Relative contribution of vasodilator prostanoids and NO to metabolic vasodilation in the human forearm

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Duffy, Stephen J., Gishel New, Binh T. Tran, Richard W. Harper, and Ian T. Meredith. Relative contribution of vasodilator prostanoids and NO to metabolic vasodilation in the human forearm. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H663–H670, 1999.—Although many factors are thought to contribute to the regulation of metabolic vasodilation in skeletal muscle vasculature, recent interest has focused on the role of the endothelium. We examined the relative roles of nitric oxide (NO) and of vasodilator prostanoids in the control of metabolically induced functional hyperemia in the forearm of humans. In 43 healthy volunteers [24 ± 5 (SD) yr] we assessed resting and functional hyperemic blood flow (FHF) in response to 2 min of isometric forearm exercise before and after inhibition of NO and/or vasodilator prostanoid production with intra-arterial Nω-monomethyl-L-arginine (L-NMMA, 2 mg/min) and aspirin (ASA, 3 mg/min), respectively. Blood flow was measured using venous occlusion plethysmography. L-NMMA and ASA decreased resting forearm blood flow by 42% (P < 0.0001) and 23% (P < 0.0001), respectively, whereas infusion of ASA followed by L-NMMA reduced flow by a further 24% (P < 0.05). L-NMMA reduced peak FHF by 18% [from 13.9 ± 1.0 to 11.4 ± 1.1 (SE) ml·100 ml forearm⁻¹·min⁻¹, P = 0.003] and the volume “repaid” after 1 and 5 min by 25% (8.9 ± 0.7 vs. 6.7 ± 0.7 ml/100 ml, P < 0.0001) and 37% (26.6 ± 1.8 vs. 16.8 ± 1.6 ml/100 ml, P < 0.0001). ASA similarly reduced peak FHF by 19% [from 14.5 ± 1.1 to 11.8 ± 0.9 ml/100 ml forearm⁻¹·min⁻¹, P < 0.001] and the volume repaid after 1 and 5 min by 14% (7.5 ± 0.6 vs. 6.4 ± 0.6 ml/100 ml, P = 0.0001) and 20% (21.2 ± 1.5 vs. 16.9 ± 1.5 ml/100 ml, P < 0.0001), respectively. The confusion of ASA and L-NMMA did not decrease FHF to a greater extent than either agent alone. These data suggest that endothelium-derived NO and vasodilator prostanoids contribute to resting blood flow and metabolic vasodilation in skeletal muscle vasculature in healthy humans. Although these vasodilator mechanisms operate in parallel in exercise-induced hyperemia, they appear not to be additive. Other mechanisms must also be operative in metabolic vasodilation.

Regional blood flow; endothelium-derived factors; exercise; eicosanoids; nitric oxide

Local regulators of blood flow, such as vasodilator metabolites and myogenic factors, are integral mechanisms by which nutritive blood flow is increased to metabolically active muscles. Oxygen tension, pH, and significant local ions and metabolites, including potassium, inorganic phosphate, lactate, and adenosine, are key factors (14, 26). These factors not only stimulate metabolic vasodilation in working skeletal muscle but must also offset the systemic neurohormonal activation that occurs to maintain or increase blood pressure and heart rate (26). A substantial body of evidence now indicates that a variety of endothelium-derived vasoactive factors also control vascular tone during changes in physiological demand (3).

Prostacyclin is the principal vasodilator prostanoid in human forearm vasculature (22). We recently demonstrated that its tonic release contributes to the control of resting forearm blood flow (8). The role of vasodilator prostanoids in the regulation of functional hyperemic blood flow (FHF) in skeletal muscle during and after exercise was first recognized by Kilbom and Wennmalm (16) more than 20 years ago. Several more recent studies have also suggested that prostanoids are important in metabolic vasodilation in humans (1, 6, 23, 28, 30). Vallance and colleagues (27) also demonstrated that nitric oxide (NO) contributes substantially to resting forearm blood flow, a finding confirmed by several other investigators (10–13, 15, 19, 29).

