Free Radical–Mediated Lipid Peroxidation and Systemic Nitric Oxide Bioavailability: Implications for Postexercise Hemodynamics

Karl J. New1, Michael E. Reilly1, Kath Templeton2, Gethin Ellis2, Philip E. James3, Jane Mceneny4, Michael Penney5, James Hooper6, Dave Hullin7, Bruce Davies1 and Damian M. Bailey1

BACKGROUND
The metabolic vasodilator mediating postexercise hypotension (PEH) is poorly understood. Recent evidence suggests an exercise-induced reliance on pro-oxidant–stimulated vasodilation in normotensive young human subjects, but the role in the prehypertensive state is not known.

METHODS
Nine prehypertensives (mean arterial pressure (MAP), 106 ± 5 mm Hg; 50 ± 10 years old) performed 30 minutes of cycle exercise and a nonexercise trial. Arterial distensibility was characterized by simultaneously recording upper- and lower-limb pulse wave velocity (PWV) via oscillometry. Systemic vascular resistance and conductance were determined by MAP/Q and Q/MAP, respectively. Venous blood was assayed for indirect markers of oxidative stress (lipid hydroperoxides (LOOH); spectrophotometry), plasma nitric oxide (NO) and S-nitrosothiols (fluorometry), atrial natriuretic peptide (ANP), and angiotensin II (ANG-II) (radioimmunoassay).

RESULTS
Exercise reduced MAP (6 mm Hg) and vascular resistance (15%) at 60 minutes after exercise, whereas conductance was elevated (20%) (P < 0.05). The hypotension resulted in a lower MAP at 60 and 120 minutes after exercise compared with nonexercise (P < 0.05). Upper-limb PWV was also 18% lower after exercise compared with baseline (P < 0.05). Exercise increased LOOH coincident with the nadir in hypotension and vascular resistance but failed to affect plasma NO or S-nitrosothiols. Exercise-induced increases in LOOH were related to ANG-II (r = 0.97; P < 0.01) and complemented by elevated ANP concentrations.

CONCLUSIONS
These data indicate attenuated vascular resistance after exercise with increased oxidative stress and unchanged NO. Whether free radicals are obligatory for PEH requires further investigation, although it seems that oxidative stress occurs during the hyperemia underlying PEH.

Keywords: blood pressure; hypertension; nitric oxide bioavailability; reactive oxygen species; vascular function.

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Exercise induces profound changes in the mechanisms that regulate and determine arterial pressure, resulting in a postexercise hypotension (PEH)1,2 that is greatest in hypertensive individuals.3 PEH results from a persistent rise in systemic vascular conductance (SVC) that is not completely corrected for by increases in cardiac output (Q).

Halliwell4 elucidated a “neural” and a “vascular” component to the alteration in sympathetic vascular regulation. The vascular component has been postulated as the attenuation of vascular responses to sympathetic vasoconstriction4 together with the influence of local and circulating vasodilator substances.4 The putative metabolic pathway is poorly understood. Halliwell et al.4 reported that PEH withstands NO blockade, whereas histamine H1 and H2 receptors have been clearly implicated in the vasodilation.4 To our knowledge no study has investigated the balance between circulating vasodilators and vasoconstrictors during PEH. Previous work from our laboratory has identified the reliance on exercise-induced free radicals for brachial artery vasodilation,7–9 whereby oral antioxidants blunt the vasodilation in response to increased shear stress in young and exercise-trained older individuals. Surprisingly, the impact of an acute exercise-induced increase in circulating free radicals and the consequent effects for nitric oxide (NO) bioavailability on PEH are unknown.

