Tolerance to isosorbide dinitrate: rate of development and reversal


ABSTRACT The circulatory response and plasma concentrations of isosorbide dinitrate (ISDN) were determined in 10 patients with chronic, stable angina after administration of 5 mg of sublingual ISDN during the control stage, after 48 hr of therapy with 15 mg of ISDN orally every 6 hr, and subsequently after a 48 hr period when ISDN was substituted by placebo four times daily. Initially, sublingual ISDN induced major reductions in both supine and standing systolic and diastolic blood pressure, but after 45 hr of therapy with oral ISDN, there was a significantly diminished vasodepressor response in both positions. Subsequently, when placebo was substituted for ISDN, the circulatory response initially seen was restored within 21 hr. Plasma ISDN concentrations after the test sublingual dose were slightly higher after 48 hr of oral ISDN dosing (i.e., the tolerant state) than at the start of the study. This suggests that tolerance is unlikely to be caused by reduced bioavailability or accelerated elimination of ISDN. It is possible that tolerance is related to accumulation of ISDN metabolites. The attenuation of the circulatory response to ISDN may be related to the altered antianginal efficacy commonly seen during sustained therapy with ISDN.


ISOSORBIDE DINITRATE (ISDN) is widely employed in the management of patients with angina pectoris and is used with increasing frequency for impendence reduction in patients with left ventricular dysfunction. The usefulness of this drug was initially questioned because of extensive first-pass metabolism in the animal liver, but recent investigations have demonstrated that orally administered ISDN is readily detected in systemic blood and induces important hemodynamic effects and is effective in the treatment of angina pectoris and left ventricular dysfunction. A significant concern in the sustained use of organic nitrates is nitrate tolerance. Studies have shown rapid development of tolerance to the organic nitrates in animals and man and cross tolerance among various nitrates. Tolerance in humans was suggested in munitions workers when headaches and postural lightheadedness, which occurred with initial exposure, cleared within a few days of continued exposure. Loss of nitrate tolerance in these workers also occurred rapidly, as indicated by the return of the original symptoms after a weekend away from exposure to nitrate, the so-called Monday disease or Monday head. Recent clinical studies have confirmed the early development of partial tolerance to the circulatory effects of ISDN and reduced antianginal efficacy after only 1 week of oral therapy.

The present investigation was designed to determine the rate of development of tolerance during initiation of therapy with oral ISDN and the rate of tolerance reversal after withdrawal of this drug.

Methods

Study population. Ten patients, nine men and one woman, ranging in age from 37 to 77 years (mean 56), with stable, exercise-induced angina pectoris were studied. Patients who had had previous exposure to ISDN or other oral or cutaneous nitrate preparations were not included. These patients had been taking nitroglycerin, but this was not used in the 24 hr before study and was not required in any patient during this period of investigation. Other exclusion characteristics included the presence of cardiomegaly, cardiac failure, cerebral vascular disease, hepatic disease, renal dysfunction, or the administration of β-blocking agents or vasodilators. Patients were also excluded if they had a history of severe headaches or symptoms of postural hypotension after nitroglycerin administration.

Study design

Day 1. After an overnight fast, control measurements were made of heart rate and blood pressure with patients in the supine and standing positions, and a 5 ml sample of venous
blood was obtained. At approximately 9 A.M., 5 mg of sublingual ISDN was administered; the patients were instructed to indicate the time of dissolution of the tablet and were asked not to swallow their saliva. The heart rate and blood pressure measurements and venous blood samples were obtained at 2, 5, 10, 20, 30, 45, and 60 min and then at 30 min intervals for a total duration of 3 hr. At approximately 12 noon, 15 mg of ISDN was given orally and heart rate and blood pressure measurements were recorded hourly for 5 hr. Subsequent doses of ISDN were given at 6 P.M. and 12 midnight.

Day 2. Fifteen milligrams of ISDN was administered orally at 6 A.M., 12 noon, 6 P.M., and 12 midnight.

Day 3. After an overnight fast, patients were studied at 9 A.M., i.e., 9 hr after the last dose of ISDN. The heart rate, blood pressure, and blood samples were taken before and after administration of 5 mg sublingual ISDN as on day 1. In addition, blood samples were collected at 60, 30, and 5 min before sublingual dosing. The plasma ISDN concentrations determined were then extrapolated by linear regression to correct for residual ISDN contributed by the last oral dose. At approximately 12 noon, 15 mg of ISDN was administered orally and heart rate and blood pressure were recorded at hourly intervals for 5 hr as on day 1. Subsequently, an ISDN placebo was administered at 6 hr intervals, but the 6 A.M. dose was withheld on days 4 and 5.