Recent studies have sought to delineate the role of NO in exercise-induced FHF in skeletal muscles in humans with contradictory results, however. Several (9, 12, 13, 15), but not all (2, 10, 29), studies have suggested a role for NO in FHF. It is possible that the failure to observe a decrease in FHF with inhibition of one vasodilator pathway might be due to compensatory upregulation of another pathway. To determine the endothelial contribution to FHF, it may be important to inhibit more than one pathway simultaneously. Moreover, the relative contribution of these paracrine factors to exercise hyperemia in humans is unknown. The objective of this study, therefore, was to determine whether vasodilator prostanoids and/or NO have complementary effects in metabolic vasodilation in the skeletal muscle of healthy humans and whether this effect remains after correction for any change in resting forearm blood flow.

METHODS

Subjects. We studied 43 healthy volunteers [23.8 ± 5.4 (SD) yr, 18 women and 25 men] recruited by advertisement at the Monash University campus and the local employment agency. All subjects were screened for cardiovascular risk factors, cardiovascular disease, or other major illness by medical history, physical examination, and fasting lipid profile. Subjects were excluded if they had cardiovascular risk factors (including a past or present history of smoking and family history of ischemic heart disease), cardiovascular disease, major noncardiac disease, or any abnormality on physical examination (including a discrepancy of ≥10 mmHg in blood pressure between the upper limbs). Subjects taking vasoc-
tive medications were also excluded. The study was approved by the National Health and Medical Research Council of Australia and the Human Research Ethics Committee of Monash Medical Centre, and all subjects gave their written informed consent.

General methods. All subjects were studied in our vascular research laboratory in the morning after a light breakfast. The room was temperature controlled at 22–23°C, a quiet atmosphere prevailed, and the lights were dimmed. Subjects were asked to refrain from caffeine-containing food and drinks and alcohol for 12 h before the study. Aspirin (ASA) and other nonsteroidal anti-inflammatory drugs were forbidden for 1 wk before the study.

After 10 min of quiet, supine rest, a 20-gauge, 5-cm polyethylene catheter (Cook, Brisbane, Australia) was introduced under local anesthesia into the brachial artery of the nondominant upper limb. The procedure was carried out reduced under local anesthesia into the brachial artery of the nondominant upper limb. The procedure was carried out under sedation. The cannula served as an infusion port for vasoactive agents and enabled blood pressure to be monitored directly and continuously. To establish a nondominant upper limb. The procedure was carried out under sedation. The cannula served as an infusion port for vasoactive agents and enabled blood pressure to be monitored directly and continuously. To establish a stable baseline, all subjects were rested for ≥30 min after arterial line insertion before the first measurement was made. During this time, isotonic glucose (5% dextrose) was infused at a rate of 0.4 ml/min intra-arterially (the same rate at which all drugs were subsequently infused).

Drug infusion protocols. ASA (Aspisol, generously supplied by Bayer), a well-known inhibitor of cyclooxygenase that acts by irreversibly acetylating this enzyme, was infused via the brachial artery at 3 mg/min for ≥10 min. This dose was calculated to achieve a local plasma concentration of 150 µg/ml with the assumption of a resting forearm blood flow of 2.5 ml·100 ml forearm⁻¹·min⁻¹ (8). On the basis of previous data from our laboratory, we anticipated that this dose would reduce the net forearm production of prostacyclin at rest by ≥70% and decrease basal forearm blood flow by ≥20% (8). N⁵-monomethyl-L-arginine (L-NMMA; Clinalfa) inhibits the production of NO by competing with L-arginine for the enzyme NO. L-NMMA was infused at 2 mg/min for ≥10 min, as in previous studies (11, 12, 19). Two criteria confirmed that the dose of L-NMMA had reduced the bioavailability of NO: 1) a reduction in resting blood flow and 2) an attenuation of ACh-stimulated forearm blood flow.

ACh chloride (Miochol, Iolab Pharmaceuticals, Sydney, Australia), an agent that causes vasodilation principally by release of endothelium-derived NO, was infused via the brachial artery for 5 min at 30 µg/min, as previously described (27). Sodium nitroprusside (Faulding, Melbourne, Australia), an NO donor that results in direct vascular smooth muscle relaxation, was administered via the brachial artery for 5 min at 1 µg/min, as described in previous studies, to assess the response to an endothelium-independent vasodilator. These drug doses have previously been shown in our laboratory not to have systemic effects (8).