Information regarding arterial pulse wave velocity (PWV) and compliance alterations at rest and after exercise provide an insight into vascular responses throughout the arterial tree. The elastic behavior of arteries converts pulsatile cardiac ejection into nearly continuous tissue perfusion and reduces systolic pressure relative to flow; this creates a reduced workload

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relative to perfusion, producing “cardiovascular efficiency.” A lowering of PWV after exercise, which could be mediated by free radicals, would augment the benefits of hypotension and lowered vascular resistance. Naka et al. have documented the postexercise reduction in peripheral arterial PWV in young adults; as yet, no study has simultaneously recorded arterial compliance during a period of PEH and related the findings to the circulating metabolic environment.

Therefore, we sought to investigate the effects of exercise-induced neurohumoral metabolites on postexercise vasodilation and hypotension. A secondary aim of our investigation was to evaluate the response of regional limb arterial PWV concomitantly with systemic hemodynamics after exercise in prehypertensives. We also compared the exercise hemodynamic response against nonexercise. This control allowed changes occurring after exercise to be ascribed to exercise per se, beyond typical changes that may occur with such labile parameters as MAP and PWV. We hypothesized (i) that PEH would occur with increased oxidative stress despite reductions in circulating NO bioavailability and augmented angiotensin II (ANG-II) concentrations and (ii) that exercise would induce reductions in regional limb PWV, which would persist for an extended time period compared with nonexercise.

METHODS

Ethical approval

Nine sedentary, prehypertensive white male subjects, volunteered to participate in the studies (Table 1) and provided written informed consent. The local Institutional Ethical Committee approved all experimental protocols.

Subjects

Subjects were free of pharmacological control of blood pressure (BP). Mean arterial pressure (MAP) assessment followed established guidelines. Fasting plasma electrolyte, osmolality, homocysteine, folate, lipid, and glucose concentrations (Table 2) were stable between study days (P = 0.78). Before beginning experimentation, subjects underwent a functional exercise stress test with peak oxygen consumption (VO2peak) determined by means of an incremental cycle ergometry test performed until volitional exhaustion.

Study design

Subjects attended the laboratory 1 week before commencement of the experiment for familiarization and refrained from exercise and alcohol for 48 hours before all testing. Subjects were then randomly assigned (single blind) to 2 further visits counterbalanced with (i) 30 minutes of cycle exercise (Monark 824e, Sweden) at 75% of VO2peak and (ii) a nonexercise day following exactly the same time course (including time of day), location, and subject positioning as the exercise day. Subjects were followed up after exercise or after the control session for 2 hours. On arrival, after an overnight fast, a 1.2 × 45-mm, 18-gauge cannula (BD, Plymouth, UK) was inserted into an antecubital vein to collect blood samples. The arm chosen for cannulation was contralateral to that being used for PWV and BP measurement; subjects underwent baseline assessment of physiological parameters after 20 minutes of seated rest. The laboratory was regulated continuously for temperature (21 ± 2 °C) and humidity (65% ± 3%).

Exercise protocol

Subjects began cycle exercise for 1 minute (70 W), at which point power output increased to 75% of Peak Oxygen Consumption. Systolic BP (SBP) (sphygmomanometer) and heart rate (HR) (3-lead ECG; Lifepulse LP10, Coventry, UK) were measured every 10 minutes during the 30-minute trial. VO2 (Medgraphics; CPX/D, Gloucester, UK) was determined midway through exercise. At cessation of exercise (and at the corresponding time point on the control day), subjects transferred to an examination bench where they remained seated; BP (photoplethysmography) (Finapres Medical Systems, Bristol, UK), HR (3-lead ECG), Rate Pressure Product (RPP), Pulse Wave Velocity (PWV) (QVL P84; SciMed, Bristol, UK; time resolution ± 2 ms), and stroke volume (SV) (Acuson; Cypress, Bristol, UK) were recorded before and immediately, 1 hour, and 2 hours after exercise. Blood samples were taken before and after exercise, contemporaneously with physiological parameters, and were immediately separated (10 minutes at 3000 rpm) with plasma stored at −80 °C until analysis.

