Aging is associated with reduced endothelial function independent of disease. Although much is known about how aging affects NO-mediated vasodilation, there is less information available about its influence on prostacyclin (PGI₂)-mediated vasodilation. In this context, indirect evidence obtained using cyclooxygenase (COX) inhibitors suggests that aging causes either reduced production of vasodilating prostanoids or perhaps reduced vasodilator responses to them. However, COX is also involved in the production of several other prostanoids (PGs), including PGE, PGF, PGD, and thromboxane, all with varying vascular effects, and there is some evidence that aging causes an increase in the production of vasoconstricting prostanoids. It is unknown whether the direct dilation caused by PGI₂ is maintained in older humans and how NO may contribute to this PGI₂-mediated dilation.

These questions are also clinically relevant in a general sense, because essential hypertension appears to cause changes in prostanoid-mediated endothelial vascular regulation similar to aging. In addition, suppression of PGI₂ caused by selective inhibition of COX-2 has been implicated in predisposing patients to myocardial infarction or thrombotic stroke. It also appears that this risk is associated with older patients who receive COX-2 inhibitors. This suggests that population differences in PGI₂ might influence these events.

With this information as a background, we sought to test the hypothesis that forearm blood flow responses to PGI₂ in healthy older adults would be reduced compared with matched young adults and to determine whether age-related differences in PGI₂-mediated vasodilation are dependent on the production of endothelial NO.

**Materials and Methods**

**Subjects**

All of the procedures and protocols for this study received previous approval by the institutional review board of the Mayo Clinic. Each subject provided written informed consent before participation. Initially, 5 younger subjects with a mean age of 32 years (range: 24 to 42 years) participated in a dose-response substudy to determine PGI₂ doses. The main protocol included 10 older subjects with a mean age of 68 years (range: 61 to 73 years) and 10 younger controls with a mean age of 29 years (range: 19 to 45 years; Table 1). The subjects were matched for body mass index (±3 kg/m²) and sex and consisted of 6 male and 4 female pairs. Participants did not require regular prescription medications (other than oral contraceptives); nonprescription medicines were stopped for 5 half-lives before the study. Subjects were physically active (but not exercise trained), nonsmokers, and presented without a diagnosis of preexisting disease (ie, cardiovascular, pulmonary, or endocrine). Younger women were studied during the low-estrogen phase of the menstrual cycle or the placebo phase of oral contraceptive use. Subjects refrained from

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**Abstract**—Aging is associated with reduced endothelial function. There is indirect evidence for reduced prostacyclin (PGI₂)-mediated vasodilation with aging, but it is unknown whether this is because of reduced dilation to PGI₂ or altered production. In addition, the contribution of endothelial NO to PGI₂-mediated dilation is unknown. Using plethysmography to determine forearm blood flow, we studied the effect of PGI₂ in 10 older (61 to 73 years) and 10 younger (19 to 45 years) subjects using 3 escalating intra-arterial doses of PGI₂ (epoprostenol). PGI₂ was also administered after NO synthase inhibition with N°-monomethyl-L-arginine acetate. The percent of change in forearm vascular conductance (mean±SEM) from baseline after PGI₂ was significantly lower (P=0.002) in the aging individuals (52±11%, 164±23%, and 221±27% versus 115±20%, 249±19%, and 370±35%). In addition, the group-by-dose interaction was also significant (P=0.018). After NO synthase inhibition, the dose-response curve to PGI₂ was blunted in the young subjects but unchanged in the older subjects; the difference between the groups was no longer significant. Our data suggest that the reduced dilator effects of PGI₂ in older individuals are attributable to a reduction in the contribution of endothelial-derived NO versus alterations in the direct effects of PGI₂ on vascular smooth muscle. (Hypertension. 2009;53:973-978.)

**Key Words:** aging | prostaglandins | NO | blood flow
foods and beverages that contain methylxanthines (caffeine and theobromine) and ethanol for 48 hours before the start of the study, and they were fasting after midnight before the study day.

