Postexercise hypotension is not explained by a prostaglandin-dependent peripheral vasodilation

Jennifer M. Lockwood, Mollie P. Pricher, Brad W. Wilkins, Lacy A. Holowatz, and John R. Halliwill
Department of Human Physiology, University of Oregon, Eugene, Oregon

Submitted 27 July 2004; accepted in final form 30 September 2004

Postexercise hypotension is not explained by a prostaglandin-dependent peripheral vasodilation. J Appl Physiol 98: 447–453, 2005. First published October 1, 2004; doi:10.1152/japplphysiol.00787.2004.—In normally active individuals, postexercise hypotension after a single bout of aerobic exercise occurs due to an unexplained peripheral vasodilation. Prostaglandin production has been suggested to contribute to the increases in blood flow during and after exercise; however, its potential contribution to postexercise hypotension has not been assessed. The purpose of this study was to determine the potential contribution of a prostaglandin-dependent vasodilation to changes in systemic vascular conductance underlying postexercise hypotension; this was done by inhibiting production of prostaglandins with the cyclooxygenase inhibitor ibuprofen. We studied 11 healthy normotensive men (aged 23.7 ± 4.2 yr) before and during the 90 min after a 60-min bout of cycling at 60% peak O₂ uptake on a control and a cyclooxygenase inhibition day (randomized). Subjects received 10 mg/kg of oral ibuprofen on the cyclooxygenase inhibition day. On both study days, arterial blood pressure (automated auscultation) and cardiac output (acetylene uptake) were measured, and systemic vascular conductance was calculated. Inhibition of cyclooxygenase had no effect on baseline values of mean arterial pressure or systemic vascular conductance (P > 0.2). After exercise on both days, mean arterial pressure was reduced (−2.2 ± 1.0 mmHg change with the control condition and −3.8 ± 1.5 mmHg change with the ibuprofen condition, both P < 0.05 vs. preexercise) and systemic vascular conductance was increased (5.2 ± 5.0% change with the control condition and 8.7 ± 4.1% change with the ibuprofen condition, both P < 0.05 vs. preexercise). There were no differences between study days (P > 0.6). These data suggest that prostaglandin-dependent vasodilation does not contribute to the increased systemic vascular conductance underlying postexercise hypotension.

Address for reprint requests and other correspondence: J. R. Halliwell, 122 Esslinger Hall, 1240 Univ. of Oregon, Eugene, OR 97403-1240 (E-mail: halliwill@uoregon.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

This study was approved by the Institutional Review Board of the University of Oregon, and each subject gave his informed, written consent before participation.

Subjects

A total of 11 healthy, nonsmoking, normotensive male subjects between the ages of 20 and 32 yr participated in this study. On the basis of their exercise habits over the prior 12 mo, subjects were classified as “normally active” (no regular endurance activity). These subjects participated in <2 h of aerobic exercise per week. Subjects were not taking any medications.

Screening Visit

Subjects reported to the laboratory for a screening visit and cycle ergometer test at least 2 h postprandial and abstained from caffeine, alcohol, and exercise for 24 h before the screening visit. Subjects performed an incremental cycle exercise test (Lode Excaliber, Groningen, The Netherlands) consisting of 1-min workload increments to determine \( V_{\text{O}_2}\text{peak} \). Specifically, after a 2-min warm-up period of easy cycling (25–30 W), workload increased at 25 or 30 W every 1 min. Selection of the workload increment was subjective, with the goal of producing exhaustion within 8–12 min. Whole body \( O_2 \) uptake was measured via a mixing chamber (Parvomedics, Sandy, UT) integrated with a mass spectrometry system (Marquette MGA 1100, MA Tech Services, St. Louis, MO). All subjects reached subjective exhaustion [rating of perceived exertion on the Borg (2) scale of 19–20] within the 8- to 12-min period. After the subjects rested for 15–20 min, they returned to the cycle ergometer for assessment of the workload corresponding to a steady-state \( V_{\text{O}_2} \) of 60% of \( V_{\text{O}_2}\text{peak} \). This workload was used on the 2 study days for the 60-min exercise bout. Subjects self-reported activity levels on two questionnaires (1, 27).

For both study days, subjects reported for the study at least 2 h postprandial and abstained from caffeine for 12 h and from exercise and ibuprofen for 24 h before the study. The second study day was at least 5 days and not more than 10 days after the first study day, providing adequate time for clearance of ibuprofen.