Arterial PWV

Arterial PWV was measured simultaneously in the arm and leg by means of oscillometry to detect wave timing (time resolution, ±2 ms) (QVL; SciMed, Bristol, UK). This method and technique have been previously validated; a novel processing algorithm performs multiple time shifts of the distal waveform relative to the proximal waveform in the region of the systolic edge. Nonocclusive cuffs were placed over the brachial and radial arteries and over the femoral and tibial arteries. Cuff inflation and characterization of the arterial pulse waves by the computer program to determine transit

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Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 ± 0.07</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88 ± 17</td>
</tr>
<tr>
<td>VO2peak (ml kg⁻¹ min⁻¹)</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>132 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>104 ± 4</td>
</tr>
<tr>
<td>Resting UL PWV (m·s⁻¹)</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Resting LL PWV (m·s⁻¹)</td>
<td>13 ± 2</td>
</tr>
</tbody>
</table>

Abbreviations: LL, lower limb; PWV, pulse wave velocity; UL, upper limb; VO2peak, peak oxygen consumption.
time have been detailed previously. Because increases in arterial pressure increase PWV, values were normalized to systemic MAP.

**Central hemodynamics**

SV was measured with Doppler echocardiography (Acuson; Cypress, Bristol, UK) at rest, immediately after exercise (i.e., within 10 seconds), and continuing for the same measurement period at 1 and 2 hours after exercise. Subjects were placed in the lateral decubitus position, and SV was calculated by measuring aortic flow:

\[ SV \text{ (ml)} = \pi D^2/4 \times VTI_{AO}, \]

where \( D \) = diameter of left ventricular outflow tract (cm or mm) and \( VTI_{AO} \) = velocity time integral of the aorta.

All measurements were recorded as the mean of 3 consecutive cardiac cycles and, together with the calculations, were performed by the same experienced investigator. Q was calculated from the product of the measured SV and HR (3-lead ECG). Systemic vascular resistance (SVR) (resistance units) and SVC (ml/min/mm Hg) were computed from the quotient of MAP and Q or Q and MAP, respectively.

**Atrial natriuretic peptide and arginine vasopressin**

Plasma atrial natriuretic peptide (ANP) and arginine vasopressin (AVP) were measured by radioimmunoassay with the method of Penney et al. The intra- and interassay Co-efficient of Variation (CVs) were both <5%.

**Catecholamines**

Plasma was assayed for adrenaline and noradrenaline via reverse-phase high-performance liquid chromatography using electrochemical detection. The intra- and interassay CVs were both <7%.

**Table 2. Fasting Plasma Concentrations of Selected Metabolic Cardiovascular Risk Factors and Electrolytes and Osmolality**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>Reference valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 (mmol·l−1)</td>
<td>288 ± 61</td>
<td>180–600</td>
</tr>
<tr>
<td>Folate (mmol·l−1)</td>
<td>8.5 ± 2.4</td>
<td>2.7–14.0</td>
</tr>
<tr>
<td>Homocysteine (mmol·l−1)</td>
<td>13.3 ± 2.2</td>
<td>&lt;16</td>
</tr>
<tr>
<td>Glucose (mmol·l−1)</td>
<td>4.5 ± 1.4</td>
<td>4.5–5.6</td>
</tr>
<tr>
<td>TG (mmol·l−1)</td>
<td>5.1 ± 0.6</td>
<td>3.5–6.5</td>
</tr>
<tr>
<td>LDL-C (mmol·l−1)b</td>
<td>3.2 ± 0.6</td>
<td>1.55–4.4</td>
</tr>
<tr>
<td>HDL-C (mmol·l−1)</td>
<td>1.3 ± 0.2</td>
<td>0.95–2.15</td>
</tr>
<tr>
<td>K⁺ (mmol·l−1)</td>
<td>1.3 ± 0.6</td>
<td>0.7–2.1</td>
</tr>
<tr>
<td>Na⁺ (mmol·l−1)</td>
<td>142.7 ± 1.2</td>
<td>135–145</td>
</tr>
<tr>
<td>Tg (mmol·l−1)</td>
<td>4.5 ± 0.2</td>
<td>3.5–5</td>
</tr>
<tr>
<td>Osmolality (mOsm·kg H₂O)</td>
<td>280 ± 9</td>
<td>280–303</td>
</tr>
</tbody>
</table>

All results are expressed as means ± SDs (n = 9). Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; Tg, triacylglycerol.

