Spatial Heterogeneity in Fasting and Insulin-Stimulated 18F-2-Deoxyglucose Uptake in Pigs With Hibernating Myocardium

James A. Fallavollita, MD

Background—Previous studies of hibernating myocardium in the fasting state have shown regionally increased 18F-2-deoxyglucose (FDG) uptake with a marked transmural gradient. We hypothesized that this adaptation to chronic ischemia might be associated with altered maximal FDG uptake.

Methods and Results—Pigs were instrumented with a 1.5-mm proximal left anterior descending artery (LAD) stenosis. Studies were conducted 106±4 days later on anesthetized animals with complete LAD occlusion and anterograde dysfunction. In fasting animals (n=9), FDG uptake in dysfunctional LAD regions was 2-fold higher than in normally perfused myocardium (7.9±1.2 versus 4.0±0.5 μmol min⁻¹ 100 g⁻¹, P<0.05), with a pronounced transmural gradient (endocardial/epicardial ratio 2.56±0.19 versus 1.25±0.03, P<0.05). Euglycemic, hyperinsulinemic clamp (insulin clamp, n=8) produced a 5- to 9-fold increase in FDG uptake, but there was no longer a regional difference in accumulation (LAD, 37.8±4.2 versus normal, 36.4±5.1 μmol min⁻¹ 100 g⁻¹, P=NS) and the transmural distribution was uniform. FDG uptake in the fasting state varied inversely with coronary flow during vasodilation. In contrast, during insulin clamp there was no relation between FDG uptake and vasodilated flow, resulting in a reduced spatial heterogeneity in individual samples (relative dispersion=SD/mean; fasting, 52±5% versus insulin, 24±2%, P<0.05).

Conclusions—In the fasting state, FDG uptake in pigs with hibernating myocardium was heterogeneous and was increased in dysfunctional regions with a marked transmural gradient and high spatial heterogeneity. In contrast, FDG uptake was more homogeneously distributed during insulin clamp with (1) uptake in dysfunctional myocardium similar to remote normal regions, (2) uniform transmural distribution, and (3) reduced spatial heterogeneity. (Circulation. 2000;102:908-914.)

Key Words: collateral circulation ■ hibernation ■ glucose ■ insulin

In the fasting state, viable chronically dysfunctional myocardium exhibits enhanced uptake of the positron-emitting glucose analog 18F-2-deoxyglucose (FDG) in comparison to normally perfused myocardium in humans1 and in pigs.2,3 Evaluating transmural FDG uptake in pigs revealed that the greatest increase in deposition was localized to the subendocardium and that local uptake varied inversely with local coronary flow reserve.2 In contrast, clinical studies performed with PET have shown that FDG uptake increases during euglycemic hyperinsulinemic clamp (insulin clamp), but it is unclear if maximal FDG uptake in dysfunctional regions is normal4 or mildly reduced.1,5 While speculative, a small reduction in full-thickness insulin-stimulated FDG uptake could be even more pronounced in the subendocardium and raise the possibility of a negative relation between chronic repetitive ischemia and maximal glucose transport.

Since transmural variations in FDG uptake can only be assessed by ex vivo counting, the present study was performed in pigs with hibernating myocardium. The primary objectives of this study were to (1) determine if FDG uptake during insulin clamp in pigs with chronically dysfunctional myocardium is similar to humans with hibernating myocardium, (2) determine the magnitude of FDG uptake during insulin clamp and to identify whether a transmural gradient favoring the subendocardium exists, (3) determine the relations between flow (both resting and vasodilated) and FDG uptake (both fasted and during insulin clamp) as indexes of flow-metabolism mismatching, and (4) determine the spatial heterogeneity of flow and FDG uptake in normal and hibernating myocardium.