Day 4. After an overnight fast, 9 hr after the last ISDN placebo and 21 hr after the last dose of ISDN, the patients were given 5 mg of sublingual ISDN and heart rate and blood pressure measurements were made over a 2 hr period, but no blood samples were obtained. If the systolic blood pressure decline approximated that seen on day 1, suggesting reversal of nitrate tolerance, the patient was studied again on the following day (day 5) with sublingual ISDN. On this occasion, in addition to measurements of heart rate and blood pressure, venous blood samples were drawn for measurement of ISDN concentration over a 3 hr period as on days 1 and 3. One patient did not show tolerance reversal until 48 hr after oral ISDN withdrawal (day 5), so the final study was carried out on day 6. The data from the patient studied on day 6 are included with the others and reported as day 5.

This study was single blind in design. All studies were carried out by one individual, with blood pressure measurements made using an sphygmomanometer; single measurements were made from the same arm in each subject.

Venous blood (5 ml) was collected in tubes containing EDTA for determination of plasma ISDN concentrations. The total blood loss was 190 ml over a period of 5 days. The plasma was immediately separated by centrifugation and stored at -20° C for later analysis.

Statistical analysis was carried out with a two-way analysis of variance with days fixed and subjects random. Multiple comparison techniques were used to compare pairs of drugs and maintain the significance level at 5%.

ISDN plasma concentrations were determined in six of the patients by means of gas-liquid chromatography as previously described. 18, 19

Results

Circulatory effects. Standing heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP) before and after sublingual ISDN on days 1, 3, 4, and 5 are shown in table 1. Similar data after oral ISDN administration on days 1 and 3 are shown in table 2.

Sublingual ISDN

SBP (table 1, figure 1)

Standing. On day 3, after 48 hr of oral ISDN therapy and 9 hr after the last oral dose, the decline in SBP after the sublingual ISDN challenge was significantly less marked than that observed on day 1 (p < .025). On days 4 and 5, after institution of placebo ISDN therapy, the decline in SBP after sublingual ISDN challenge was similar to that observed on day 1 and significantly greater than that seen on day 3 (p < .025). The duration of the hypotensive effect after sublingual ISDN also differed during the study period. On day 3, SBP had returned to normal at the end of the 3 hr observation period, while the blood pressure had remained depressed at this time on days 1 and 5. No data are available beyond 2 hr on day 4.

Supine. The changes in supine SBP were less marked than those seen in the standing position. However, a similar pattern of change occurred, with a decrease on day 3 that was significantly less than that observed on days 1, 4, and 5 (p < .02).

The duration of the hypotensive effect on supine SBP was also different during the four study days. On day 3 the supine SBP had returned to normal after 3 hr, but this remained depressed at this time on days 1, 4, and 5.

DBP and heart rate (table 1). The changes in DBP followed a trend similar to that for changes in SBP, but there were no significant changes seen between the various study days in either the supine or standing positions. With the decline in SBP after sublingual ISDN, there were increases in heart rate, but there were no significant differences among the four study days.

Oral ISDN (table 2, figure 2). The changes in SBP after oral ISDN were similar during days 1 and 3, the only days when this was studied. It is important to point out that the SBP when the initial oral ISDN was administered on day 1 was still depressed by the sublingual ISDN given 3 hr previously. Thus the study design does not permit a comparison between the changes in SBP during initial and sustained therapy.

Plasma ISDN levels. Plasma concentrations before and after sublingual ISDN administration on days 1, 3, and 5 were determined for six patients in the study (figure 3). The peak plasma ISDN concentration and the area under the time-concentration curve were higher on day 3 than on day 1 (p < .05).

Adverse effects. After the initial sublingual ISDN administration, two patients had moderately severe headaches, which decreased in severity with subsequent ISDN therapy. Three patients experienced postural
TABLE 1

Circulatory effects of sublingual ISDN (mean ± SD)

