respectively), and sensitivity to ACh was similar between the 4 groups (pEC 50: control/breeding diet, 7.04±0.10 [n=10]; diabetic/breeding diet, 7.00±0.10 [n=9]; control/30%-fat diet, 7.08±0.08 [n=10]; diabetic/30%-fat diet, 7.08±0.19 [n=8]; Figure 2A).

Offspring (15 Days). Maximal relaxation to ACh was significantly reduced in the femoral arteries from the offspring of normal dams fed a 30%-fat diet compared with offspring of controls fed the breeding diet (76.5±3.6% of NE-induced contraction, n=8 versus 85.0±3.4%, n=10; P<0.01) and was further impaired if offspring were from diabetic rats fed the 30%-fat diet (41.1±10.2%, n=4; P<0.001). Maximal relaxation to ACh was normal in arteries from offspring of diabetic animals on a breeding diet (85.4±4.5%, n=8; Figure 2B). Arteries from offspring of diabetic dams fed the 30%-fat diet also showed decreased sensitivity to ACh compared with
those from offspring of controls fed the breeding diet (pEC50: 6.45±0.20, n=4 versus 7.13±0.12, n=10; P<0.01). Sensitiv-
ity to ACh was similar to controls in arteries from offspring
of normal animals fed the 30%-fat diet (pEC50: 6.98±0.12, n=8) and from offspring of diabetic animals fed the breeding
diet (7.19±0.18, n=8; Figure 2B).

**Endothelium-Independent Relaxation**

Maximum relaxation to spermine NONOate was similar in all groups of maternal rats, but sensitivity was significantly
reduced in the offspring of both controls and diabetic dams
fed the 30%-fat diet (pEC50: 5.39±0.07, n=8 and 5.29±0.15, n=4, respectively) compared with offspring of control dams fed the breeding diet (5.84±0.13, n=10; P<0.05 for both groups). Relaxation was not different from controls in the offspring of diabetic dams (pEC50: 5.80±0.13, n=9; Figure 3).

---

**Figure 2.** ACh-induced relaxation in (A) maternal small mesen-
teric arteries 16 days postpartum and (B) femoral arteries of
15-day-old offspring. ○, Control dams fed breeding diet; ●, dia-
betic dams fed breeding diet; ▼, control dams fed high-fat diet;
and ▲, diabetic dams fed high-fat diet. A. **P<0.01 maximum response to ACh in diabetic dams fed high-fat diet vs diabetic
dams fed breeding diet. B. **P<0.01 maximum relaxation to ACh in offspring of high-fat-fed dams vs offspring of controls fed breeding diet; ***P<0.001 maximum relaxation in offspring of high-fat-fed diabetic dams vs offspring of controls fed breeding diet. When absent, error bars lie within symbols.

---

**Figure 3.** Relaxation to the NO donor spermine NONOate in femoral arteries of 15-day-old offspring. ○, Offspring of control dams fed breeding diet; ●, offspring of diabetic dams fed breeding diet; ▼, offspring of control dams fed high-fat diet; and ▲, offspring of diabetic dams fed high-fat diet. *P<0.05 pEC50 for offspring of control dams fed high-fat diet and for offspring of diabetic dams fed high-fat diet vs offspring of control dams fed breeding diet. When absent, error bars lie within symbols.

---

**Study 2: Vascular Function in 60-Day-Old Offspring**

**Weight and Plasma Analyses**

None of the dams fed the 20%-fat diet had difficulty in labor, and the size of the litters was not significantly different from those of dams fed the breeding diet. However, mortality among the neonates remained higher in the offspring of the rats fed the 20%-fat diet (18.6% versus 5.3% for controls). Plasma triglycerides were signif-
icantly raised in the 60-day-old offspring of rats fed 20% fat
compared with 60-day-old controls (1.22±0.08 mmol/L, n=7 versus 0.92±0.11 mmol/L, n=7; P<0.05). No significant differ-
ences were observed in any of the other plasma analyses.

**Vascular Function**

**Constrictor Responses**

Sensitivity to NE was enhanced in arteries from the 60-day-old offspring of dams fed the 20%-fat diet compared with offspring of dams fed the breeding diet (pEC50: 6.36±0.05, n=7 versus 5.92±0.13, n=7; P<0.05). Maximal responses to NE were not significantly different from controls (119.90±9.66% of contraction to KPSS, n=7 versus 95.55±6.33%, n=7; Figure 4A). There were also no significant differences in responses to U46619 between offspring of dams fed 20% fat or of controls fed the breeding diet (pEC50: 6.27±0.15, n=7 versus 6.08±0.07, n=7; maximal response 110.66±8.98%, n=7 versus 99.25±17.90%, n=7).

**Endothelium-Dependent Relaxation**

Arteries from the 60-day-old offspring of 20%-fat-fed dams demonstrated decreased maximal relaxation to ACh (56.47±1.36% of NE-induced preconstriction, n=7 versus 72.99±4.08%, n=7 for controls; P<0.001), whereas sensitivity was similar to controls (pEC50: 7.07±0.28, n=7 versus 7.19±0.19, n=7 for controls; Figure 4B).
Endothelium-Independent Relaxation

Relaxation to spermine NONOate was similar in 60-day-old offspring of controls and 20%-fat-fed dams (pEC50: 6.19±0.40, n=7 versus 6.08±0.39, n=7 for controls; maximum relaxation 55.63±5.40% of NE-induced constriction, n=7 versus 59.00±6.01%, n=7 for controls; Figure 4C).