*Typical laboratory reference levels.

bLDL-C was derived via the Friedewald equation.

**ANG-II concentrations**

ANG-II concentrations were determined in plasma with Euria-Angiotensin II (Euro-Diagnostica AB, Malmo, Sweden). After extraction, ANG-II was measured by a double-antibody radioimmunoassay. The intra- and interassay CVs were <2% and <5%, respectively.

**Nitrate and nitrite**

Plasma nitrate (NO₃⁻) and nitrite (NO₂⁻) (NOx) were measured using fluorimetry (PerkinElmer Fluorescence Spectrophotometer, Llantrisant; emission, 450 nm; excitation, 365 nm). This assay offers specificity, sensitivity, and versatility with detection limits as low as 1030 nmol/l (10–30 pmol/ml) and can be used to quantify NO₃⁻ and NO₂⁻ generated under physiologically relevant conditions.

**S-Nitrosothiol**

The S-nitrosothiol (RSNO) concentration was quantified in a separate duplicate aliquot of plasma by the Saville reaction. The intra- and interassay CVs for all NO metabolites were 7% and 10%, respectively.

**Free radical–mediated lipid peroxidation**

Serum lipid hydroperoxides (LOOH) were determined with the ferrous iron–xylenol orange assay with modification. The intra- and interassay CVs were <2% and <4%, respectively.

**Antioxidants**

After stabilization and deproteinization (900 µl of 5% metaphosphoric acid to 100 µl of K-EDTA plasma) ascorbic acid was assayed by fluorimetry. Concentrations of lipid
soluble antioxidants were determined by high-performance liquid chromatography. The intra- and interassay CVs were both <5%.

Percentage of change in plasma volume

Arterialized capillary blood was collected from a hyperemic ear lobe to determine packed cell volume (ultracentrifugation) and hemoglobin (reflectance photometry). Blood metabolites were corrected for the percentage of change in plasma volume after exercise.

Statistical analysis

Power calculations, based on our previous studies that have incorporated the same measurement techniques, indicated that 8 subjects were required to achieve a power of >0.7 at the P < 0.05 level (2-tailed test). Statistical analysis was performed using SPSS software (version 18; Surrey, UK). Data were analyzed using parametric or nonparametric statistics after mathematical determination of distribution normality by repeated Shapiro-Wilk W tests. Data were analyzed using a 2-way (trial: active vs. passive; time: pre- vs. postexercise) repeated-measures analysis of variance (ANOVA). After a significant interaction, grouped means for trial and time were analyzed using a 1-factor repeated-measures ANOVA with a posteriori Bonferonni-corrected paired-samples t tests. Q and metabolic data were analyzed with a 1-factor repeated-measures ANOVA with a posteriori Bonferonni-corrected paired-sample t tests. The nonparametric equivalent was the Friedman test with Bonferonni-corrected Wilcoxon signed rank tests. The relationship between selected variables was identified using a Pearson product moment or Spearman rank-order correlation. The α level was established at P < 0.05 for all 2-tailed tests, and values are reported as means ± SDs.

RESULTS

Response to exercise

Exercise intensity equated to 75% ± 5% of VO\textsubscript{2peak}, with an average workload of 112 ± 35 W and HR of 140 ± 17 beats/min during exercise.