**Brachial Arterial Catherization**

After local anesthesia with 2% lidocaine hydrochloride and using an aseptic technique, each subject underwent catheterization of the brachial artery. This was performed in the nondominant arm using a 5-cm-long, 20-gauge Teflon arterial catheter. This catheter was connected to a 3-port connector to allow for continuous measurement of arterial pressure (1 port) and for the administration of study drugs (2 ports).11

**Blood Flow Measurement**

Forearm blood flow (FBF) was determined 4 times each minute using venous occlusion plethysmography with mercury-in-silicone strain gauges placed around the nondominant forearm at its greatest circumference.12 During measurement of FBF, blood flow to the hand was excluded by inflation of a wrist cuff to 250 mm Hg. In addition to FBF, heart rate and mean arterial blood pressure were measured continuously.

**Data Acquisition**

Arterial blood pressure, heart rate, and FBF were monitored, stored, and analyzed offline using an automated electronic data acquisition system. FBF was determined from the derivative of the forearm plethysmographic tracing. To adjust for changes in systemic pressures, strain gauge-derived FBF was calculated by using FBF and mean arterial pressure (MAP) with the equation FVC=(FBF/MAP×100) and expressed as arbitrary units. In general, the last 2 minutes of each dose were used to determine FBF (an average of 4 measurements) and FVC.

**Brachial Artery Infusions**

To standardize drug concentrations, forearm volume (FVC) was determined in each subject by water displacement, and drugs were administered on the basis of FAV. Normal saline (0.9% sodium chloride) was infused during baseline measurements to maintain hemodynamic variables (heart rate and mean arterial pressure) were not significantly different between groups. Statistical significance was set at P<0.05.

**Infusion Protocol**

After instrumentation, a control infusion of 0.9% sodium chloride was initiated during baseline measurements and to compensate for total flow rate throughout the protocol as new infusions were added. A 3-point PGI2 dose response consisting of 2.5, 5.0, and 10.0 ng dL−1 of FAV min−1 (low, medium, and high) was performed followed by a 20-minute washout period and 3 doses of the endothelium-independent vasodilator SNP (0.25, 0.50, and 1.00 μg dL−1 of FAV min−1). The objective of this portion of the protocol was to determine whether aging was associated with a blunted vasodilator response to PGI2.

On completion of the first set of dose-response curves to PGI2 and SNP, the NOS 1-NMMA was infused at 5 mg/min for 10 minutes (50-mg loading dose), followed by an infusion of 1 mg/min for the remainder of the experiment (maintenance dose). A second set of dose responses (low, medium, and high doses) to PGI2 and SNP was then performed. The objective of this portion of the protocol was to determine whether the NO contribution to the vasodilation caused by PGI2 differed in the older subjects compared with the younger control subjects.

**Plasma Cholesterol Measurement**

In an attempt to examine the effect of plasma cholesterol on the PGI2-mediated dilatation, subject cholesterol data were either gathered from the patient’s medical chart (closest available total cholesterol level to study day) or subjects without a recent value were requested to return to have lipids drawn.

**Statistical Analysis**

Hemodynamic variables (heart rate and mean arterial pressure) were determined from the electronic records. Changes in FBF and FVC were determined with each subject serving as their own control using a paired analysis. Repeated-measures ANOVA were completed using SAS software version 9. The dependent variable was the percentage of change from baseline, and the repeated factor was dose. The independent cross-classification variable was age group. This model also included a group-by-dose interaction effect to assess whether differences between the comparative groups were dose dependent. In cases where dose-by-group interactions were identified, supplemental group comparisons were performed at each dose level without adjusting for multiple comparisons. Data are expressed as mean±SEM. Statistical significance was set at P<0.05.

**Results**

Table 1 shows the baseline data for the younger and older subjects. Systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure were not significantly different between groups.

Mean FBF responses to the interventions are shown in Table 2, and the calculated FVC responses are shown in Table 3. There were no statistical differences in the baseline data between the 2 groups before the administration of each of the PGI2 or SNP trials.