All drugs were diluted in an isotonic glucose solution (5% dextrose) and were infused at 0.4 ml/min by using a syringe pump (Terumo, Tokyo, Japan); this rate of infusion is the same as the rate of vehicle infusion during the initial resting and exercise-induced blood flows.

Hemodynamic measurements. Forearm blood flow was measured in the nondominant arm by venous occlusion plethysmography by using calibrated mercury-in-Silastic strain gauges (D. E. Hokanson, Bellevue, WA) and expressed in milliliters per 100 ml of forearm tissue per minute, as previously described (8). Resting blood flow measurements were taken for ≥2 min, and an average of a minimum of five results was used for analysis. Forearm vascular resistance (expressed as units indicating mmHg·ml⁻¹·100 ml tissue⁻¹·min) was calculated from mean arterial blood pressure and forearm blood flow, whereas minimum resistance after exercise was calculated from mean arterial blood pressure and peak FHB.

The exercise protocol consisted of rhythmic, isotonic exercise at the wrist. The level and type of exercise were chosen to avoid systemic effects. Maximal flexion-extension wrist movement at 45 cycles/min was encouraged and timed with a metronome. Exercise-induced FHB was measured continuously for 5 min after the metabolic stimulus to examine the time course and volume of the hyperemic response, and this commenced immediately after the cessation of exercise. Short venous occlusion cycles were used for at least the 1st min, with 12-s cycles thereafter. Peak FHB was determined and usually occurred in the first 10–15 s after completion of the workload. The blood volume ("debt") repaid during the 1st min and subsequent 4 min after exercise was calculated from the area under the curve (Fig. 1). Figure 1 demonstrates a typical individual example of the blood flow-time relationship after the cessation of exercise. Hyperemia declines rapidly over the 1st min. Although blood flow appears to plateau over the subsequent 4 min, flow usually does not return to baseline by 5 min. Figure 1 shows the reduction in FHB with ASA infusion. In addition, the "absolute" peak FHB (change from baseline to maximal blood flow) and the absolute volumes repaid at 1 and 5 min were calculated by subtraction of resting forearm blood flow to eliminate the effect that any change in resting flow may have had on the results. Similarly, the absolute change in blood flow in response to ACh and nitroprusside was calculated to account for any change in basal blood flow due to L-NMMA or ASA.

Reproducibility of FHB. Reproducibility of FHB in response to this stimulus was established in our laboratory in 12 subjects (32.5 ± 10.4 yr, 5 women and 7 men) by three successive exercise periods with assessment of the blood flow response. There was a seven- to eightfold increase in forearm blood flow compared with baseline. Peak FHB after the three bouts of exercise was similar: 16.0 ± 1.4, 16.2 ± 2.2, and 17.1 ± 1.9 (SE) ml·100 ml⁻¹·min⁻¹ (P = 0.71). The difference between the measurements of peak FHB was −0.2 ± 1.7 and −1.0 ± 1.4 ml·100 ml⁻¹·min⁻¹. The volume "repaid" was 9.8 ± 0.9, 9.5 ± 1.2, and 9.9 ± 0.9 ml·100 ml⁻¹·min⁻¹ during the 1st and subsequent 4 min after exercise.
Experimental protocols. To test the hypothesis that vasodilator prostanoids and/or NO contributes to basal and stimulated blood flow in the human forearm, resting forearm blood flow and exercise-induced FHBF were measured before and after ASA and/or L-NMMA infusion, respectively. The study design included two protocols (Fig. 2). Baseline flow was reestablished after each intervention. FHBF was assessed with vehicle infusion (isotonic glucose infusion), repeated with L-NMMA (n = 25) or ASA (n = 18) infusion alone, and then (in 18 subjects) with ASA + L-NMMA infusion. After the initial baseline resting flow was recorded, all subjects received ACh. The response to ACh was reassessed after infusion of L-NMMA or coinfusion of L-NMMA + ASA. Sodium nitroprusside response was assessed before and after L-NMMA.