Methods

All experimental procedures and protocols conform to institutional guidelines for the care and use of animals in research. The initial instrumentation and experimental protocol have been previously published in detail.2,3,6 Briefly, juvenile pigs (n=19) were instrumented with a 1.5-mm Delran stenosis on the proximal left anterior descending artery (LAD). Three to 4 months later, the pigs were fasted overnight. Anesthesia was induced with a Telazol (50 mg/mL tiletamine and 50 mg/mL zolazepam)/100 mg/mL xylazine) mixture (0.022 mL/kg IM) and maintained with isoflurane (1% to 3%) supplemented with additional Telazol/xylazine (0.011 mL/kg IM PRN). Catheters were placed retrograde from the carotid arteries into the left atrium for pressure monitoring and microsphere injection and...
TABLE 1. Hemodynamic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Fasting (n=9)</th>
<th>Insulin Clamp (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>88±3</td>
<td>97±5</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>80±3</td>
<td>113±2*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>13.9±1.4</td>
<td>13.3±2.3</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; LVEDP, left ventricular end-diastolic pressure.

Values are mean±SEM.

*P<0.05, fasting vs insulin values; †P<0.05 vs rest values.

FDG Quantification by Ex Vivo Tissue Counting

One hour after the last pharmacological intervention, blood was obtained for metabolic substrate levels. Glucose and lactate were quantified by an automated oxidation analysis (all animals, ABL System 625, Radiometer Medical A/S). An enzymatic colorimetric assay was used to quantify nonesterified fatty acids (fasting, n=8; insulin, n=5; NEFA C, Wako Chemicals USA, Inc), and a radioimmunoassay was used to quantify insulin (fasting, n=8; insulin, n=5; Biotrak, Amershams International). Ten animals received FDG in the fasting state, and in 9 animals glucose (and FDG) uptake was stimulated with a euglycemic hyperinsulinemic clamp. Insulin (regular purified pork insulin, 100 U/mL, Novo Nordisk) was infused at 1 mU · kg⁻¹ · min⁻¹ with a 20% dextrose infusion adjusted to maintain glucose at preinsulin levels. Insulin and glucose infusions were continued until the heart was removed for sampling.

FDG (1 to 2 mCi, Department of Nuclear Medicine, University at Buffalo; Buffalo, NY)⁸ was injected as a bolus, and an arterial sample was withdrawn (1 mL/min) for 45 minutes to determine the integrated FDG time-activity curve. After FDG accumulation, the heart was fixed with intravenous KC1 and rapidly excised. A mid-ventricular ring was divided into 12 full-thickness wedges, which were subdivided into subendocardial, mid-myocardial, and subepicardial layers. Samples were placed into tared vials, weighed, and annihilation γ-radiation at 511 keV measured in a γ-counter (model 1470, EG&G Wallac Inc). The same samples were used for microsphere flow determinations. The average sample weight (n=491) was 0.89±0.01 g.

FDG deposition was determined by dividing FDG activity in individual samples by the integrated arterial input curve.² The rate of FDG uptake (RFDGU in μmol · min⁻¹ · 100 g⁻¹) was estimated as the product of FDG deposition · glucose · 100².

Histology

Myocardial rings apical and basal to the ring used for microsphere and FDG analyses were incubated in triphenyl tetrazolium chloride to exclude myocardial necrosis. Additional samples were trichrome stained to quantify connective tissue by standard point-counting techniques.² Two animals had gross evidence of myocardial infarction (1 fasting and 1 insulin-stimulated) that encompassed >1% of the left ventricular mass. They were excluded from further analysis; therefore, the final results compare 9 animals receiving FDG in the fasting state and 8 animals that were injected during insulin clamp.

Data Analysis

Data are presented as mean±SEM. Flow and FDG in the LAD and normal regions represent weighted means for all samples within a given region after the perfusion boundaries were determined from the distribution of flow during vasodilation.² Relative dispersion (RD; SD/mean) was determined on a regional basis per pig. Measurements in the LAD and normal regions were compared by means of paired t tests. Differences between pharmacological interventions were assessed by means of ANOVA and t tests with the Bonferroni correction for multiple comparisons. Fasting and insulin-stimulated groups were compared by means of unpaired t tests. A value of P<0.05 was considered significant.