<table>
<thead>
<tr>
<th>Day</th>
<th>C</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Standing</td>
<td>±22</td>
<td>±17</td>
<td>±15</td>
<td>±12</td>
<td>±9</td>
<td>±12</td>
<td>±8</td>
<td>±10</td>
<td>±12</td>
<td>±12</td>
<td>±12</td>
</tr>
<tr>
<td></td>
<td>Supine</td>
<td>125</td>
<td>119</td>
<td>114</td>
<td>110</td>
<td>106</td>
<td>109</td>
<td>110</td>
<td>112</td>
<td>115</td>
<td>116</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±22</td>
<td>±20</td>
<td>±15</td>
<td>±13</td>
<td>±14</td>
<td>±15</td>
<td>±13</td>
<td>±11</td>
<td>±12</td>
<td>±12</td>
<td>±16</td>
</tr>
<tr>
<td></td>
<td>DBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Standing</td>
<td>±17</td>
<td>±15</td>
<td>±16</td>
<td>±14</td>
<td>±12</td>
<td>±33</td>
<td>±12</td>
<td>±13</td>
<td>±11</td>
<td>±13</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Supine</td>
<td>118</td>
<td>105</td>
<td>96</td>
<td>94</td>
<td>90</td>
<td>92</td>
<td>95</td>
<td>98</td>
<td>105</td>
<td>106</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±14</td>
<td>±11</td>
<td>±13</td>
<td>±11</td>
<td>±9</td>
<td>±13</td>
<td>±14</td>
<td>±12</td>
<td>±14</td>
<td>±15</td>
<td>±15</td>
</tr>
</tbody>
</table>

|     | SBP (mm Hg) |     |     |     |     |     |     |     |     |     |     |     |
|     | Standing | ±22 | ±23 | ±19 | ±14 | ±15 | ±13 | ±14 | ±15 | ±15 | ±15 | ±15 |
|     | Supine  | 130 | 126 | 121 | 116 | 114 | 115 | 116 | 117 | 120 | 122 | 123 |
|     |         | ±22 | ±21 | ±18 | ±15 | ±15 | ±14 | ±13 | ±12 | ±14 | ±15 | ±13 |
|     | DBP (mm Hg) |     |     |     |     |     |     |     |     |     |     |     |
|     | Standing | ±18 | ±16 | ±16 | ±15 | ±17 | ±13 | ±12 | ±14 | ±9  | ±14 | —   |
|     | Supine  | 123 | 118 | 109 | 103 | 99  | 98  | 104 | 103 | 110 | 108 | 116 |
|     |         | ±18 | ±15 | ±16 | ±14 | ±12 | ±11 | ±14 | ±15 | ±13 | ±10 | ±15 |

**C** = control; **HR** = heart rate.

*Day 1, short-term ISDN therapy; day 3, after 45 hr of ISDN therapy; day 4, after 15 hr of placebo therapy (21 hr after last ISDN); day 5, after 39 hr of placebo therapy (45 hr after last ISDN).

light-headedness and nausea in the upright position after the initial sublingual ISDN administration. The symptoms disappeared promptly on resumption of the supine position and did not recur during this investigation.

**Discussion**

This study shows that partial tolerance develops to the hemodynamic effects of ISDN within 48 hr of initiating oral therapy and that reversal of tolerance begins within 24 hr and is complete within 48 hr of drug withdrawal. The demonstration of rapid circulatory tolerance is in agreement with our earlier findings but is contrary to the results of other studies. No other data are available that relate to rate of tolerance reversal in patients taking this drug.

The reduction in SBP after the initial sublingual ISDN challenge was not only greater than that seen after 48 hr of oral ISDN therapy but also persisted for a longer period. Thus 3 hr after the initial sublingual ISDN dose, the hypotensive effect remained, but after 48 hr of oral ISDN therapy, blood pressure returned to control values within 60 to 90 min.

This prolonged vasodepressor effect with initial sublingual ISDN exposure is similar to that observed in our earlier investigations with oral ISDN. With short-term, single doses of 15 to 120 mg of ISDN, SBP fell and remained below control for at least 8 hr. However, after 1 week of sustained oral therapy (four times daily), the decline in blood pressure after a given dose was less marked and persisted for only 4 hr.

This study also demonstrates that tolerance to the
hemodynamic effects of ISDN was quickly reversed after drug withdrawal. Only 21 hr after the last dose of oral ISDN, the response to sublingual ISDN was similar to that initially observed. This reversal of tolerance was complete within 45 hr, as demonstrated by the maximal hypotensive effect, which was almost identical to the observations on day 1.

The development of hemodynamic tolerance may have important clinical implications, since the therapeutic effects of the nitrates in angina pectoris and left ventricular dysfunction are dependent on their vascular effects. The organic nitrates dilate coronary arterial vessels but their major site of action appears to be peripheral. They dilate the peripheral arterial bed and thus reduce left ventricular afterload, which augments ventricular ejection while diminishing myocardial oxygen requirements. The dilating effects on capacitance vessels reduces venous return and left ventricular preload. This also results in decreased myocardial oxygen requirements. The reduced left ventricular filling pressure may, in itself, increase subendocardial perfusion. Both the arterial and venous effects of the nitrates are considered to be beneficial in the management of patients with cardiac failure, while the venous effects are generally considered to be of paramount importance in the control of angina pectoris.