Statistical analysis. Baseline subject data are means ± SD. All physiological measurements are means ± SE. Two-sided Student’s t-test was used for comparison of paired data such as the responses to ACh and nitroprusside before and after L-NMMA and the effects of ASA or L-NMMA given alone on hemodynamic variables. The local and systemic hemodynamic effects of ASA and L-NMMA were assessed by repeated-measures ANOVA. Where a statistical difference was detected by ANOVA, the Bonferroni multiple-comparison procedure was used to define differences between the results. Statistical significance was accepted where P < 0.05.

RESULTS

A total of 43 subjects [23.8 ± (SD) 5.4 yr, range 18–37 yr, 18 women and 25 men] were recruited for these studies. Their mean total cholesterol was 4.7 ± 0.7 mmol/l, low-density-lipoprotein cholesterol was 2.8 ± 0.6 mmol/l, body mass index was 22.8 ± 3.1 kg/m², and waist-to-hip ratio was 0.83 ± 0.06.

Resting hemodynamics. Infusion of L-NMMA (n = 25) reduced resting forearm blood flow by 42% after 10 min. Forearm blood flow at baseline and after 5 and 10 min of L-NMMA infusion was 2.9 ± 0.2, 1.9 ± 0.1, and 1.7 ± 0.1 (SE) ml·100 ml forearm⁻¹·min⁻¹, respectively (P < 0.0001; Fig. 3A). There was a corresponding 77% increase in forearm vascular resistance from 32.6 ± 2.3 to 49.5 ± 3.4 units after 5 min and 57.8 ± 4.3 units after 10 min (P < 0.0001; Fig. 3B). Post hoc analysis revealed a significant difference in resistance at 5 and 10 min after initiation of L-NMMA. There was no change in mean arterial blood pressure with L-NMMA: 82.5 ± 1.6 vs. 83.5 ± 1.7 mmHg after 5 min and 83.6 ± 1.5 mmHg after 10 min (P = 0.28).

ASA infusion at 3 mg/min reduced resting forearm blood flow by 23%. Forearm blood flow at baseline and after 10 min of ASA infusion was 2.4 ± 0.2 and 1.9 ± 0.2 ml·100 ml forearm⁻¹·min⁻¹, respectively (P < 0.0001; Fig. 3C).
The decrement in peak hyperemic blood flow occurred despite a small, but significant rise in mean arterial blood pressure with L-NMMA (Table 1; P = 0.0001). The absolute peak hyperemic blood flow (peak resting blood flow) with L-NMMA was also lower (Table 1).

Intra-arterial ASA infusion resulted in a 19% decrease in peak hyperemic blood flow (P < 0.001; Table 2, Fig. 5). The decrement in peak hyperemic blood flow with ASA remained significant after the effect of change in resting blood flow was eliminated (P < 0.003; Table 2). There was a concordant 24% increase in minimum forearm vascular resistance after exercise with ASA (P = 0.0002; Table 2, Fig. 5). Mean arterial blood pressure after exercise was unaffected by ASA (P = 0.13; Table 2).

When ASA and L-NMMA were coinfused, there was no further reduction in peak hyperemic blood flow compared with the effect seen with ASA alone (Table 2, Fig. 5). Further analysis using the Bonferroni multiple-

### Table 1. Exercise hemodynamics in response to L-NMMA

<table>
<thead>
<tr>
<th></th>
<th>NoDrug</th>
<th>L-NMMA</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td>Peak FBF, ml·100 min⁻¹</td>
<td>13.9 ± 1.0</td>
<td>11.4 ± 1.1</td>
<td>&lt;0.01</td>
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<tr>
<td>∆Peak FBF, ml·100 min⁻¹</td>
<td>11.3 ± 9.7</td>
<td>9.7 ± 1.1</td>
<td>&lt;0.05</td>
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<tr>
<td>Blood volume repaid in 1 min, ml/100 ml</td>
<td>8.9 ± 0.7</td>
<td>6.7 ± 0.7</td>
<td>&lt;0.0001</td>
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<tr>
<td>∆Blood volume repaid in 1 min, ml/100 ml</td>
<td>6.4 ± 0.8</td>
<td>5.2 ± 0.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Blood volume repaid in 5 min, ml/100 ml</td>
<td>26.6 ± 1.8</td>
<td>16.8 ± 1.6</td>
<td>&lt;0.0001</td>
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<tr>
<td>∆Blood volume repaid in 5 min, ml/100 ml</td>
<td>13.7 ± 1.9</td>
<td>9.1 ± 1.5</td>
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<tr>
<td>Minimum FVR, units</td>
<td>6.7 ± 0.5</td>
<td>10.0 ± 1.2</td>
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<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>81.9 ± 1.6</td>
<td>86.7 ± 1.6</td>
<td>0.0001</td>
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</tbody>
</table>