BP response

Exercise decreased MAP by 6 mm Hg at the 1-hour postexercise time point (P = 0.01) (Table 3). The PEH persisted for the entire 120-minute recovery, with a nadir at 60 minutes. The reduction in MAP resulted from reduced SBP whereas diastolic BP (DBP) was unchanged. SBP was attenuated by ~10% at 1 hour after exercise, remaining ~7% below baseline for the rest of recovery (P = 0.03). SBP values were 13% and 11% lower (P = 0.02) at 1 and 2 hours after exercise, respectively, compared with the control condition.

Postexercise hemodynamics

Exercise increased both HR and Q (P = 0.02), which returned to resting values after 60 minutes of recovery. SVR was attenuated by ~30% after exercise and remained blunted by ~13% and 8% after 60 and 120 minutes of recovery, respectively (P = 0.02) (Figure 1). SVC was augmented by ~28 ml/min/mm Hg, remaining elevated until 120 minutes after exercise (P = 0.02).

Table 3. Cardiovascular Responses to Either 30 Minutes of Submaximal Exercise or Control Condition in Prehypertensive Subjects

<table>
<thead>
<tr>
<th></th>
<th>Passive</th>
<th>Recovery</th>
<th>Active</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-30 minute exercise</td>
<td>Post-30 minute exercise</td>
<td>+60 min</td>
<td>+120 min</td>
</tr>
<tr>
<td>HR</td>
<td>70 ± 7</td>
<td>69 ± 8</td>
<td>70 ± 7</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>SBP</td>
<td>132 ± 10</td>
<td>131 ± 8</td>
<td>133 ± 11**</td>
<td>133 ± 9**</td>
</tr>
<tr>
<td>DBP</td>
<td>90 ± 5</td>
<td>89 ± 4</td>
<td>89 ± 4</td>
<td>89 ± 5</td>
</tr>
<tr>
<td>MAP</td>
<td>104 ± 4</td>
<td>103 ± 4</td>
<td>104 ± 4**</td>
<td>104 ± 3**</td>
</tr>
<tr>
<td>UL PWV</td>
<td>12 ± 2</td>
<td>13 ± 3**</td>
<td>13 ± 3</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>LL PWV</td>
<td>13 ± 3</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>UL PWVn</td>
<td>0.14 ± 0.05</td>
<td>0.12 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>0.24 ± 0.36</td>
</tr>
<tr>
<td>LL PWVn</td>
<td>0.13 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>0.23 ± 0.33</td>
</tr>
</tbody>
</table>

All results are expressed as means ± SDs (n = 9). Passive indicates 30-minute seated rest; active, 30-minute upright cycle exercise. Abbreviations: DBP, diastolic blood pressure (mm Hg); HR, heart rate (beats/min); LL PWV, lower-limb pulse wave velocity (m/s); LL PWVn, normalized LL PWV (m·s\(^{-1}\)/torr); MAP, mean arterial pressure (mm Hg); SBP, systolic blood pressure (mm Hg); UL PWV, upper-limb pulse wave velocity; UL PWVn, normalized UL PWV (PWV/MAP) (m·s\(^{-1}\)/torr).

*P < 0.05 (vs. rest within condition).

**P < 0.05 (vs. corresponding time point between conditions).
Upper- and lower-limb PWV

Exercise reduced upper-limb (UL) PWV (23%; \( P = 0.02 \)) compared with passive controls (Table 3). However, normalizing for differences in MAP between the active and passive conditions removed this difference (Table 4). Normalizing UL PWV to MAP from rest to postexercise failed to change the 18% reduction in UL PWV (Table 3). Hence, the modulation of UL PWV after exercise is probably due to inherent mechanisms of arterial wall propagation independent of MAP and SBP per se. Lower-limb (LL) PWV responses were unremarkable (\( P > 0.05 \)) across the protocol.

