Effects of Dynamic Exercise on Plasma Arachidonic Acid Epoxides and Diols in Human Volunteers

Rose M. Giordano, John W. Newman, Theresa L. Pedersen, Marisa I. Ramos, and Charles L. Stebbins

Metabolites of the cytochrome P450 (CYP) pathway may contribute to vasodilation of the vasculature. However, it is not known whether exercise affects their circulating concentrations. The authors determined effects of exercise intensity and duration on plasma concentrations of epoxy and dihydroxy metabolites of arachidonic acid. Their goal was to delineate the threshold workload, optimal workload, and duration required to produce increases in plasma concentrations of these vasoactive substances. Healthy volunteers (N = 14) performed maximal exercise testing on a bicycle ergometer during Visit 1. On separate days, subjects cycled for 20 min at 30%, 60%, and 80% of their maximal exercise intensity. The last day consisted of 40 min of exercise at 60% of maximal exercise intensity. Venous blood was obtained before, during, and after exercise for analysis.

Compared with rest, increases were observed during the 80% workload at 20 min postexercise —14,15-DHET (0.77 ± 0.21 vs. 0.93 ± 0.27 nM)—and at 2 min postexercise: 11,12-DHET (0.64 ± 0.22 vs. 0.71 ± 0.24 nM; p < .05). Also compared with rest, 40-min values during the 60% workload were 14,15-DHET 0.79 ± 0.22 vs. 0.91 ± 0.31 nM and at 2 min post 14,15 EET 0.12 ± 0.06 vs. 0.21 ± 0.16 nM (p < .05). Results suggest the CYP metabolites (i.e., DHETs) are released during short-term high-intensity and long-term moderate-intensity exercise.

Keywords: epoxyeicosatrienoic acid, dihydroxyeicosatrienoic acid, cytochrome P450

Previous research has identified vasoactive substances that are released from contracting skeletal muscle and vascular endothelium and may contribute to vascular smooth-muscle relaxation (e.g., prostaglandins, nitric oxide, adenosine, ATP; Clifford & Hellsten, 2004). However, other vasoactive substances may also be involved. In this regard, arachidonic acid (AA) can be converted to vasoactive metabolites via the cyclo-oxygenase, lipoxygenase, and cytochrome P450 enzymatic pathways (Figure 1). In particular, the cytochrome P450 pathway converts AA to four distinct epoxyeicosatrienoic acid (EET) regioisomers: 4,6-; 8,9-; 11,12-; and 14,15-EET (epoxides). EETs can cause hyperpolarization of vascular smooth-muscle cells (Spector, 2009) and induce vascular relaxation mediated by activation of Ca++-dependent K+ (K_Ca) channels in phospholipid membranes (Zhu, Schieber, McGiff, & Balazy, 1995). Activation of these channels is partially or totally unresponsive to inhibitors of cyclo-oxygenases that metabolize AA to 5-, 12-, and 15-hydroxyeicosatetraenoic acid; prostaglandins; prostacyclin; thromboxane; and leukotrienes and to those that block nitric oxide synthases (Busse et al., 2002). Based on these properties, EETs are considered candidates for endothelial-derived hyperpolarizing factors (Campbell, Gebremedhin, Pratt, & Harder, 1996), whose release is triggered by Ca++-induced activation of the cytochrome P450 pathway (Graber, Alfonso, & Gill, 1997).

It has been suggested that the endothelial-derived hyperpolarizing factors can contribute to increases in skeletal-muscle blood flow that occur during exercise (Hillig et al., 2003). In addition, dihydroxyeicosatrienoic acids (DHETs, diols), the stable metabolites of EET metabolism produced by the action of epoxide hydrolase, have been found to cause vasodilation in some regional circulations, including the heart (Hercule et al., 2009; Larsen et al., 2006). Thus, increases in their concentrations during exercise may also contribute to corresponding increases in blood flow. At present, there is no direct evidence demonstrating that production of these cytochrome P450 metabolites actually increases in response to exercise. Therefore, we tested the hypothesis that plasma concentrations of 8,9-, 11,12-, and 14,15-EETs and their stable metabolites, 8,9-; 11,12-; and 14,15-DHET, increase in response to dynamic exercise and that these increases depend on work intensity and exercise duration.