Values are means ± SE. L-NMMA, N⁶-monomethyl-L-arginine; ∆, hyperemic blood flow less resting flow; FBF, forearm blood flow; FVR, forearm vascular resistance. *Pre- vs. post-L-NMMA.

### Table 2. Exercise hemodynamics in response to ASA and L-NMMA

<table>
<thead>
<tr>
<th></th>
<th>NoDrug</th>
<th>ASA</th>
<th>P*</th>
<th>L-NMMA</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak FBF, ml/100 min⁻¹</td>
<td>14.5 ± 1.1</td>
<td>11.8 ± 0.9</td>
<td>&lt;0.05</td>
<td>11.3 ± 1.1</td>
<td>NS</td>
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<tr>
<td>∆Peak FBF, ml/100 min⁻¹</td>
<td>12.1 ± 1.1</td>
<td>9.9 ± 0.8</td>
<td>&lt;0.05</td>
<td>9.9 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Blood volume repaid in 1 min, ml/100 ml</td>
<td>7.5 ± 0.6</td>
<td>6.5 ± 0.6</td>
<td>&lt;0.05</td>
<td>6.1 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>∆Blood volume repaid in 1 min, ml/100 ml</td>
<td>5.2 ± 0.5</td>
<td>4.7 ± 0.6</td>
<td>NS</td>
<td>4.8 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Blood volume repaid in 5 min, ml/100 ml</td>
<td>21.2 ± 1.5</td>
<td>16.9 ± 1.5</td>
<td>&lt;0.05</td>
<td>14.7 ± 1.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>∆Blood volume repaid in 5 min, ml/100 ml</td>
<td>9.3 ± 0.9</td>
<td>7.7 ± 1.0</td>
<td>&lt;0.05</td>
<td>7.8 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Minimum FVR, units</td>
<td>6.1 ± 0.5</td>
<td>7.5 ± 0.5</td>
<td>&lt;0.05</td>
<td>9.0 ± 1.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>79.5 ± 1.2</td>
<td>81.3 ± 1.7</td>
<td>NS</td>
<td>84.5 ± 1.8</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. ASA, aspirin; NS, not significant. P < 0.01 by ANOVA for all measurements (except †). *Pre- vs. post-ASA; †post-ASA vs. post-L-NMMA by post hoc analysis (Bonferroni).
comparison procedure indicated that although peak flows with ASA and ASA + L-NMMA were significantly lower than without drug treatment, they were not different from each other. Minimum forearm vascular resistance was slightly higher with ASA + L-NMMA (Table 2, Fig. 5). There was a small, but significant, rise in mean arterial blood pressure with the two drugs (P < 0.05; Table 2).

Blood volume repaid after exercise. Infusion of L-NMMA significantly reduced the total volume of blood repaid after 1 and 5 min by 25 and 37%, respectively (Table 1, Fig. 4). This reduction of volume repaid remained significant after accounting for the change in resting forearm blood flow volume with L-NMMA. The absolute change in blood volume repaid after 1 and 5 min was reduced by 19 and 34%, respectively (Table 1). Similarly, infusion of ASA alone significantly reduced the total volume of blood repaid after 1 and 5 min by 14 and 20%, respectively (Table 2, Fig. 6). The reduction of volume repaid over 5 min remained significant after accounting for the change in basal forearm blood flow with ASA, with the absolute change in blood volume repaid after 1 and 5 min being reduced by 10 and 18%, respectively (Table 2).

When ASA and L-NMMA were coinfused, there was no further reduction in the volume of blood repaid after 1 min compared with the effect seen with ASA alone. There was a further reduction in the volume of blood repaid after 5 min, but this was no longer apparent when the change in resting flow was accounted for. Analysis using the Bonferroni multiple-comparison procedure indicated that although the absolute volumes repaid after 5 min with ASA and ASA + L-NMMA were significantly lower than those without drug treatment, they were not different from each other (Table 2).