Experimental Protocol

Subjects reported for parallel experiments on 2 separate days. The order of experiments was randomized between a cyclooxygenase inhibition (ibuprofen) and a control day. On study days, subjects were given a snack with or without cyclooxygenase inhibition 90 min before the start of exercise. The subjects were then instructed to lie in the supine position for instrumentation. A venous catheter was inserted into the right arm in the antecubital region to obtain blood samples. Exercise consisted of a 60-min period of seated upright cycling at 60% \( V_{\text{O}_2}\text{peak} \). Exercise of this intensity and duration produces a sustained (~2 h) postexercise hypotension (15). During exercise, subjects received 10 ml of water for every kilogram of body weight to replace water loss due to sweating. Measurements were taken for 30 min before and throughout the 90 min after a 60-min bout of exercise. Baseline (preexercise), 30 min, 60 min, and 90 min postexercise measurements included cardiac output, heart rate, arterial pressure, and a blood sample for assessment of cyclooxygenase inhibition. All pre- and postexercise measurements were made in the supine position.

Measurements

Heart rate and arterial pressure. Heart rate and arterial pressure were monitored throughout all experimental procedures. Heart rate was monitored using a five-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA). Arterial pressure was measured in the arm with an automated auscultometric device (Dinamap Pro100 vital signs monitor, Critikon, Tampa, FL).

Cardiac output. We estimated cardiac output using an open-circuit acetylene washin method as developed by Stout et al. (44), modified by Gan et al. (14), and validated in humans vs. the direct Fick approach (23). This method allows the noninvasive estimation of cardiac output. We chose an open-circuit method vs. rebreath techniques because subjects are exposed to stable oxygen and carbon dioxide levels throughout the measurement. Subjects breathed a gas mixture containing 0.6% acetylene-9.0% helium-20.9% oxygen- balance nitrogen for 8–10 breaths via a two-way nonbreathing valve. During the washin phase, breath-by-breath acetylene and helium uptakes were measured by a respiratory mass spectrometer (Marquette MGA 1100) and tidal volume was measured via a pneumotach (model 3700, Hans Rudolph, Kansas City, MO) linearized by the technique of Yeh et al. (49) and calibrated by the use of test gas before each study. The pneumotach and valve system had a combined dead space of 24 ml. Cardiac output calculations have been described previously (23). Stroke volume was determined from cardiac output/heart rate. Systemic vascular conductance was calculated as cardiac output/mean arterial pressure (expressed as ml min\(^{-1}\) mm Hg\(^{-1}\)).

Leg blood flow. In three subjects, we measured leg blood flow as described previously (16). We measured femoral artery blood velocity using a 4-MHz pulsed Doppler ultrasound probe (model 500M, Multigon Industries, Yonkers, NY) placed above the femoral bifurcation. The entire width of the artery was insonated with an angle of 60°. A 7-MHz linear array ultrasound probe (Acuson 128XP/10-ART Ultrasound System, Mountain View, CA) was used to obtain femoral artery diameter measurements immediately after each femoral artery blood velocity measurement. Leg blood flow was calculated as artery cross-sectional area multiplied by femoral mean blood velocity, doubled to represent both legs (reported here in ml/min). Leg vascular conductance was calculated as flow for both legs per mean arterial pressure (in ml min\(^{-1}\) mm Hg\(^{-1}\)).

Cyclooxygenase inhibition and biochemical analyses. Cyclooxygenase was inhibited with ibuprofen. Subjects received 10 mg/kg of oral ibuprofen, with a maximum dosage of 1,000 mg. Inhibition of cyclooxygenase prevents the breakdown of arachidonic acid, the precursor for prostaglandins (45). A primary branch of this arachidonic acid cascade produces prostacyclin, a potent but unstable vasodilator (half-life of ~3 min) that breaks down into the stable but less active 6-keto-prostaglandin-F\(_{1\alpha}\) (6-keto-PGF\(_{1\alpha}\)) (33, 40). To confirm inhibition of cyclooxygenase in our study, blood samples were taken with an intravenous catheter before exercise, immediately at the end of the exercise bout, and after exercise. Whole blood samples were centrifuged, separated, and stored at ~80°C until analyses. The adequacy of the cyclooxygenase blockade was assessed by measuring plasma concentrations of 6-keto-PGF\(_{1\alpha}\) with a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI), expressed in picograms per milliliter (32, 36, 39). The reported lower limit for detection of 6-keto-PGF\(_{1\alpha}\) is 4.3 pg/ml. Across the range of values in this study, interassay and intra-assay coefficients of variation are 8.0 and 10.4%.

Data Analyses and Statistics

The individual analyzing the data was blinded to the drug condition for each study day.