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Methods

Demographic Data

Our subject population consisted of 14 lightly to moderately active, nonsmoking, healthy, normotensive adult men (n = 6) and women (n = 8) ranging from 28 to 56 years in age (36.9 ± 8.4 years), height 167.2 ± 35.3 cm, weight 173.5 ± 8.9 kg, percentage body fat 26.5% ± 2.2%, and percentage lean body mass 73.5% ± 2.2%. Activity levels were established relative to the exercise recommendations from the Dietary Guidelines for Americans, 2005 (www.health.gov/dietaryguidelines/pubs.asp). The subjects’ activity level was considered light if they participated in physical activity for 30 min at least 1–2 days/week. Moderate activity was classified as participating in 30 min of physical activity 3–4 days/week.

The study was approved by the University of California, Davis, institutional review board and the CTSC Clinical Research Center in the Sacramento Veterans Affairs Medical Center in Rancho Cordova, CA, on the use of humans in research. Recruitment was primarily via person-to-person interview and through advertisements posted throughout the University of California, Davis, campus and the University of California, Davis, Medical Center. All subjects were properly informed of study procedures and gave signed written consent. All completed a lifestyle questionnaire to determine their eligibility for enrollment in the study. Individuals were excluded if they had a medically documented history of cardiovascular or respiratory disease, any skeletal-muscle disorders, or other organ disease. Those who were unable to perform exercise on a stationary bike were also excluded from the study. An additional exclusionary criterion was the use of any medication for treatment of cardiovascular disease (e.g., antihypertensives).

Subjects were asked to refrain from nonsteroidal medications (e.g., ibuprofen and aspirin) for 4 days before each scheduled test to minimize any potential effects of these drugs on the measured variables.

Exercise-Testing Protocol

During the first visit to the laboratory, the subjects performed a maximal exercise test on a bicycle ergometer (Monark Ergomedic 839E) for the purpose of determining their maximal heart rate. A modified Bruce protocol was used in which workload and resistance were progressively increased over a period of 8–10 min until the subject could no longer maintain the workload at a given cadence (50 rpm). Target heart rates for workloads used in subsequent exercise testing were determined using the Karvonen formula: [(HR
\text{max} - HR
\text{rest}) \times \% \text{ intensity}] + HR
\text{rest}. Immediately before testing (time = 0), baseline heart rate was measured. Once the target heart

Figure 1 — Pathways for metabolism of arachidonic acid via the cyclo-oxygenase (COX), cytochrome P450, and lipooxygenase (LOX) pathways. DHET = dihydroxyeicosatrienoic acid; seH = soluble epoxide hydrolase; PGs = prostaglandins; PGI
\text{2} = prostacyclin; TXA
\text{2} = thromboxane A
\text{2}. 

[Diagram of Arachidonic Acid Metabolism]
rate was achieved during exercise, it was maintained within narrow range limits (e.g., 5–6 beats/min). In the event that heart rate exceeded the desired goal range, the subject was asked to reduce cadence. On two occasions, the wheel resistance had to be reduced because of fatigue.

During Visits 2, 3, and 4, the subjects randomly performed submaximal bicycle tests for 20 min at intensities of 30%, 60%, and 80% of their maximal workload, respectively. During Visit 5, subjects cycled at 60% of their maximal work capacity for 40 min. During Visits 2–4, 10 ml of blood was obtained from a catheter placed in a forearm vein (i.e., the antecubital vein). Samples were taken immediately before exercise, during the 20th min of exercise, and 2 min after exercise. During Visit 5, 10 ml of blood was obtained at rest and during the 39th min of exercise. In addition, heart rate was monitored by a heart-rate monitor worn around the subject’s torso (Polar S725 heart-rate-monitor watch).