Stimulated endothelium-dependent and -independent responses. Twenty-five subjects received ACh before and after inhibition of NO production with L-NMMA. In 14 of these subjects, L-NMMA significantly decreased the maximal hyperemic response to ACh from 18.4 ± 2.8 to 11.0 ± 2.1 ml·100 ml forearm⁻¹·min⁻¹ (P < 0.001), our predefined indicator of effective NO inhibition. Minimum forearm vascular resistance with ACh was increased by L-NMMA from 8.2 ± 2.2 to 19.6 ± 6.3 units (P = 0.02). In the 11 subjects in whom L-NMMA did not produce a significant decrease in the maximal response to ACh, there was a 35% reduction in resting forearm blood flow from 3.1 ± 0.3 to 2.0 ± 0.2 ml·100 ml forearm⁻¹·min⁻¹ (P < 0.0001), our second predefined indicator of effective NO inhibition. The effects of L-NMMA on exercise blood flow were similar in those in whom L-NMMA did or did not alter the ACh response.

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Fig. 5. Effect of ASA and L-NMMA on peak exercise hyperemia. A: peak flow; B: minimum resistance. *P < 0.001 by ANOVA for all measures.

Fig. 6. Effect of ASA and L-NMMA on exercise hyperemia, i.e., volume repaid during 1 min (A) and 5 min (B) after exercise. *P < 0.0001 by ANOVA for all measures.
ASA + L-NMMA attenuated the maximal hyperemic response to ACh from 13.1 ± 1.6 ml·100 ml forearm⁻¹·min⁻¹ before to 10.4 ± 1.4 after these agents (n = 18, P = 0.005), despite an increase in mean arterial blood pressure from 76.5 ± 1.4 to 86.2 ± 1.5 mmHg between the two measurements (P < 0.0001). The absolute change in blood flow with ACh (peak − baseline) was also reduced by ASA + L-NMMA from 11.1 ± 1.6 to 8.9 ± 1.5 ml·100 ml forearm⁻¹·min⁻¹ (P < 0.02).

Seventeen subjects received sodium nitroprusside before and after L-NMMA infusion. The absolute change in blood flow from baseline with sodium nitroprusside before and after L-NMMA was similar: 4.7 ± 0.4 and 4.8 ± 0.5 ml·100 ml forearm⁻¹·min⁻¹, respectively (P = 0.34). Forearm vascular resistance with nitroprusside was higher after L-NMMA (11.9 ± 0.9 and 19.5 ± 3.1 units before and after L-NMMA, respectively, P = 0.04). Mean arterial blood pressure increased between these two measurements from 80.2 ± 1.4 to 87.3 ± 1.3 mmHg (P < 0.0001).

**DISCUSSION**

This study demonstrates that the vasodilator prostacyclins and endothelium-derived NO are important in the regulation of resting skeletal muscle blood flow, in the peak hyperemic FHBF achieved, and in the volume repaid after exercise. Although their contribution to resting forearm blood flow was additive, this was not apparent with functional hyperemia after exercise. Although both paracrine factors appear to contribute to peak FHBF and volume repaid, inhibiting both resulted in no greater change than inhibiting either factor alone. These results indicate that the two factors operate in parallel in exercise hyperemia.

Resting blood flow. From previous studies we anticipated that inhibiting NO and vasodilator prostacyclins with L-NMMA and ASA, respectively, would result in a decline in resting forearm blood flow of ~20–40%. Vallance and colleagues (27) were able to reduce resting blood flow by 50% with L-NMMA, suggesting that NO contributes substantially to the maintenance of peripheral arteriolar tone in humans. Their findings have been confirmed by others (10–13, 15, 19, 20) reporting reductions of resting forearm blood flow of 25–40%. Our observations in this study are consistent with these findings.