Results

Flow and Function in Pigs With Hibernating Myocardium

Animals were studied 106±4 days after instrumentation, at which time they were in good health and weighed 85±5 kg. Blood gases and hematocrits were no different between the groups and averaged: pH, 7.41±0.00; Pco₂, 46±1 mm Hg, Po₂, 511±21 mm Hg; Hct, 33±1%. Baseline hemodynamics (Table 1) were slightly lower in the fasting group; however, resting microsphere flow measurements were not different.

Figure 1 illustrates the transmural distribution of flow in the dysfunctional LAD region versus the normally perfused region at rest, during submaximal epinephrine infusion, and during adenosine vasodilation. Subendocardial flow and the endocardial/epicardial (endo/epi) ratio were regionally reduced at rest (Table 2). In contrast to the increases in flow to remote normal regions during inotropic (epinephrine) and vasodilatory stimuli (adenosine), flow was significantly lower than baseline in the LAD subendocardium during these interventions and accompanied by a fall in the endo/epi ratio.

Anterograde wall motion was reduced in all animals, with an average wall motion score of 0.9±0.2 (normal=3) and a corresponding centerline score of −1.85±0.14 (normal=0). Both scores improved during epinephrine infusion (wall motion score 0.9±0.2 to 1.2±0.2, P=0.10; centerline score −1.85±0.14 to −1.52±0.19, P<0.05). Connective tissue staining by point counting was slightly increased in the dysfunctional LAD region in comparison to normally perfused myocardium (8.0±0.8% versus 3.8±0.2%, P<0.05).
FDG Uptake: Fasting Versus Euglycemic Hyperinsulinemic Clamp

Hemodynamics, substrate, and insulin levels obtained immediately before FDG administration are presented in Table 3. Hemodynamics had returned to baseline in each group. Insulin clamp resulted in a 20-fold increase in serum insulin, a slight increase in glucose, and reduced free fatty acids. Figure 2 illustrates the RFDGU in viable, dysfunctional LAD regions (left) was compared with flow in normally perfused remote regions (right). Normal region demonstrated significant increases in flow over resting values during epinephrine infusion and adenosine vasodilation in each layer of myocardial wall. In contrast, subendocardium (Endo) of LAD region actually showed reduced perfusion during each intervention, demonstrating critical reduction in flow reserve. Although flow to subepicardium significantly increased with metabolic and pharmacological stimuli, mid-myocardial (Mid) and full-thickness weighted averages (FT) did not change in comparison to resting values. Epi indicates epicardium.

Better correlations were found for fasting FDG uptake with the use of maximal flow during pharmacological vasodilation as an index of the susceptibility to develop myocardial ischemia (Figure 5, upper graph). In contrast to a weak relation in the normally perfused remote region ($r^2=0.03$), the hibernating region demonstrated a steep inverse relation with dramatic increases in FDG uptake at reduced levels of vasodilated flow ($r^2=0.50$). During insulin clamp (Figure 5, lower graph), similar FDG uptake in hibernating and normal fasting conditions was completely abolished during insulin clamp.

Correlation of Flow and FDG Uptake: Fasting Versus Euglycemic Hyperinsulinemic Clamp

As a method of assessing the flow-metabolism mismatch in hibernating myocardium, flow and FDG uptake were correlated in individual myocardial samples (Figures 4 and 5). Under resting conditions in the fasting state (Figure 4, upper graph), there was no correlation between flow and FDG uptake. However, during insulin clamp (Figure 4, lower graph), weak inverse correlations were present between resting flow and FDG uptake in both hibernating and normally perfused regions ($r=0.04$ to 0.07).