Figure 1. Relative change in systemic vascular resistance (\( \Delta SVR \)) (a) and in systemic vascular conductance (\( \Delta SVC \)) (b) from rest to immediately (~10 s) after exercise and 60 and 120 minutes into recovery. Values are mean ± Sds. *\( P = 0.02 \) (vs. exercise). †\( P = 0.04 \) (vs. 60 minutes).
Redox Regulation of Postexercise Hemodynamics

Metabolic response

**Oxidative stress: free radical mediated lipid peroxidation and antioxidants.** Exercise increased LOOH by 60 minutes after exercise ($P = 0.03$) (Table 4). No significant relationships were observed between the exercise-induced ($\Delta$ exercise − rest) LOOH concentration and $\Delta$MAP, $\Delta$SVR, and $\Delta$SVC. There were also no changes in the concentrations of ascorbic acid or lipid-soluble antioxidants (Table 3).

**NO metabolites.** Exercise failed to affect NOx and RSNO across the trial (Table 3). However, UL PWV was inversely related to venous NOx ($r = -0.48; P = 0.01$).

**Circulating catecholamines.** Plasma noradrenaline and adrenaline concentration were unmodified ($P = 0.25$) by exercise.

**ANG-II response.** ANG-II concentrations increased ($P = 0.03$) after exercise and continued to be elevated by ~25% after 60 minutes of recovery ($P = 0.03$) (Table 4). ANG-II concentrations (pooled values at each time point) were significantly associated with systemic venous LOOH concentrations ($r = 0.97; P = 0.01$), with the strongest association displayed at the 2-hour time point ($r = 0.83; P = 0.01$) (Figure 2).

**AVP and ANP response.** Exercise increased AVP concentration 2-fold ($P = 0.02$) (Table 4). ANP was increased by ~37% ($P = 0.03$) after exercise, with values decreasing by 33% ($P = 0.03$) below baseline after 120 minutes of recovery (Table 3). $\Delta$ANP was significantly related to $\Delta$AVP ($r = 0.64; P = 0.01$) and $\Delta$ANG-II ($r = 0.66; P = 0.01$).

**DISCUSSION**

Our major findings are as follows: (i) after acute dynamic exercise, attenuated SVR and MAP persists, with increased oxidative stress but unchanged NO bioavailability; (ii) the hypotension and blunted resistance appear contemporaneously alongside increased ANP and LOOH, outweighing increases in ANG-II and AVP; and (iii) MAP and UL PWV are lowered after acute exercise, beyond the nonexercising diurnal variation. This simple, though critical, design concept emphasizes the importance of the active intervention for vascular function.

**PEH, systemic hemodynamics and arterial compliance**

The present study investigated the hemodynamic and PWV response to an acute bout of exercise in prehypertensive humans. The time frame and magnitude of the hemodynamic changes are similar to findings of previous studies.1-3,6,24,25 Both the active and inactive muscle vascular beds are sites of pronounced increases in vascular conductance, contributing ~56% to the rise in

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**Table 4. Metabolic Data**

<table>
<thead>
<tr>
<th>Endocrine markers</th>
<th>Rest</th>
<th>Exercise</th>
<th>+60 min</th>
<th>+120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline (nmol/l)</td>
<td>0.77 ± 0.4</td>
<td>0.5 ± 0.3</td>
<td>0.45 ± 0.3</td>
<td>0.25 ± 0.1</td>
</tr>
<tr>
<td>Noradrenaline (nmol/l)</td>
<td>1.84 ± 0.9</td>
<td>2.67 ± 1.3</td>
<td>2.87 ± 1.6</td>
<td>2.65 ± 1.1</td>
</tr>
<tr>
<td>Angiotensin II (pmol/l)</td>
<td>25.1 ± 4.1</td>
<td>27.7 ± 5.5*</td>
<td>31.5 ± 7.1*</td>
<td>27.4 ± 7.2</td>
</tr>
<tr>
<td>Angina vasopressin (pg/ml)</td>
<td>0.91 ± 0.6</td>
<td>2.2 ± 1**</td>
<td>1.2 ± 0.8</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>Atrial natriuretic peptide (pg/ml)</td>
<td>27.6 ± 6.1</td>
<td>37.5 ± 17.1*</td>
<td>35.1 ± 15.9</td>
<td>18.5 ± 3.7*</td>
</tr>
<tr>
<td>NOx (µmol/l)</td>
<td>11.94 ± 3.84</td>
<td>13.07 ± 5.2</td>
<td>11.34 ± 2.89</td>
<td>9.28 ± 2.9</td>
</tr>
<tr>
<td>RSNO (µmol/l)</td>
<td>7.47 ± 5.28</td>
<td>11.13 ± 5.25</td>
<td>16.43 ± 6.78</td>
<td>16.29 ± 15.43</td>
</tr>
</tbody>
</table>