The results were analyzed with a repeated-measures two-way ANOVA (drug vs. time). Significant effects were further tested with Fisher’s least significant difference test, and differences were considered significant at \( P < 0.05 \). All values are reported as means ± SE unless otherwise noted.
RESULTS

Subject characteristics are shown in Table 1. VO2 peak values were within the normal range for this population (3.614 ± 744 ml/min, mean ± SD).

Exercise

The goal was to have subjects exercise for 60 min at 60% VO2 peak. On both days, the average workload was 157.1 ± 10.4 W. On the control day, heart rate increased from 57.7 ± 3.9 beats/min at supine rest to 142.0 ± 5.0 beats/min during exercise (mean for entire 60 min of exercise; P < 0.05). This represented on average, 66.4 ± 3.5% heart rate reserve (heart rate reserve is defined as maximal heart rate achieved during VO2 peak testing minus the resting supine heart rate) and is consistent with the target workload. On the cyclooxygenase inhibition day (ibuprofen day), heart rate increased from 3.9 beats/min at supine rest to 142.0 ± 10.4 W. On the control day, heart rate increased from 57.7 ± 10.4 beats/min to 139.4 ± 4.6 beats/min during exercise (mean for entire 60 min of exercise; P < 0.05). This represented, on average, 63.3 ± 3.7% heart rate reserve and is consistent with the target workload. There were no differences in baseline heart rate (P > 0.2) or percent heart rate reserve (P > 0.2) between the 2 study days. There were also no differences in the arterial pressure response to exercise (97.1 ± 2.8 mmHg for control condition and 96.2 ± 1.9 mmHg for ibuprofen condition; P > 0.6).

6-keto-PGF1α Concentration

Figure 1 shows plasma concentrations of the stable cyclooxygenase product 6-keto-PGF1α. Exercise increased the concentration of 6-keto-PGF1α from baseline values on the control day (P < 0.05). Ibuprofen attenuated this exercise-induced increase in 6-keto-PGF1α concentration, and the concentrations remained lower at 30 min postexercise on the cyclooxygenase inhibition day (P < 0.05 vs. control day; Fig. 1A). The change in 6-keto-PGF1α from baseline to 30 min postexercise was greater on the control day than on the cyclooxygenase inhibition day (P < 0.05 vs. control day; Fig. 1B); the change in 6-keto-PGF1α concentration was not different from zero (no change) on the cyclooxygenase day. Combined, these results are consistent with the inhibition of the cyclooxygenase enzyme and the reduction in prostaglandin concentration.

Postexercise Hemodynamics

Table 2 shows postexercise vs. preexercise hemodynamics on both study days. Heart rate was higher postexercise compared with preexercise on both study days (P < 0.05). There were no differences in heart rate, stroke volume, or cardiac output between the 2 study days (P > 0.10). Figure 2 shows mean arterial pressure, systemic vascular conductance, and femoral vascular conductance values preexercise to 90 min postexercise on both study days. Mean arterial pressure was reduced from baseline during recovery from exercise on both study days (P < 0.05; Fig. 2A). Systemic vascular conductance increased after exercise on both study days (P < 0.05; Fig. 2B). Femoral vascular conductance increased after exercise and remained elevated through 90 min postexercise on both study days; however, this trend was not tested statistically due to the small number of subjects. There were no differences in mean arterial pressure and systemic vascular conductance between the 2 study days (P > 0.4). There were no apparent differences in femoral vascular conductance between the 2 study days.

In Fig. 3, the reduction in mean arterial pressure and the rise in systemic and femoral vascular conductances from baseline to 30 min postexercise are shown. The cyclooxygenase inhibition day and the control day exhibited a similar degree of postexercise hypotension (~3–4 mmHg; P < 0.05 vs. preexercise) and increase in systemic vascular conductance (~6–9%; P < 0.05 vs. preexercise). There were no differences in

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.7 ± 4.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181.4 ± 6.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88.2 ± 19.9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.6 ± 4.7</td>
</tr>
<tr>
<td>VO2 peak, ml/kg·min⁻¹</td>
<td>41.8 ± 8.4</td>
</tr>
<tr>
<td>Workload at 60% of VO2 peak, W</td>
<td>157.1 ± 32.9</td>
</tr>
<tr>
<td>Baeeke sport index, arbitrary units</td>
<td>10.3 ± 2.2</td>
</tr>
<tr>
<td>Index of physical activity, MET·h⁻¹·wk⁻¹</td>
<td>159.6 ± 94.0</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 11 subjects. VO2 peak, peak oxygen consumption; MET, metabolic equivalents.