### TABLE 2. Subendocardial Perfusion and Endo/Epi Ratios

<table>
<thead>
<tr>
<th></th>
<th>Endo</th>
<th>Endo/Epi</th>
<th>Endo</th>
<th>Endo/Epi</th>
<th>Endo</th>
<th>Endo/Epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD</td>
<td>0.92±0.05*</td>
<td>1.03±0.05*</td>
<td>0.64±0.08†</td>
<td>0.57±0.07*</td>
<td>0.60±0.12†</td>
<td>0.31±0.04†</td>
</tr>
<tr>
<td>Normal</td>
<td>1.03±0.06</td>
<td>1.24±0.02</td>
<td>1.38±0.12†</td>
<td>1.17±0.04</td>
<td>4.10±0.28†</td>
<td>0.84±0.03†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Endo values are in mL·min⁻¹·g⁻¹.

*P<0.05, LAD vs normal values; †P<0.05 vs rest values.
myocardium resulted in no correlation between FDG uptake and local coronary flow reserve. Thus, the strongest correlation was between vasodilated flow and FDG uptake under fasting conditions, suggesting that the propensity of a region to develop ischemia is associated with increased FDG uptake in the fasting state.

Spatial Heterogeneity of FDG Accumulation
The spatial heterogeneity of FDG uptake and flow was determined in dysfunctional and normal regions from both fasting and insulin-stimulated animals. RD are shown in Table 5. Under fasting conditions, the heterogeneity of FDG uptake in the LAD region (RD=0.45±0.03) was 3-fold higher than in normal myocardium (0.15±0.02, P<0.05), reflecting both the marked transmural variation in FDG uptake and the variability among samples within a given region. In contrast, during insulin stimulation, not only was FDG uptake similar in dysfunctional and normal regions, but uptake among individual samples was also more homogeneously distributed. The RD of FDG uptake during insulin stimulation was similar in dysfunctional (0.21±0.03) and normal regions (0.17±0.03) and not significantly different from the RD of resting flow (0.15±0.01). To directly compare the spatial heterogeneity of resting flow, adenosine flow, and FDG uptake, all samples from the normal regions of both groups of animals were combined (Figure 6). The RD of resting flow (0.15±0.01), adenosine flow (0.15±0.01), and FDG uptake (0.16±0.02) were very similar.

Discussion
There are several important new findings from this investigation. First, like humans, pigs with hibernating myocardium had a 2-fold regional increase in the FDG uptake in the fasting state but normal FDG uptake when assessed during euglycemic hyperinsulinemic clamp. Second, stimulation of fasting state but normal FDG uptake when assessed during

FDG Administration

<table>
<thead>
<tr>
<th>Heart rate, bpm</th>
<th>MAP, mmHg</th>
<th>Glucose, mmol/L</th>
<th>Lactate, mmol/L</th>
<th>Free Fatty Acids, meq/L</th>
<th>Insulin, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>81.4±4</td>
<td>87.4±6</td>
<td>4.0±0.7</td>
<td>2.2±0.2</td>
<td>0.62±0.06</td>
</tr>
<tr>
<td>Insulin clamp</td>
<td>85±8</td>
<td>88±3</td>
<td>6.2±0.7*</td>
<td>2.0±0.2</td>
<td>0.09±0.01*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure.
Values are mean±SEM.
*P<0.05, insulin vs fasting.

TABLE 4. Subendocardial and Full-Thickness RFDGUs (μmol·min⁻¹·100 g⁻¹) and Endo/Epi Ratios

<table>
<thead>
<tr>
<th>Subendocardial</th>
<th>Full-Thickness</th>
<th>Endo/Epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>12.0±2.0*</td>
<td>7.9±1.2*</td>
</tr>
<tr>
<td>Normal</td>
<td>4.5±0.5</td>
<td>4.0±0.5</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>37.2±4.4†</td>
<td>37.8±4.2†</td>
</tr>
<tr>
<td>Normal</td>
<td>38.9±6.8†</td>
<td>36.4±5.1†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*P<0.05, LAD vs normal; †P<0.05, fasting vs insulin.

Figure 3. Circumferential distribution of FDG uptake during insulin clamp. Regional FDG activity for contiguous wedges (full-thickness weighted average) is expressed as percentage of mean value of each animal. There were no regional variations in FDG accumulation in pigs with hibernating myocardium during insulin clamp.