At least 72 hr elapsed between visits, and every subject completed all visits within 6 weeks. Subjects were instructed to maintain their current level of activity and not to make any changes during the course of the study.

Measurement of AA Metabolites in Plasma

Sample Collection

After a 10-hr fast, blood was collected from an intravenous catheter (placed in a forearm vein) during all but the maximal exercise test (Visit 1). At the previously described specified time intervals, 10 ml of blood were withdrawn through the venous catheter into either an ethylene diamine tetra-acetic acid (EDTA) -coated syringe and then immediately transferred into EDTA plastic Vacutainer tubes or directly into an EDTA Vacutainer tube. Samples were then immediately placed on ice until completion of the exercise-testing protocols. Subsequently, they were centrifuged at 1,500 g for 15 min. Plasma was isolated and stored at –80 °C until extraction and analysis.

Sample Extraction

The extraction protocol was modified from that described by Luria et al. (2007). Briefly, samples were randomized and extracted by solid-phase extraction (SPE) using 60-mg Oasis HLB cartridges (Waters, MA) and a 20-port vacuum manifold. SPE cartridges were prewashed with 2 × 3 ml of methanol and conditioned with 2 × 3 ml of 0.1% acetic acid in 5% methanolic water. Valves were closed when the meniscus reached the sorbent bed. Next, 10 μl of 0.17-mg/ml EDTA and butylated hydroxytoluene in methanol:water (1:1) and 10 μl of 2,000-nM analytical surrogates in methanol, including d6 20-HETE (Caymen Chemical, Ann Arbor, MI) and d8 11,12-EET (BioMol Research Laboratories, Inc., Plymouth Meeting, PA), were added to the SPE cartridge. Plasma samples (250 μl) and replicates (10%) were aliquotted into extraction cartridges and allowed to equilibrate for 2–5 min. Samples were then diluted with 1.25 ml of wash solution (0.1% acetic acid:5% methanol) and loaded onto the SPE sorbent via gravity. Subsequently, the extraction-cartridge sorbent bed was washed with 3.5 ml of a 0.1% acetic acid:5% methanol solution and dried for 20 min with approximately 25 psi vacuum.

Samples were eluted with 0.5 ml methanol followed by 2.0 ml of ethyl acetate into 5-mL polypropylene cryotubes containing 6 μl of 30% glycerol in methanol. Solvents were removed via evaporation by speedvac over a 2-hr period, leaving residues in a glycerol plug. Vials with sample residue were then capped and stored in a –20 °C freezer until analysis. At that time, residues were thawed and reconstituted with 50 μl of an 800-nM internal standard solution containing 1-cyclohexyl-ureido-3-dodecanoic acid (Sigma-Aldrich, St. Louis, MO).

A surrogate normalization solution was prepared at the time of extraction by spiking 10 μl of the analytical surrogate solution into a glass insert containing 40 μl of internal standard solutions and used to confirm surrogate-solution concentrations. The sample batch, replicate, and norm solution were stored at –20 °C, along with the prepared samples, and analyzed within 3 days.

Sample Analysis

Ten μl-volume sample aliquot injections were separated on a 2.1 × 150-mm, 5-micron HSS-C18 analytical column (Waters) on an Aquity UPLC and detected by negative-mode electrospray ionization tandem quadrupole mass spectroscopy on a Waters Quattro Micro using a complex gradient of H2O with 0.1% acetic acid and 90:10 acetonitrile:methanol (v:v) at 250 μl/min. All DHETs were quantified relative to d6-20-HETE recoveries, and epoxy fatty acids were tagged to the d8-11,12-EET. The d6-20-HETE and d8-11,12-EET surrogates had mass transitions of 325.2 > 281.15 and 327.2 > 171.15, respectively, and retention times of 17.77 and 20.32 min, respectively. Mass transitions and retention times for other analytes can be found in supplemental tables in Luria et al. (2007).