The percentage of change in FVC from baseline after each dose of FAV was statistically different (P=0.002) in the older individuals (52±11%, 164±23%, and 221±27%) compared with younger controls (115±20%, 249±19%, and 272±26%).
Without Coinfusion of L-NMMA baseline caused by both the SNP 0.25 and 0.5 comparisons suggested that the percentage of change from prehoc analyses that did not adjust for multiple dependent (dose-by-group interaction: suggesting that differences between groups were dose longer significantly different between younger and older groups (P=0.60). In addition, the group-by-dose interaction was no longer significant (P=0.87) with the combination of PGI2 and L-NMMA (Figure 2).

After L-NMMA and SNP, there was some evidence suggesting that differences between groups were dose dependent (dose-by-group interaction: P=0.047). Subsequent posthoc analyses that did not adjust for multiple comparisons suggested that the percentage of change from baseline caused by both the SNP 0.25 and 0.5 μg dL⁻¹ of FAV min⁻¹ doses did not differ significantly between age groups (P=0.817 and P=0.340, respectively). However, the older group experienced a greater change (P=0.036) at the highest dose (SNP 1 μg dL⁻¹ of FAV min⁻¹).

In subsequent examination of total cholesterol, the mean value was 179±6 mg/dL for 9 younger subjects and 206±16 mg/dL for 10 of the older subjects (P=0.154 by t test), showing no significant difference between groups. In addition, there was no correlation between total cholesterol and maximum PGI-mediated dilation (10 ng dL⁻¹ of FAV min⁻¹) in the younger individuals (r²=0.0041) or older individuals (r²=0.0062).

**Discussion**

The main findings of this study are that forearm vasodilator responses to PGI2 are reduced with aging and that these differences in PGI2-mediated vasodilation are likely attrib-

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### Table 2. FBF Responses to Infusion of PGI2 and SNP With and Without Coinfusion of L-NMMA

<table>
<thead>
<tr>
<th>Drug</th>
<th>FBF (mL dL⁻¹ FAV min⁻¹)</th>
<th>Young</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline coinfusion</td>
<td>PGI (ng dL⁻¹ FAV min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.86±0.27</td>
<td>2.12±0.23</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3.93±0.72</td>
<td>3.04±0.30</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>6.19±0.93</td>
<td>5.12±0.47</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>7.81±0.86</td>
<td>6.23±0.57</td>
<td></td>
</tr>
<tr>
<td>SNP (μg dL⁻¹ FAV min⁻¹)</td>
<td>Baseline</td>
<td>2.69±0.35</td>
<td>2.75±0.31</td>
</tr>
<tr>
<td>0.25</td>
<td>6.63±0.85</td>
<td>5.11±0.65</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>9.82±0.83</td>
<td>9.07±0.79</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>12.72±0.93</td>
<td>11.55±0.98</td>
<td></td>
</tr>
</tbody>
</table>

| l-NMMA coinfusion | PGI (ng dL⁻¹ FAV min⁻¹) |       |       |
| Baseline | 1.71±0.16 | 1.67±0.13 |
| 2.5 | 2.47±0.29 | 2.26±0.19 |
| 5.0 | 4.14±0.49 | 4.06±0.53 |
| 10.0 | 5.69±0.54 | 5.34±0.72 |
| SNP (μg dL⁻¹ FAV min⁻¹) | Baseline | 1.93±0.19 | 1.67±0.16 |
| 0.25 | 5.34±0.80 | 4.87±0.56 |
| 0.50 | 8.38±0.82 | 8.51±0.72 |
| 1.00 | 9.96±0.92 | 11.53±1.02 |

Values are mean±SEM. PGI indicates epoprostenol.