TABLE 5. Under fasting conditions, the heterogeneity of FDG accumulation in pigs with hibernating myocardium during insulin clamp. Second, stimulation of glucose uptake by insulin clamp abolished the marked transmural gradient in FDG uptake present in viable, dysfunctional myocardium under fasting conditions and abolished the inverse relation between FDG uptake and local coronary flow reserve. Finally, the strongest correlation between perfusion and FDG uptake was evident between vasodilated flow and FDG uptake under fasting conditions. This suggests that the most important determinant of regional alterations in FDG uptake in hibernating myocardium is the propensity of a region to develop ischemia.

Insulin-Stimulated FDG Accumulation in Hibernating Myocardium
In the fasting state, the finding of regionally increased FDG uptake in pigs with hibernating myocardium is in agreement with patients with coronary artery disease. However, previous studies with insulin clamp have shown FDG uptake to be reduced or unchanged in hibernating as compared with normal remote regions. Using segmental analysis, Mäki et al found a small but significant reduction in FDG uptake in dysfunctional regions of 7 patients with collateral-dependent myocardium and no history of prior myocardial infarction (72±22 versus 79±21 μmol·min⁻¹·100 g⁻¹ in normal remote regions, P<0.05). Gerber et al reported similar results in viable segments of patients with and those without prior infarction. FDG uptake in dysfunctional regions was 20% lower as compared with remote regions (38±20 versus 47±18 μmol·min⁻¹·100 g⁻¹, P<0.05). In contrast, when Marinho et al accounted for potential regional differences in myocardial fibrosis by quantifying water-perfusable tissue, no regional variations in FDG uptake were found.
FDG uptake in viable segments of patients with prior myocardial infarction was the same as normally perfused remote regions (44.14 ± 61.19 m mol−1 min−1 g−1, P = NS). These results are qualitatively and quantitatively similar to the results of the present study in which FDG uptake during insulin clamp was the same in hibernating and normal regions. Thus, the finding of homogeneous FDG uptake during insulin clamp by Marinho et al.4 and the present study support a role of regional fibrosis as the explanation for reduced FDG uptake in some clinical studies, as suggested by Shilvalkar et al.10

Increased FDG uptake in the fasting state has been speculated to reflect alterations in myocardial glucose utilization, resulting in altered glucose transporter expression and increased transport capacity. Quantification of mRNA from biopsies of patients with hibernating myocardium has demonstrated induction of the glucose transporter primarily responsible for basal glucose uptake, GLUT1, with no change in the other major myocardial glucose transporter, GLUT4.11 However, the finding that FDG uptake was similar in hibernating and normal myocardium during insulin clamp argues against altered levels of recruitable glucose transport capacity. Thus, the present study favors a simpler hypothesis for the enhanced basal glucose uptake in hibernating myocardium, that is, a chronic translocation of a portion of intracellular GLUT1 or GLUT4. Further studies will be required to specifically address this issue.

The present data in fasting animals confirms our previous report of a pronounced transmural gradient in FDG uptake in hibernating myocardium (endo/epi ratio = 2.56 ± 0.19) and an inverse correlation of FDG uptake and local flow reserve in individual samples.6 Insulin clamp increased FDG uptake in both hibernating and normal regions, resulting in a more homogeneous distribution throughout the left ventricle. This was associated with a loss of the transmural gradient in viable, chronically