Oxylipids were quantified using internal standard methodologies against a minimum 5-point calibration curve that bracketed all reported concentrations. Sensitivity was <0.4 nM for the EETs and <0.25 nM for the DHETs. Recovery was 69% ± 7% for 6d-20-HETE and 80% ± 9% for d8-11,12-EET.

Statistical Analysis

Descriptive statistics were calculated and variables were examined for meeting assumptions of normal distribution without skewness or kurtosis. All data are presented as
$M \pm SEM$. A general linear-model repeated-measures analysis was used to test differences across the different exercise trials within each intensity group. Differences across the subject groups were examined using general linear-model multifactor analyses of variance (ANOVA). When significant differences were found ($p < .05$), Fisher’s least-significant-difference post hoc test was used for pairwise comparisons. All statistical analyses were conducted using SPSS software (version 1600, SPSS Inc., Chicago, IL).

**Results**

**Heart-Rate Responses**

Heart rates achieved after 20 min of the 30%, 60%, and 80%max workloads were 99 ± 4, 138 ± 3, and 163 ± 3 beats/min, respectively. During the 40 min of exercise (60%max), heart rate was 120 ± 14 beats/min after 40 min of exercise.

**Effects of Exercise Intensity**

Significant changes in AA concentrations from baseline values were seen only in response to the 80%max workload ($p < .05$). In this regard, AA levels were reduced by 23% ± 8% at 20 min of exercise but returned to baseline values at 2 min postexercise (Figure 2).

Representative chromatograms of the epoxygenase metabolites during exercise are shown in Figure 3. No significant exercise-induced increases in EETs were seen at any work intensity or at 2 min postexercise ($p < .05$; Table 1). A small decrease in 14,15-EET was observed at 20 min of exercise during the 80% work intensity. Increases in DHETs during exercise were seen only for 8,9-DHET and 14,15-DHET, and only in response to the 80% work intensity ($p < .05$; Table 1). Increases from baseline values at 2 min postexercise were found for 11,12-DHET after the 80% workload and for 14,15-DHET after the 30% and 80% workloads ($p < .05$; Table 1).

**Effects of Exercise Duration**

AA levels decreased from baseline conditions by 28% ± 9% ($\Delta = 1,089 \pm 0.40 \mu M$) at 40 min of exercise at the 60%max workload ($p < .05$) but were not statistically significantly different from baseline values at 2 min postexercise (3,344 ± 326 vs. 2,996 ± 91 μM).

![Figure 2](image-url) — Changes in plasma concentrations of arachidonic acid (AA) from resting values to 20 min of cycling at workloads of 30%, 60%, and 80% of maximal exercise intensity and at 2 min postexercise. Mean resting value of AA was 3,158 ± 224 μM. *$p < .05$ vs. rest.
Figure 3 — Representative chromatograms of 14,15-DHET; 11,12-EET; and 8,9-EET in plasma of a single subject at 0, 10, and 20 min of exercise at 80% of maximal overlaid with a 1-nM calibration standard.