Circulatory tolerance to the organic nitrates has been frequently demonstrated in animal experiments. After repeated dosing, reductions in the vasodepressor effects of nitroglycerin have been observed in rats, dogs, and rabbits. This phenomenon has not been restricted to nitroglycerin, since tolerance has been shown to develop to many organic nitrates, including sodium nitrate, erythrityl tetranitrate, penterythrityl tetranitrate, and ISDN. In addition, cross tolerance among the nitrates has been documented. It has long been recognized among workers involved in the industrial manufacture of nitrates that tolerance to headaches and lightheadedness, experienced upon initial exposure to the work environment, develops quickly. Workers found that despite prolonged exposure to nitrates, their tolerance to the vascular effects of nitrates rapidly dissipated during periods of nonexposure. Indeed, these employees were known to rub nitrates into their clothes and skin to maintain tolerance over weekends or during holiday periods. Failure to do so resulted in symptoms similar to those initially experienced in the nitrate environment.

The first clinical evidence of tolerance development was reported in 1888 and occurred in hypertensive patients who required increasing doses of nitroglycerin to exert a hypotensive effect. Recently, studies have demonstrated the development of circulatory tolerance during therapy with oral ISDN and the presence of cross tolerance to glyceryl trinitrate. Subsequent studies demonstrated that long-term therapy with oral ISDN led to diminished antianginal efficacy in addition to circulatory tolerance.

There is, however, controversy regarding the occurrence of nitrate tolerance. Danahy and co-workers showed similar blood pressure changes with oral ISDN during initial dosing and after sustained therapy. They also found no change in antianginal efficacy during this period. However, these responses were measured as long as 12 to 18 hr after the last dose of ISDN and reversal of tolerance may have occurred by that time. Franciosa and Cohn have shown that ISDN continues to produce significant hemodynamic effects in patients with left ventricular failure after 3 months of regular nitrate therapy, although these changes were less marked than those after the initial dose. Thus 90 min after the first dose of 40 mg of ISDN, pulmonary capillary wedge pressure fell 8 mm Hg. Wedge pres-
sure measured 8 to 10 hr after a dose during sustained ISDN therapy was 2.5 mm Hg below the initial control levels. Ninety minutes after oral administration of ISDN, there was a decline of 5.4 mm Hg and the wedge pressure was then similar to that seen at that time during short-term therapy. Although these changes were significantly different from those seen during placebo therapy, the absolute level of pulmonary capillary wedge pressure 90 min after placebo or active drug differed by only 2 mm Hg. This small difference, at a time when the drug effect would be maximal, suggests that tolerance had developed to ISDN. In a recent study by Leier et al., patients with left ventricular failure were assessed clinically and hemodynamically before and after 3 months of therapy with ISDN (40 mg four times daily). The decline in SBP seen during short-term dosing did not occur during sustained therapy, a finding that is in agreement with our current and previous studies. These workers did, however, demonstrate that the pulmonary capillary wedge pressure, measured 5 to 6 hr after the last dose during sustained ISDN therapy was significantly lower than the initial control values. This declined further with the next dose and the levels recorded were similar to that seen 60 to 90 min after the initial dosing. This suggests that tolerance may develop to the arterial- but not the venous-dilating effects of the nitrates, which is contrary to the conclusions of Zelis and Mason in their plethysmographic study.

The demonstration of increased plasma ISDN levels during sustained therapy in this and previous studies indicates that ISDN tolerance is not the result of diminished circulating drug levels. The data suggest that plasma concentrations of ISDN per se bear no apparent direct relationship to the phenomenon of circulatory tolerance.

The metabolites, 2-isosorbide mononitrate and 5-isosorbide mononitrate, are less potent vasodilators than the parent compound. Thus denitration of ISDN renders it biologically less active. Relative receptor affinity is unknown, but it is possible that these metabolites may alter the binding of ISDN to nitrate receptors, and this could account for the diminished vasodilator response observed during sustained therapy.

Vascular receptors for organic nitrates have been

TABLE 2
Circulatory effects of oral ISDN during short-term and sustained therapy (mean ± SD)