<table>
<thead>
<tr>
<th>TABLE 5. Relative Dispersions of FDG and Resting Flow in Fasting and Insulin-Stimulated Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>LAD</td>
</tr>
<tr>
<td>Normal</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05, LAD vs normal; †P<0.05, fasting vs insulin.
A close correlation between flow and metabolism during resting conditions (as evidenced by reductions in subendocardial ischemia,12 but the relative dispersion of flow and FDG has not been previously examined. The RD of resting flow, adenosine flow, and FDG uptake in the present study were nearly equivalent (RD=0.15 to 0.16, mean sample weight 0.92±0.02 g, n=276) and similar to that reported for resting flow in 1-g samples with radioactive microspheres in awake baboons (RD=0.17).13,14 Under fasting conditions, a modest transmural gradient in FDG uptake was present in normally perfused myocardium (endo/epi ratio=1.25±0.03), closely approximating the gradient in resting perfusion (endo/epi ratio=1.24±0.02). Similar findings have been previously reported.15 Although the transmural gradients in resting flow and FDG uptake suggest that they may be matched, absolute resting flow and FDG uptake were not correlated under fasting conditions (Figure 4, upper graph). However, when resting flow and FDG uptake were normalized to the average value per region,16 a significant correlation was present (Figure 7). Nevertheless, this relation accounted for only a small portion of the variability (r²=0.13), suggesting that factors other than those responsible for the local regulation of perfusion determine myocardial glucose uptake. A similar normalized result was reported in anesthetized dogs with the use of ²H₂-deoxyglucose and radioactive microspheres (mean r²=0.24, range 0.11 to 0.59).16 These findings in normal and chronically dysfunctional myocardium appear to be consistent with the weak relation between flow and FDG uptake at moderate levels of acute ischemia17 and support the contention that in contrast to other metabolic substrates, delivery is not the primary determinant of FDG/glucose uptake.17

Methodological Limitations

All animals underwent interventions that resulted in subendocardial ischemia (as evidenced by reductions in subendo-cardial perfusion) before FDG administration. Since ischemia is known to stimulate glucose (and FDG) uptake, regional and transmural differences in the FDG uptake could simply reflect antecedent ischemia. However, PET studies in pigs with hibernating myocardium provided quantitatively similar regional variations in FDG uptake when compared with animals that underwent a protocol nearly identical to the present fasting studies (LAD/normal, 1.8±0.2 by PET versus 1.9±0.1 by ex vivo counting).2 This similarity suggests that a 1-hour interval after pharmacological interventions is adequate for glucose (and FDG) uptake to return to baseline. Chronically dysfunctional myocardium with the physiological features of hibernating myocardium developed in normal pigs in the absence of risk factors known to be associated with coronary artery disease. Specifically, insulin resistance, which is present in patients with coronary artery disease,18 is unlikely to be a feature of this porcine model. Further studies will be required to determine the impact of insulin resistance on FDG distribution in viable, chronically dysfunctional myocardium in humans.

Clinical Implications

FDG uptake in the fasting state was regionally increased in viable, chronically dysfunctional myocardium in comparison to regions with normal coronary flow reserve. In contrast, FDG uptake during insulin clamp resulted in homogeneous uptake. Thus, imaging in the fasting state would accentuate the “flow-metabolism mismatch” between hibernating and normal myocardium.1 However, imaging in the fasting state would limit the technical quality of the images and complicate the placement of regions of interest caused by the similarity of FDG activity between normal regions and the blood pool. In addition, FDG uptake in the fasting state was associated with greater spatial heterogeneity in viable, chronically dysfunctional myocardium, potentially limiting the size of regions of interest. Since insulin-stimulated FDG uptake is
normal in hibernating myocardium, our data would support the assessment of myocardial viability with imaging during insulin clamp.

Acknowledgments
This study was supported by Clinician Scientist and Affiliate Grant-in-Aid awards from the American Heart Association and a Merit Review Award from the Department of Veterans Affairs. The author would like to thank Dr John Canty for his constructive critiques of the manuscript and Deana Grettka, Jennifer Mortellaro, Amy Johnson, Susan Popeano, Anne Coe, Felicia Bosinski, and Randy Bassett for technical assistance.

References
Spatial Heterogeneity in Fasting and Insulin-Stimulated $^{18}$F-2-Deoxyglucose Uptake in Pigs With Hibernating Myocardium

James A. Fallavollita

_Circulation_. 2000;102:908-914
doi: 10.1161/01.CIR.102.8.908

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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/102/8/908

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/