<table>
<thead>
<tr>
<th>Workload</th>
<th>30%</th>
<th>60%</th>
<th>80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>8,9-EET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.30 ± 0.06</td>
<td>0.34 ± 0.04</td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>20 min</td>
<td>0.29 ± 0.05</td>
<td>0.30 ± 0.04</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td>Post (2 min)</td>
<td>0.36 ± 0.06</td>
<td>0.28 ± 0.03</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>8,9-DHET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.37 ± 0.12</td>
<td>0.37 ± 0.12</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>20 min</td>
<td>0.26 ± 0.05</td>
<td>0.37 ± 0.05</td>
<td>0.48 ± 0.06*†</td>
</tr>
<tr>
<td>Post (2 min)</td>
<td>0.39 ± 0.05</td>
<td>0.41 ± 0.08</td>
<td>0.41 ± 0.08</td>
</tr>
<tr>
<td>11,12-EET</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>0.35 ± 0.14</td>
<td>0.35 ± 0.17</td>
<td>0.40 ± 0.13</td>
</tr>
<tr>
<td>20 min</td>
<td>0.29 ± 0.14</td>
<td>0.34 ± 0.15</td>
<td>0.33 ± 0.19</td>
</tr>
<tr>
<td>Post (2 min)</td>
<td>0.34 ± 0.17</td>
<td>0.31 ± 0.16</td>
<td>0.34 ± 0.17</td>
</tr>
<tr>
<td>11,12-DHET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.35 ± 0.14</td>
<td>0.65 ± 0.19</td>
<td>0.64 ± 0.22</td>
</tr>
<tr>
<td>20 min</td>
<td>0.29 ± 0.14</td>
<td>0.64 ± 0.22</td>
<td>0.68 ± 0.23</td>
</tr>
<tr>
<td>Post (2 min)</td>
<td>0.34 ± 0.17</td>
<td>0.66 ± 0.16</td>
<td>0.71 ± 0.24*†</td>
</tr>
<tr>
<td>14,15-EET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.15 ± 0.09</td>
<td>0.22 ± 0.17</td>
<td>0.18 ± 0.09</td>
</tr>
<tr>
<td>20 min</td>
<td>0.11 ± 0.08</td>
<td>0.17 ± 0.08</td>
<td>0.11 ± 0.05*</td>
</tr>
<tr>
<td>Post (2 min)</td>
<td>0.16 ± 0.09</td>
<td>0.14 ± 0.08</td>
<td>0.15 ± 0.11</td>
</tr>
<tr>
<td>14,15-DHET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.65 ± 0.17</td>
<td>0.75 ± 0.22</td>
<td>0.77 ± 0.21</td>
</tr>
<tr>
<td>20 min</td>
<td>0.73 ± 0.23</td>
<td>0.79 ± 0.32</td>
<td>0.93 ± 0.27*†</td>
</tr>
<tr>
<td>Post (2 min)</td>
<td>0.75 ± 0.23</td>
<td>0.75 ± 0.20</td>
<td>0.89 ± 0.32*†</td>
</tr>
</tbody>
</table>

*p < .05 vs. rest. †p < .05 vs. corresponding 30%.
The values of each EET and DHET during the 40-min, 60%\textsubscript{max} workload are shown in Figure 4. An increase in 14,15-EET concentrations was seen when the resting value was compared with the 2-min postexercise condition ($p < .05$; Figure 4). An increase in 14,15-DHET concentrations was also found when the baseline value was compared with that at 40 min of exercise ($p < .05$; Figure 4).

**Discussion**

To our knowledge, this is the first study to assess the impact of exercise on individual EET and DHET regio-isomers at rest and during exercise in human plasma. Moreover, results compare well with resting plasma concentrations recently reported in a survey of 70 subjects evenly distributed among men and women (8,9-DHET, 0.24 ± 0.08 nM; 11,12-DHET, 0.57 ± 0.02 nM; 14,15-DHET, 0.60 ± 0.18 nM; 14,15-EET, 0.44 ± 0.59 nM; Psychogios et al., 2011). Reports for plasma DHETs in a separate cohort also correspond with our reported values (i.e., 11,12-DHET, 0.74 ± 0.08 nM; 14,15-DHET, 0.75 ± 0.08 and 1.3 ± 0.1 nM; Goodfriend et al., 2007).

Although we did not confirm our hypothesis that the plasma concentrations of EETs increase during exercise, we did find increases in plasma concentrations of DHETs. In this regard, plasma concentrations of 8,9- and 14,15-DHET increased from baseline to exercise at a work intensity of 80%\textsubscript{max}, and an increase in 11,12-DHET was seen 2 min postexercise. Because AA, the precursor of EETs and DHETs, is continuously metabolized by the cyclo-oxygenase, lipooxygenase, and cytochrome P450 pathways, it is possible that the specific pathway for AA metabolism shifts based on tissue needs (e.g., resting vs. exercise metabolism in skeletal muscle).