<table>
<thead>
<tr>
<th>Day</th>
<th>C</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>105</td>
<td>104</td>
<td>101</td>
<td>98</td>
<td>96</td>
<td>100</td>
<td>104</td>
<td>111</td>
</tr>
<tr>
<td>Standing</td>
<td>±10</td>
<td>±11</td>
<td>±12</td>
<td>±10</td>
<td>±9</td>
<td>±9</td>
<td>±9</td>
<td>±8</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>127</td>
<td>118</td>
<td>114</td>
<td>110</td>
<td>113</td>
<td>116</td>
<td>120</td>
<td>121</td>
</tr>
<tr>
<td>Standing</td>
<td>±16</td>
<td>±13</td>
<td>±10</td>
<td>±11</td>
<td>±14</td>
<td>±16</td>
<td>±20</td>
<td>±16</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>70</td>
<td>68</td>
<td>68</td>
<td>65</td>
<td>65</td>
<td>67</td>
<td>69</td>
<td>71</td>
</tr>
<tr>
<td>Standing</td>
<td>±5</td>
<td>±5</td>
<td>±5</td>
<td>±6</td>
<td>±7</td>
<td>±8</td>
<td>±7</td>
<td>±7</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>78</td>
<td>73</td>
<td>70</td>
<td>70</td>
<td>71</td>
<td>73</td>
<td>74</td>
<td>75</td>
</tr>
<tr>
<td>Standing</td>
<td>±4</td>
<td>±7</td>
<td>±7</td>
<td>±8</td>
<td>±7</td>
<td>±8</td>
<td>±6</td>
<td>±6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>88</td>
<td>90</td>
<td>91</td>
<td>88</td>
<td>90</td>
<td>85</td>
<td>84</td>
<td>85</td>
</tr>
<tr>
<td>Standing</td>
<td>±1</td>
<td>±1</td>
<td>±1</td>
<td>±1</td>
<td>±1</td>
<td>±1</td>
<td>±1</td>
<td>±1</td>
</tr>
</tbody>
</table>

C = control; HR = heart rate.

*Day 1, short-term ISDN therapy; day 3, after 45 hr of ISDN therapy.

FIGURE 2. Standing SBP and standing heart rate (SHR) after sublingual (SL) ISDN challenge and oral (PO) ISDN administration on days 1 and 3. Values shown are mean ± SD.
demonstrated in many tissues in both animals and man.\textsuperscript{27} In vitro tolerance to the vasodilator effect of ISDN and other organic nitrates has been established by a number of investigators. Needleman and Johnson\textsuperscript{2} proposed that organic nitrates react with a specific population of vascular receptors that possess reduced sulfhydryl groups. This interaction results in the oxidation of these receptors to the oxidized form, which has a much lower affinity for organic nitrates. It is postulated that the decreased attraction between these receptors and the circulating organic nitrate molecules accounted for tolerance. Ignarro et al.\textsuperscript{28} proposed that not one but two sulfhydryl receptor sites are present in the blood vessel wall. They suggest that through these two independent sites, the organic nitrates induce the formation of S-nitrosothiols within the muscle cell. The S-nitrosothiols, in turn, act on muscle cell cyclic guanosine monophosphate to induce muscle relaxation and vasodilatation.\textsuperscript{28} Sustained high-dose ISDN therapy could reduce the formation of S-nitrosothiols as a consequence of receptor oxidation and thereby inhibit cyclic guanosine monophosphate-mediated smooth muscle relaxation and vasodilatation.

In addition, vasodilator therapy has been shown to activate the sympathetic nervous and renin-angiotensin systems.\textsuperscript{29,30} The activation of these systems limits the hypotensive response, induces tachycardia, and may produce rebound changes after sudden withdrawal of vasodilator therapy. Therefore partial activation of counteracting vasoconstrictor forces during prolonged vasodilator treatment may account, in part, for the development of tolerance to the therapeutic effects of these drugs. The demonstration that tolerance to vasodilators of one type is not associated with cross tolerance to other classes of agents is against the hypothesis that increased vasoconstrictor forces are responsible for nitrate tolerance.\textsuperscript{30}

The present investigation has demonstrated the rapid development of tolerance during therapy with ISDN and prompt reversal of tolerance after drug withdrawal. The mechanisms involved are unknown, but it does not appear to be caused by decreased absorption or accelerated elimination of ISDN. Because the beneficial clinical effects of the nitrate groups of drugs in angina pectoris and left ventricular dysfunction are secondary to their vasodilator effects, it would be expected that circulatory tolerance would be associated with changes in antianginal efficacy.

These observations relating to the rapid onset and offset of circulatory tolerance to ISDN may have particular significance in relation to dosing schedules. Thus, perhaps the drug should be administered throughout the 6 to 10 hr period of the working day and so allow a period of 14 to 18 hr before the next dose. This type of schedule might minimize the development of tolerance and allow time for any tolerance that did develop to be reversed.

References


CIRCULATION
Tolerance to isosorbide dinitrate: rate of development and reversal.
J O Parker, H L Fung, D Ruggirello and J A Stone

Circulation. 1983;68:1074-1080
doi: 10.1161/01.CIR.68.5.1074

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 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/68/5/1074

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/