### Table 3. FVC Responses to Infusion of PGI2 and SNP With and Without Coinfusion of L-NMMA

<table>
<thead>
<tr>
<th>Drug</th>
<th>FVC, Arbitrary Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline coinfusion</td>
<td>PGI (ng dL⁻¹ FAV min⁻¹)</td>
</tr>
<tr>
<td>Baseline</td>
<td>2.11±0.30</td>
</tr>
<tr>
<td>2.5</td>
<td>4.64±0.86</td>
</tr>
<tr>
<td>5.0</td>
<td>7.22±1.17</td>
</tr>
<tr>
<td>10.0</td>
<td>9.34±1.35</td>
</tr>
<tr>
<td>SNP (μg dL⁻¹ FAV min⁻¹)</td>
<td>Baseline</td>
</tr>
<tr>
<td>0.25</td>
<td>7.56±1.10</td>
</tr>
<tr>
<td>0.50</td>
<td>11.29±1.00</td>
</tr>
<tr>
<td>1.00</td>
<td>14.85±1.14</td>
</tr>
</tbody>
</table>

| l-NMMA coinfusion | PGI (ng dL⁻¹ FAV min⁻¹) |       |       |
| Baseline | 1.86±0.18 | 1.83±0.18 |
| 2.5 | 2.68±0.30 | 2.44±0.23 |
| 5.0 | 4.50±0.53 | 4.53±0.71 |
| 10.0 | 6.27±0.61 | 5.99±0.93 |
| SNP (μg dL⁻¹ FAV min⁻¹) | Baseline | 2.01±0.18 | 1.81±0.21 |
| 0.25 | 5.80±0.92 | 5.21±0.58 |
| 0.50 | 9.10±0.90 | 9.33±0.98 |
| 1.00 | 11.06±1.15 | 12.89±1.33 |

Values are mean±SEM. PGI indicates epoprostenol.
Aging effects have also been studied using other endothelial-dependent dilators. In a study by DeSouza et al., acetylcholine-induced vasodilation was impaired in aging individuals; however, the forearm endothelial vasodilations in response to bradykinin, substance P, and isoproterenol were well preserved in older men (50 to 76 years) when compared with younger men (23 to 35 years). These findings suggested that agonist-stimulated, endothelium-dependent vasodilation is not universally impaired with age and depends on the agonist. However, similar to acetylcholine, our data indicate that PGF2α-mediated dilation is impaired with age. Although age-related decreases in endothelial-dependent NO release or NO bioavailability appear to be agonist specific, vascular smooth muscle responsiveness to the direct effects of NO remains relatively unchanged in otherwise healthy subjects. In addition, the current data suggest that vascular smooth muscle responsiveness to the direct effects of PGF2α is unchanged with aging.

**Prostacyclin**

To the best of our knowledge, PGF2α has not been used in forearm studies focused on vascular aging in escalating doses. However, in a previous forearm trial, Kamper et al. demonstrated that the NOS inhibitor L-NMMA blunted the vasodilation evoked by the PGF2α analog iloprost. This early finding is consistent with our observation that NO mediates a portion of PGF2α-induced vasodilation.

On the basis of previous studies with COX inhibition, we hypothesized that the direct vasodilatory effects of PGF2α would be reduced in the older compared with the younger subjects. This idea came from studies that used an indirect approach to identify the role of PGs in regulating vascular tone with aging. In these studies, the effects of nonselective inhibitors of COX (ie, ketorolac and aspirin) on blood flow were determined. In the study by Singh et al., the COX inhibitor aspirin (3, 9, and 30 μmol/min) was administered to 18 young and 15 older healthy subjects. Aspirin caused a greater dose-related reduction in FBF in the younger versus the older subjects. This reduction was interpreted to suggest that the role played by PGs in the regulation of vascular tone diminishes with age.

However, using this approach, it is not possible to determine whether aging alters vascular responsiveness to PGF2α or shifts the production from dilating to constricting prostanoids. In addition, there is the possibility that the direct smooth muscle effects of PGF2α might increase because of the chronically low circulating PGF2α levels in older individuals with a resultant vascular receptor upregulation over time. Under any of these circumstances, determination of the contribution of dilating PGs indirectly via COX inhibition would be difficult. However, the effects of PGF2α on forearm vasodilation were reduced in our older subjects compared with the younger individuals clearly demonstrating a loss of PGF2α-mediated vasodilation with aging.