We and others (8, 30) recently showed that inhibition of cyclooxygenase with ASA or indomethacin decreases resting forearm blood flow by 20–30%. These findings were confirmed in this study. The inhibition of NO with L-NMMA after suppression of prostacyclin production with ASA resulted in an additional 24% reduction in basal flow. The maximum combined contribution of these endothelium-derived vasodilators to resting forearm blood flow, therefore, appears to be 40–50%. Prostaglandin E₂ production should also have been inhibited by this dose of ASA (30), but whether endothelium-derived hyperpolarizing factor contributes to basal blood flow or functional hyperemia in humans is uncertain (5). The remaining fraction of resting skeletal muscle blood flow appears to be regulated by factors not controlled by these pathways, including the sympathetic nervous system, local vasodilator metabolites, myogenic factors, and endothelium-derived contracting factors (14, 26).

Exercise (functional) hyperemia. Although some previous investigators demonstrated a significant role of NO in exercise hyperemia (9, 12, 13, 15), these studies have focused on maximal (or peak) hyperemia at each level of exercise. Node et al. (21) recently presented data to suggest that acute exercise increased circulating plasma NO levels. Three additional studies (2, 10, 29), however, were unable to show any significant effect of NO inhibition on metabolic vasodilation. Endo and colleagues (10) suggested that the small reduction in exercise hyperemia that they observed could be explained by the reduction in resting forearm blood flow with NO inhibition and noted that ANG II resulted in a similar reduction in metabolic vasodilation. Our data confirm that NO does contribute to peak functional hyperemia and the maintenance of FHBF in response to exercise. Peak FHBF was reduced by 21%, while the volume of blood repaid over 5 min was decreased by 36%, when NO production was inhibited with L-NMMA. This significant effect was maintained even after accounting for the fall in basal blood flow. This occurred despite a modest increase in mean arterial blood pressure.

Similar reductions in peak functional hyperemia and the maintenance phase of FHBF were seen when vasodilator prostacyclins were inhibited. Peak FHBF was reduced by 20%, while the volume of blood repaid over 5 min was decreased by 18%, when ASA was infused. These results confirmed the changes in peak flow observed in previous studies (6, 16, 30), but we were also able to show that these reductions occurred after correction for the change in resting flow with ASA. Whether a higher dose of ASA (8) would have resulted in a further decrement in FHBF warrants further investigation. The fact that we could not produce any further decrement in FHBF (after accounting for the reduction in resting forearm blood flow) when NO and vasodilator prostacyclins were inhibited indicates that the maximum contribution of these endothelium-derived vasodilators to metabolic vasodilation is ~20% of peak FHBF and 35% of the recovery hyperemia, although a myogenic response may have masked any further reduction in flow when both factors were inhibited. Other vasodilators may include endothelium-derived hyperpolarizing factor (5) and local vasodilator metabolites (26).

Methodological differences from previous studies may account for our findings. We were able to study larger numbers than previous investigators and were able to assess the entire recovery phase of FHBF. We chose a "no-load" form of isotonic exercise to minimize systemic effects and thereby minimize effects on systemic arterial reflexes. We also chose drug doses estimated to produce the maximal local effect on blood flow without any systemic effect, although L-NMMA did produce a modest increase in mean arterial blood pressure with exercise. In addition, we infused ASA and L-NMMA for...
The mechanisms responsible for the release of NO and prostacyclin with exercise are unclear. The most important stimulus appears to be shear stress, which has been shown to release both substances from the endothelium (17, 24). Thus we are unable to determine whether the greater FHBF observed before inhibition of NO and prostacyclin was due to their release in response to exercise hyperemia-induced shear stress or whether NO and prostacyclin are a necessary part of metabolic vasodilation.

Our findings are consistent with studies of reactive hyperemia induced by an ischemic stimulus. It has previously been noted that endothelium-derived vasodilators contribute significantly to the reactive hyperemia seen after 3–5 min of arterial occlusion in the forearm of healthy volunteers. Kilbom and Wennmalm (16) noted that they could reduce postischemic reactive hyperemia with indomethacin, an inhibitor of prostaglandin production. More recently, Meredith and colleagues (19) demonstrated a similar reduction in reactive hyperemia after inhibition of NO with L-NMMA. Engelke and colleagues (11) were able to detect a modest additive effect of vasodilator prostanoi and NO inhibition on postischemic reactive hyperemia, although there were significant methodological differences from our investigation.