A decrease in the plasma concentration of AA was observed at 20 min of acute exercise (80%\textsubscript{max}), followed by an increase at 2 min postexercise. This decrease is consistent with reported increases in fatty-acid metabolism and utilization that occur when blood flow in exercising skeletal muscle is increased (Friedberg & Estes, 1962). In this regard, exercise accelerates the reduction of plasma free-fatty-acid concentrations beginning with an initial fall, followed by a gradual rise with continued activity (Friedberg, Sher, Bogdonoff, & Estes, 1963). This initial fall is seen even during short periods (~15 min) of higher submaximal work intensities (Friedberg, Harlan, Trout, & Estes, 1960). This phenomenon may represent activation of neural and hormonal mechanisms that increase the utilization of AA (Friedberg et al., 1963). Our results may also reflect an augmentation of exercise-induced increases in AA oxidation (Friedberg et al., 1963).
Turnover rates of AA in healthy subjects are faster than those of other free fatty acids (Zhou & Nilsson, 2001). Thus, there may be an exchange of AA with other free fatty acids between the plasma and endothelium (Hagenfeldt & Wahren, 1975). As a result, increases in muscle activity likely increase AA utilization to a greater extent within the endothelial layer. This likelihood is supported by the fact that increases in cyclo-oxygenase and lipooxygenase activity during exercise are known to produce eicosanoids such as prostacyclin and prostaglandins (Langberg, Boushel, Skovgaard, Risum, & Kjaer, 2003).

Our data also suggest that AA metabolism may shift toward the cytochrome P450 pathway, in particular the epoxygenases, during moderate- to high-intensity exercise. This apparent shift is associated with an overall decrease in AA plasma concentrations and increases in the epoxygenase metabolites, namely, 8,9-; 11,12-; and 14,15-DHET.

Release of DHETs During Exercise

The lower workloads did not evoke any increases in DHETs during exercise. This limited response could indicate that epoxygenase metabolites have more involvement during heavy than during light activity or that metabolites are cleared quickly into the urine (Newman, Watanabe, & Hammock, 2002). Regardless, the increased levels of these metabolites seen at the higher workload may have been caused by greater increases in skeletal-muscle oxygen demand or elevated shear stress (Furchgott & Vanhoutte, 1989). On the other hand, shear stress produced during the lower intensity workloads may not have created an adequate stimulus for release of 14,15-DHET.

It should be noted that the concentration of 14,15-DHET observed 2 min after the 30% workload was higher than the corresponding resting value ($p < .05$). In spite of this increase, no differences in concentration were found between these two time points for the 60% workload or between the two workloads for the 2-min postexercise values. There was, however, a tendency for a lower resting value when the 30% and 60% workloads were compared ($0.65 \pm 0.17$ vs. $0.75 \pm 0.22$ nM). This tendency may have indirectly accounted for the increase seen in 14,15-DHET after completion of the lower workload as opposed to a direct increase caused by exercise alone.

Our longer duration, moderate workload only caused an increase in the release of 14,15-DHET. This response may be related to an increased utilization of plasma fatty acids that becomes prominent during prolonged exercise at moderate work intensities (Romijn et al., 1993). Consequently, increased metabolism of AA, characterized by a shift toward the cytochrome P450 pathway, may have occurred, resulting in an increase in metabolism of 14,15-EET to 14,15-DHET.

Exercise-induced increases in DHETs have implications for regulation of blood flow in the coronary circulation and possibly in skeletal muscle. The 8,9-; 11,12-; and 14,15-DHET regioisomers induce vasodilation and can cause vascular smooth-muscle relaxation via stimulation of Ca++-activated K+ channels in coronary arteries (Larsen et al., 2006) or via modulation of endothelial NO release (Hercule et al., 2009).