**NOS Inhibition**

The older individuals demonstrated that the vasodilation caused by the PGF2α was similar before and after the L-NMMA administration. Because L-NMMA inhibits the endothelial production of NOS, this suggests that older subjects produce less NO in response to PGF2α. This finding is consistent with the observation that the effects of L-NMMA diminish with advanced age, indicating a generalized blunting of basal endothelial function in older people. By contrast, the NO contribution to PGF2α-mediated vasodilation was larger in younger individuals. In this context, NOS inhibition also facilitated the comparison of the smooth muscle effects of PGF2α and showed that the smooth muscle effects of PGF2α appeared similar in both populations once the NO component of dilation was absent.

Although PGF2α caused an NO-mediated dilation in the younger group only (as evidenced by the L-NMMA effect), administration of SNP also increased flow, but NOS inhibi-
tion with L-NMMA had little effect on the dilator responses to SNP. Along these lines, many agonists that increase FBF show a 20% to 40% reduction in flow after NOS inhibition, and a question that always arises is whether this reduction is because of a nonspecific blunting of shear/flow-related NO release or whether it is likely a receptor-mediated response. The current data indicate that a general flow-related effect on endothelium-dependent NO release is unlikely to explain the interactions of L-NMMA and PGI2 on vasodilator responses between the 2 groups.

Limitations
There are limitations to this study. First, the forearm vasculature is indicative but not necessarily representative of all human blood vessels, because other vascular beds may be more or less sensitive to the effects of these agents because of differences in receptor density or signaling. Second, although all of the subjects denied a history of hypertension, we did find that the older subject group displayed a greater mean arterial pressure than the young group (87.0±3.5 versus 79.7±2.5 mm Hg), which may have affected our interpretation. Third, we later assessed plasma cholesterol. Although the lipid results were not contemporaneous with our blood flow study, we feel that these results are likely representative of the lipid status of our study subjects. On analysis, the mean total cholesterol was higher in the older subject group (206±16 versus 179±6 mg/dL). Although these values did not reach significance, we acknowledge the possibility of a type II error because of the small sample size. However, no correlation between total cholesterol and maximum PGI-mediated dilation (10 ng dL$^{-1}$ of FAV min$^{-1}$) was observed in either group. Finally, there was some evidence suggesting that older individuals may dilate more compared with the younger subjects with the highest dose combination of SNP and L-NMMA. Only the SNP 1 dose was significant between groups ($P=0.036$ unadjusted for multiple comparisons), unlike the other 2 doses after posthoc analysis. Because an improvement in the vasodilator effects of SNP after NOS inhibition was not expected, we believe that the overall dose-by-age-group interaction differences ($P=0.047$) are not meaningful.

Perspectives
The importance of NO and PGI2 as vasodilators is well known. We believe that our findings have several areas of physiological and/or clinical significance. First, there is evidence that the aging endothelium appears to produce more vasoconstricting prostanoids. The current findings demonstrate that the blood vessels of healthy older subjects are also less responsive to the overall vasodilating effects of PGI2. In addition, these age-related changes in PGI-mediated dilation do not appear to be easily explained by differences in plasma cholesterol. Finally, the vasodilating effects of PGI appear to be greater when NO is available, and NO release from the vascular endothelium by PGI2 plays an essential role in our observations. Of potential clinical importance, PGI2 inhibition has been implicated in the toxicity of the selective COX-2 inhibitors, which might explain the increased rate of adverse cardiovascular effects with COX-2 inhibitors in older individuals.

Conclusions
Aging blunts the vasodilator effects of PGI2, and NOS inhibition significantly reduces PGI2-mediated vasodilation in young but not older subjects. This suggests that the reduced vasodilator effects of PGI2 in aging individuals may be attributed to a reduction in the contribution of endothelial-derived NO when compared with younger individuals.

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Disclosures
None.

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Aging Is Associated With Reduced Prostacyclin-Mediated Dilation in the Human Forearm
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