Study limitations. A potential limitation of this study is that the combined inhibition of NO and vasodilator prostanoïds was not performed in randomized order. It is thus possible that the additive effect of ASA and L-NMMA infusion on resting blood flow over and above the effect of ASA alone may not have been apparent if the drugs were infused in the reverse order. We chose this order of infusions, because we found that, unlike ASA, L-NMMA infusion at 2 mg/min had a small, but significant, systemic hemodynamic effect during exercise, which may have masked any effect that ASA would have had if given after L-NMMA. It is also possible that inhibition of prostanoïds may have produced a compensatory increase in NO production; however, stimulation of NO production by shear stress should have been less owing to the lower resting blood flow (24).

Although we have attempted to compensate for any effect that reduction in resting forearm blood flow with inhibition of vasodilator prostanoïds or NO may have had on FHBF by calculating the absolute increase in blood flow after exercise, it is possible that returning resting flow to baseline levels with a vasodilator (such as sodium nitroprusside) may have attenuated the effect of ASA and L-NMMA on FHBF. This possibility warrants further investigation.

We measured blood flow after, rather than during, exercise, inasmuch as measurement of blood flow during exercise with this technique is difficult. Nevertheless, we found that peak FHBF reproducibly occurred in the first 10–15 s after the cessation of exercise. We were particularly interested in the factors that contribute to the peak and the maintenance phases of FHBF, inasmuch as metabolites are unlikely to be involved in the latter (26). Although we did not measure oxygen utilization during exercise in this study, previous investigators have shown that resting and exercise oxygen extraction is increased with NO inhibition, whereas oxygen consumption is unaffected (10, 12). In a previous study using ASA to assess changes in resting flow, we found a nonsignificant 30% increase in oxygen extraction but no change in oxygen consumption (8).

Clinical implications. Although our findings are applicable to young, healthy individuals, it is likely that these endothelium-derived vasodilators contribute to resting blood flow and exercise-induced hyperemia in other healthy age groups (12, 15). It is known that endothelium-dependent vasodilation in response to pharmacological stimuli and shear stress is impaired in patients with atherosclerosis and in those with risk factors for atherosclerosis (3, 20). Moreover, in the forearm of patients with congestive cardiac failure, there is recent evidence that NO-mediated metabolic vasodilation is impaired (15). In this situation, patients may become more dependent on vasodilator prostanoïds for metabolic vasodilation, as suggested recently by Lang and colleagues (18). Whether the apparent reduced bioavailability of NO in atherosclerosis (or in those with risk factors for atherosclerosis) would result in impairment of exercise hyperemia is unknown.

Recent studies have shown that interventions that increase the bioavailability of NO can improve the response to endothelium-dependent vasodilators such as ACh and shear stress (3). Although speculative, the possibility that these same interventions could improve NO-mediated metabolic vasodilation may lead to new therapeutic modalities in some cardiovascular diseases. Similarly, inasmuch as stimulated production of prostacyclin may be impaired in atherosclerosis (25), treatments that enhance prostacyclin production may improve exercise capacity in these conditions (18).

Release of NO and prostacyclin is stimulated by bradykinin (7). The reduction of metabolism of this kinin is thought to be part of the therapeutic effect of angiotensin-converting enzyme inhibitors (4). Thus part of this therapeutic benefit, especially in heart failure, may be to increase the bioavailability of these vasodilators for metabolic vasodilation. Cyclooxygenase inhibitors such as ASA and indomethacin have been shown to impair the beneficial hemodynamic effects and prognostic benefits of angiotensin-converting enzyme inhibitors in patients with heart failure (4) and reduce metabolic vasodilation in this group of patients (18). The observed role of vasodilator prostanoïds in resting blood flow and exercise-induced hyperemia suggests a possible mechanism to explain these observations.

Conclusion. This study has demonstrated that endothelium-derived NO and vasodilator prostanoïds contribute to resting skeletal muscle blood flow and exercise-induced functional hyperemia in healthy young humans and that this effect is maintained after correction for the change in basal blood flow. Although sequential inhibition of these two vasodilators has an additive effect on resting forearm blood flow, there was
no apparent additional effect on functional hyperemia. Other factors are also important and may be recruited if one or the other mechanism is rendered inoperable.

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