**Oxidative stress–LOOH**

| LOOH (µmol/l) | 0.69 ± 0.1 | 0.73 ± 0.1 | 0.86 ± 0.2* | 0.81 ± 0.1* |

**Antioxidants**

| Ascorbic acid (µmol/l) | 42.9 ± 18.9 | 44.8 ± 27.4 | 55.6 ± 24 | 47.3 ± 29.8 |
| α-Tocopherol (µmol/l) | 31 ± 6.96 | 33 ± 7.60 | 36.6 ± 7.50 | 33 ± 10.09 |
| γ-Tocopherol (µmol/l) | 3.5 ± 0.80 | 3.5 ± 0.70 | 3.8 ± 0.70 | 3.3 ± 0.90 |
| l-α-Carotene (µmol/l) | 0.28 ± 0.18 | 0.26 ± 0.09 | 0.30 ± 0.13 | 0.26 ± 0.90 |
| Retinol (µmol/l) | 2.17 ± 0.37 | 2.32 ± 0.53 | 2.42 ± 0.47 | 2.2 ± 0.62 |
| Lycopene (µmol/l) | 0.31 ± 0.21 | 0.44 ± 0.29 | 0.29 ± 0.17 | 0.30 ± 0.11 |
| Lutein (µmol/l) | 0.2 ± 0.07 | 0.22 ± 0.08 | 0.23 ± 0.07 | 0.19 ± 0.06 |

**Abbreviations:** LOOH, lipid hydroperoxides; NO, nitric oxide; NOx, nitrate and nitrite; RSNO, S-nitrosothiol.

*P = 0.03 (vs. rest).

**P** = 0.02 (vs. rest).
SVC. It is important to recognize that these prior studies have shown that ~36% of the total systemic response still remains unaccounted for. It could be speculated that, with a reduced SVR, vasodilation of the microcirculation takes place, which would in turn affect DBP. Wilkins et al. directly assessed the contribution of the cutaneous circulation (i.e., a microvascular bed) to the SVC response, using laser-Doppler flowmetry of red blood cell flux at 4 skin sites. The authors conclude that although exercise increases cutaneous vascular conductance it rapidly returns to baseline before SVC; hence sustained vasodilation of the microcirculation is not obligatory for PEH. The authors documented only MAP responses, so it is unknown whether DBP was changed.

A novel aspect of the present study is the simultaneous measurement of PWV during PEH and in comparison with a passive control condition. The results indicate that immediately after exercise PWV is significantly attenuated in the UL region but not the LL region, when compared with a nonexercise control. Previous findings—obtained using exactly the same methodology used in the present study but in young, normotensive subjects—highlighted a modest decline in UL and LL PWV from 10 to 60 minutes after exercise, with a concomitant decline in SBP but not DBP or MAP. This PWV methodology has also been shown to be responsive to endothelial-dependent and endothelial-independent stimulation, but endogenous NO bioavailability was not reported. In agreement with the current findings, prior data indicate that the UL vascular bed, with this methodology, is more responsive than the LL vascular bed after exercise and pharmacological provocation. Our unique finding is that we have simultaneously quantified the vasoconstrictor-vasodilator milieu in relation to PWV and PEH.