Discussion

This study has shown that excessive saturated fat intake in pregnant rats can lead to disturbances of vascular function in weanlings and young adult offspring. This is a novel observation that provides support for the hypothesis that in utero events related to maternal nutrition may adversely affect specific pathways of vascular control and may predispose the offspring to vascular disease in later life.1

The femoral arteries of both young and older offspring of normal pregnant rats fed a diet enriched with lard showed poor endothelium-dependent relaxation. In the young weanlings, this was likely to be attributable in part to reduced vascular smooth muscle sensitivity to NO or to more rapid NO degradation, because the response to spermine NONOate was also blunted. Reduced relaxation to ACh and the NO donor in vitro provide evidence for abnormalities that, in vivo, could affect many aspects of normal vascular function. The enhanced sensitivity to NE in arteries from the young adults could also contribute to elevation of peripheral vascular resistance.

To the best of our knowledge, no study has previously directly investigated vascular function in the offspring of rats fed a high-saturated-fat diet, although raised blood pressure has been recorded9 in young adult offspring of dams fed a diet rich in coconut oil. Abnormal cholesterol metabolism has also been reported in the offspring of rats fed a diet rich in corn oil when the young animals were challenged with the same diet.10 In humans, the high incidence of fatty streaks in human fetal arteries and the correlation with maternal cholesterol also provides some evidence for the acquisition of vascular disease in utero.11

The origin of the vascular dysfunction induced in the offspring cannot be directly inferred from the present study. None of the plasma lipids measured were overtly raised in the 15-day-old offspring, but plasma triglycerides were elevated in the young adults. Hypertriglyceridemia may be indicative of insulin resistance, and triglycerides have been implicated in endothelial dysfunction.12 Hypercholesterolemia, frequently implicated in endothelial dysfunction, was not observed in the offspring; indeed, cholesterol was reduced in the weanlings. The low plasma cholesterol in the fat-fed dams agrees with previous studies in fat-fed normal rats13 and is characteristic of the rodent response to a saturated-fat diet. Free fatty acids could also play a role, because saturated fats interfere with the metabolism of fatty acids,14 some of which are precursors of vasoactive prostanoids.15

The poor reproductive performance in the dams fed the high-fat diet agrees with a similar study of fat-fed rats 16 in which a 33% reduction in pregnancy rate and high pup mortality were observed. Fat-induced reproductive dysfunction is proposed to result from energetic inhibition of reproduction,17 inability of pups to metabolize the longer-chain fatty acids in the dams’ milk,18 or maternal behavioral disorder, leading to cannibalism.19

Severe maternal diabetes together with high-fat feeding led to higher mortality among the pups. Those that survived were small
but apparently healthy. However, the weanlings’ femoral arteries were more responsive to NE. There was also deterioration of endothelium-dependent relaxation compared with offspring of nondiabetic dams fed the fat diet, whereas the response to the NO donor was similar. These offspring had acquired an endothelial defect similar to that observed in arteries of diabetic adult rats27 and in patients with diabetes.2,12 Because maternal diabetes per se was not associated with offspring vascular malfunction, the data imply that diabetes increased the susceptibility of the offspring vasculature to high maternal fat intake. We have reported both the abnormal transfer of certain free fatty acids across the isolated placenta in diabetic rats fed a 30%-saturated-fat diet23 and raised fetal blood glucose, the latter probably a reflection of fetal insulin resistance.24 The resultant fatty acid imbalance and/or fetal hyperglycemia potentially could lead to irreversible vascular dysfunction, maintained or “imprinted” in the vasculature of the newborn. The growth retardation in these offspring, sometimes observed in severe human diabetes, could also have contributed to cardiovascular dysfunction, because offspring of nutritionally deprived2,3 dams are reported to have raised blood pressure. Moreover, fetuses of severely diabetic dams are insulinopenic6 and may have lower insulin-like growth factor-1, both of which may lead to reduced growth and blunted NO synthesis.25,26

All offspring were suckled by their dams, and a contribution of the composition of the dam’s milk to offspring vascular malfunction cannot be discounted. The abnormal nutrient composition, particularly of fatty acids, described in the milk of diabetic rats27 is likely to be further disturbed by a saturated fat diet. Additional studies in which offspring are cross-fostered with normal or diabetic dams would determine the relative importance of the suckling period compared with the influence of the in utero environment.

Diabetes was not associated with abnormal maternal vascular function, which contrasts with the endothelial dysfunction reported in previous studies in virgin diabetic animals28 and may reflect a pregnancy-induced “protection” against endothelial damage. However, when pregnant animals were fed the high-fat diet, vascular dysfunction was evoked in the maternal mesenteric arteries and was similar to that in the arteries of the offspring. Alone, this group of dams demonstrated an elevation of plasma triglycerides, which is also implicated in endothelial dysfunction.12

In conclusion, the data presented in this study suggest strongly that excessive maternal fat intake can play an important role in the development of cardiovascular disorders in the offspring and that maternal diabetes may present an additional confounding influence.

Acknowledgments
The authors thank the trustees of St. Thomas’ Hospital and Tommy’s Campaign for financial support.

References
Offspring of Normal and Diabetic Rats Fed Saturated Fat in Pregnancy Demonstrate Vascular Dysfunction
E. Koukkou, P. Ghosh, C. Lowy and L. Poston

Circulation. 1998;98:2899-2904
doi: 10.1161/01.CIR.98.25.2899

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/98/25/2899

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