Fig. 1. A: prostaglandin concentrations before exercise (Pre), at the end of 60 min of exercise, and at 30, 60, and 90 min postexercise (Post). ○, Control day; ●, ibuprofen day. B: change (Δ) in prostaglandin concentration from preexercise to 30 min postexercise. Open bar, control day; filled bar, ibuprofen day. Values are means ± SE. *P < 0.05 vs. preexercise. †P < 0.05 vs. control day at same time point.
Postexercise hypotension after a single bout of aerobic exercise is due to an unexplained peripheral vasodilation. Prostaglandins have been suggested to contribute to increases in blood flow during and immediately after exercise. The goal of this study was to determine the potential contribution of a prostaglandin-dependent vasodilation to increases in systemic vascular conductance underlying postexercise hypotension. We found that the postexercise fall in arterial pressure and increase in systemic vascular conductance remained unchanged despite the adequate inhibition of cyclooxygenase. Thus it does not appear that a prostaglandin-dependent vasodilation plays a role in the peripheral vasodilation that underlies postexercise hypotension.

Postexercise hypotension is characterized by a persistent rise in systemic vascular conductance that is not completely offset by increases in cardiac output. Forearm and calf vascular conductances are increased in parallel with systemic vascular conductance; thus the vasodilation that underlies postexercise hypotension is not restricted to the sites of active skeletal muscles. This peripheral vasodilation includes both a neural and vascular component. Previously, Halliwill et al. showed in humans that the baroreflex is reset to a lower pressure after exercise, creating a reduction in sympathetic vasoconstrictor outflow. In addition, vascular responsiveness to sympathetic vasoconstrictor outflow is impaired so that vascular resistance is reduced for a given level of sympathetic nerve activity, independent of changes in \( \alpha \)-adrenergic receptor responsiveness. This suggests a presynaptic inhibition of norepinephrine release from sympathetic vasoconstrictor nerves after exercise. However, in another study by Halliwill et al., the increase in vascular conductance after exercise was greater than that observed after \( \alpha \)-adrenergic receptor blockade, suggesting the existence of a superimposed vasodilator signal. We considered the possibility that prostacyclin or a related prostaglandin could be that signal.

Prostacyclin is one of several potent vasodilators produced by arachidonic acid in vascular endothelial cells in response to exercise, increased blood flow, and shear wall stress. Although prostaglandins have a clear role in reactive hyperemia, their contribution to exercise hyperemia is controversial. Ritter et al. showed increased 6-keto-PGF\(_{1\alpha}\) concentrations immediately after a 10-min sprint workout. Wilson and Kapoor showed that the exercise hyperemia induced by isometric exercise of the forearm was attenuated by cyclooxygenase blockade. Boushel et al. showed that exercise hyperemia, induced by knee extension exercise, was reduced by the combination of cyclooxygenase and nitric oxide synthase inhibition. Farouque and Meredith also found that a combination of cyclooxygenase and nitric oxide synthase inhibition reduced forearm blood flow during wrist exercise. In contrast to these findings, Shoemaker et al. reported that inhibition of cyclooxygenase did not reduce arm blood flow during arm exercise.

Table 2. Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>58 ± 4</td>
<td>62 ± 4*</td>
<td>60 ± 3</td>
<td>59 ± 4</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>5.9 ± 0.4</td>
<td>5.7 ± 0.4</td>
<td>5.5 ± 0.4</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Stroke volume, ml/beat</td>
<td>97.4 ± 7.0</td>
<td>87.6 ± 6.5</td>
<td>92.0 ± 5.9</td>
<td>94.3 ± 7.7</td>
</tr>
<tr>
<td>Femoral blood flow, ml/min</td>
<td>310 ± 31</td>
<td>420 ± 56</td>
<td>425 ± 78</td>
<td>392 ± 52</td>
</tr>
</tbody>
</table>

| Ibuprofen           |             |        |        |        |
| Heart rate, beats/min| 61 ± 3      | 65 ± 4*| 61 ± 3 | 61 ± 4 |
| Cardiac output, l/min| 5.9 ± 0.4   | 6.0 ± 0.4| 5.6 ± 0.4| 5.3 ± 0.4|
| Stroke volume, ml/beat| 98.6 ± 9.5 | 94.7 ± 9.0| 95.3 ± 9.1| 91.1 ± 8.2|
| Femoral blood flow, ml/min| 295 ± 31 | 443 ± 54| 398 ± 27| 312 ± 42|