Activity and Location of CYP Enzymes

The cytochrome P450 enzyme CYP2J2, which is instrumental in the synthesis of EETs, has been found in the heart, coronary circulation, and skeletal muscle (Node et al., 1999). Other important P450 enzymes (i.e., CYP2C8 and CYP2C9) have been identified in skeletal-muscle resistance arteries (Boël, de Wit, & Pohl, 1999). Therefore, it is tempting to assume that the primary sources of DHETs and EETs during exercise are the heart, skeletal muscle, or the vascular endothelium in these organs. However, red blood cells have been postulated to be a major source of EETs and DHETs in the blood (Jiang, Anderson, & McGiff, 2010), and lipoprotein fractions themselves carry substantial EET concentrations (Karara et al., 1992). Because exercise causes increases in plasma ATP (Wood, Wishart, Walker, Askew, & Stewart, 2009) and ATP enhances EET and DHET production in red blood cells (via stimulation of P2× receptors; Jiang et al., 2010), an augmented release of DHETs from these cells may have contributed to their elevated levels in the plasma. On the other hand, EETs are readily incorporated into phospholipids in red blood cells and then converted to DHETs via epoxide hydrolase (Spector, 2009). Accordingly, an increased release of DHETs from red blood cells might not be accompanied by a concomitant release of EETs. This outcome would explain, at least in part, why we did not observe any increases in plasma EETs.

Although our lower work intensity did not cause detectable increases in the DHETs or EETs we measured, this does not exclude the possibility that they were produced. An important source of these metabolites is the vascular endothelium (Clifford & Hellsten, 2004; Haddy & Scott, 1975), so it is likely they are produced by endothelial cells and released, in part, abluminally into the vascular smooth muscle. As a result, only a limited amount of these metabolites may have entered the circulation. Consequently, a greater work intensity may be required for concentrations of these metabolites to reach detectable levels in the plasma.

It remains unclear whether endothelial-derived hyperpolarizing factor is in fact an epoxygenase metabolite. The study of the epoxygenases and endothelial-derived hyperpolarizing factor still remains in its infancy (Clifford & Hellsten, 2004). Thus, more research is needed to determine the possible contribution of EETs and DHETs to regulation of exercise-induced increases in coronary- and/or skeletal-muscle blood flow (Fleming & Busse, 2006; Fleming et al., 2001).
Exercise-Protocol Considerations

The 20-min exercise duration for the intensity protocol was selected to ensure that all subjects were able to complete the highest intensity workload without fatiguing. For consistency, we also used the 20-min duration for the two lower workloads. For the longer duration exercise protocol (40 min), we chose the moderate work intensity (i.e., 60% of VO2max) to ensure that all subjects could complete the test without fatiguing.

Limitations

We obtained venous blood from an arm vein, although the source of release of these CYP450 metabolites, if derived from the exercising muscle and its vasculature, suggests that the leg would provide a different measure. However, blood samples were taken from the arm vein because of the greater difficulty associated with obtaining blood from a vein in dynamically contracting leg muscles. Therefore, higher concentrations may have been present in the venous effluent of the exercising muscle.

Conclusion

Our results suggest that dynamic exercise increases the levels of nonesterified DHETs in the systemic circulation. The magnitude of these increases depends on both the duration and the intensity of exercise and may be related to a shift in AA metabolism toward the cytochrome P450 pathway or to the release of preformed EETs and their subsequent degradation to DHETs. The sources of these substances are not clear, but they may include contracting skeletal muscle, the vascular endothelium of the heart or skeletal muscle, red blood cells, or circulating lipoproteins. Because the downstream metabolites of EETs (i.e., DHETs) have vasodilatory effects (Zhou & Nilsson, 2001), exercise-induced increases in their production may contribute to concomitant increases in skeletal-muscle and myocardial blood flow.

Acknowledgments

The authors would like to thank the CTSC Clinical Research Center nursing staff, as well as Barbara Gale, MS; Jeffery Southard, MD; and Martin Hoffman, MD, for their technical assistance. The use of the clinical research facility was made possible by a grant (UL1 RR024146) from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. Additional support was provided for J.W.N. and T.L.P. by USDA-ARS CRIS 5306-51530-019-00D.

References


Plasma Arachidonic Acid