**Metabolic vasodilation or vasoconstriction**

The present study documented the response of key vasoactive metabolites together with the systemic hemodynamic and limb arterial compliance response to acute exercise. Its results confirm the findings of Paulev et al., who demonstrated increased ANG-II concentrations during sustained PEH. An important finding of the present study is the direct relation between exercise-induced LOOH concentration and ANG-II. To our knowledge this is the first in vivo data, in contrast to earlier in vitro studies reporting free radicals (ROS) as important mediators of ANG-II induced vascular dysfunction. We suggest that in our cohort a clear vaso-dilatory mechanism, capable of buffering vasoconstriction, mediates exercise-induced hyperemia.

In the present study, AVP concentrations returned to baseline by 60 minutes after exercise. This corroborates the findings of Paulev et al., who showed a lack of change in AVP during PEH. ANP returned to baseline levels after 1 hour of recovery.
in the present study, similar to earlier data. The temporal profile of ANP concentrations during recovery was analogous to prior data, showing reductions below baseline during an episode of PEH. The significant decline in ANP values after exercise occurs over a similar time frame as the blunting of systemic vasodilation and arterial distensibility. ANP secretion or its residual effects may be required to initiate and maintain a degree of hyperemia but cannot be solely responsible for the reductions noted in MAP during this time period.

Free radical-mediated regulation of vascular tone: implication for PEH

Exercise induced an increase in venous LOOH, a marker of free-radical mediated damage to lipid membranes, during a sustained period of vasodilation and hypotension. Although correlational analysis failed to show a relationship between lipid peroxidation, MAP, and SVR in the present study, increased oxidative stress in this cohort fails to affect vasodilation negatively. The finding of increased lipid peroxidation occurring contemporaneously with vasodilatation emphasizes the complex nature of oxidative stress in vivo, where vascular tone is critically determined by the balance between pro- and antioxidants. Our group and others have outlined the evidence for nonmuscle mitochondrial respiration and ROS regulating flow-mediated dilation. No previous study has investigated the role of proantioxidant balance as a mediator of metabolic vasodilatation underlying PEH. These results support the concept that ROS may play a central role in regulating the hyperemia and hypotension.

It is well accepted that free radicals probably limit vasodilatation by reducing NO bioavailability; however, our prior results implicate ROS in mediating hyperemia via direct vaso-active properties in normotensive. Evidence also points to a reduced arterial BP in rats after acute exposure to peroxyl radicals, which reverses after subsequent exposure to antioxidants. Although in the current study we chose to document LOOH only as an oxidative stress marker, our previous work indicates that this assay is a robust probe of systemic oxidation state and corresponds to more direct markers, such as electron paramagnetic resonance spectroscopy–detected ascorbate radical and α-phenyl-tert-butyl nitrotrone spin adduct. To date, no study has evaluated the circulating concentration of NO or ROS during a period of PEH in hypertensives. Our data show venous NOx and RSNO unchanged after exercise per se or oxidative stress. We have previously used the current NO assay to evaluate differences in NOx or RSNO in clinical populations, so we are confident that this method was sensitive enough to detect changes in circulating NO bioavailability. The present study endorses NO blockade findings by Hilliwell et al. in normotensives, indicating that augmented circulating NO bioavailability is not obligatory for the PEH in our prehypertensive subjects.

CONCLUSIONS

Our findings demonstrate that acute exercise induces postexercise hyperemia in prehypertensives, which lasts up to 2 hours, together with an immediate decrease in UL PWV. Lowered PWV after exercise has important ramifications for circulatory efficiency. PEH and hyperemia occur during increased oxidative stress and unchanged NO bioavailability. Whether ROS are obligatory for PEH requires further investigation, although it seems that oxidative stress occurs during the hyperemia underlying PEH.

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DISCLOSURE

The authors declared no conflict of interest.

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