Values are means ± SE; \( n = 11 \) subjects (except \( n = 3 \) subjects for femoral blood flow). \(* P < 0.05 \) vs. preexercise.
However, the primary question addressed here is whether there is a vasodilatory role for prostaglandins after exercise ends. The few studies that have addressed the potential contribution of prostaglandins after exercise completion have focused on the immediate postexercise period (i.e., <30 min postexercise). In the presence of a cyclooxygenase inhibitor, Cowley et al. (8, 9) found calf blood flow was reduced up to 30 min after a bout of treadmill exercise. Karamouzis et al. (24) found exercise to increase muscle interstitial concentrations of 6-keto-PGF$_{1\alpha}$; however, 30 min after exercise, the concentrations returned to baseline levels. In agreement, in the present study, we found that, 30 min after exercise, the plasma concentration of 6-keto-PGF$_{1\alpha}$ had returned to baseline levels although systemic vascular conductance was still elevated above baseline levels. Thus it appears that prostaglandins play a role in the immediate postexercise vasodilation; however, the response is short lived and therefore does not contribute importantly to postexercise hypotension. The substance responsible for the persistent postexercise vasodilation contributing to postexercise hypotension remains elusive.

**Limitations**

The magnitude of postexercise hypotension seen in this study was slightly less than seen in our previous studies, despite tight experimental control over most of the factors recognized to modulate postexercise hypotension (15). Thus it is unclear what factor(s) is responsible for this observation. In the present study, our subjects were young, healthy normotensive men who were recreationally active, as we have studied in the past. However, the present subjects were larger and thus worked at higher absolute workloads than the men of similar fitness in our prior studies (41). It is unclear if this in some way explains the absence of a rise in cardiac output during postexercise hypotension in the present study or the trend for cardiac output to decline at 90 min postexercise. Nonetheless, we were able to document vasodilation in terms of both leg and systemic vascular conductances in this protocol, supporting the notion that our results are comparable with our prior studies on postexercise hypotension. However, it should be noted that our conclusions regarding the role of prostaglandins in postexercise hypotension remain elusive.
cise hypotension may be limited to healthy individuals of average fitness; it could be that, in different populations (e.g., older hypertensives), prostaglandins contribute to the vasodilation that underlies postexercise hypotension.

It is possible that cyclooxygenase inhibition prevented localized vasodilation in the legs that was masked by vasoconstriction elsewhere and was, thus, undetectable via assessment of systemic vascular conductance. To address this possibility, we also determined leg vascular conductance in a subset of subjects \( (n = 3) \). Although based on a limited number of observations, the results are inconsistent with a localized effect of cyclooxygenase inhibition in the legs during the first hour of postexercise hypotension. Interestingly, these observations suggest that prostaglandins could be involved in the resolution of postexercise hypotension near 90 min postexercise. This delayed component merits further study but cannot be adequately addressed in the present 90-min protocol.

Another limitation to this study is the nonselective nature of cyclooxygenase inhibition. Ibuprofen prevents the enzyme cyclooxygenase from converting arachidonic acid to prostacyclin, prostaglandins, and thromboxanes. Some of these products act as vasodilators, whereas others cause vasoconstriction. Thus ibuprofen could be inhibiting vasoconstricting prostaglandins and thromboxanes as well as the vasodilatory prostaglandins of interest here. This could explain our observation that ibuprofen had no effect on systemic vascular conductance after exercise, despite reduced circulating prostaglandin levels. Nonetheless, if vasodilators produced by cyclooxygenase are offset by vasoconstrictors also produced by cyclooxygenase, then it is unlikely that this mechanism is the principal vasodilator during postexercise hypotension.

Furthermore, we used the measurement of the representative prostaglandin 6-keto-PGF\(_{1\alpha}\). Because prostacyclin has a half-life in the blood of 3 min \( (40) \), we measured the concentration of its stable metabolite 6-keto-PGF\(_{1\alpha}\) which has a half-life of 30 min \( (40) \). Thus we do not have a direct measure of prostacyclin, which is the biologically active vasodilator. Nonetheless, we believe that we demonstrated adequate cyclooxygenase inhibition.

In conclusion, we found that the sustained peripheral vasoconstriction underlying postexercise hypotension is unaffected by the inhibition of cyclooxygenase. Thus it does not appear that postexercise hypotension is due to a prostaglandin-dependent vasoconstriction. The substance responsible for the persistent peripheral vasodilation during postexercise hypotension remains to be identified.

ACKNOWLEDGMENTS

We extend our appreciation to subjects who volunteered for this study. We also thank Carolyn Snarski and Jay Williams for technical assistance.

This study was conducted in partial fulfillment of the requirements for the degree of Masters of Science at the University of Oregon for J. M. Lockwood.

GRANTS

This research was supported by a grant from American Heart Association, Northland Affiliate, Scientist Development Grant 30403Z and National Heart, Lung, and Blood Institute Grant HL-65305.

REFERENCES


J Appl Physiol • VOL 98 • FEBRUARY 2005 • www.jap.org


