Fenofibrate Improves Vascular Endothelial Function by Reducing Oxidative Stress While Increasing Endothelial Nitric Oxide Synthase in Healthy Normolipidemic Older Adults

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Abstract—Vascular endothelial dysfunction develops with aging, as indicated by impaired endothelium-dependent dilation, and is related to increased cardiovascular disease risk. We hypothesized that short-term treatment with fenofibrate, a lipid-lowering agent with potential pleiotropic effects, would improve endothelium-dependent dilation in middle-aged and older normolipidemic adults by reducing oxidative stress. Brachial artery flow-mediated dilation, a measure of endothelium-dependent dilation, was assessed in 22 healthy adults aged 50 to 77 years before and after 7 days of fenofibrate (145 mg/d; n=12) or placebo (n=10). Brachial flow-mediated dilation was unchanged with placebo, but improved after 2 and 7 days of fenofibrate (5.1±0.7 versus 2 days: 6.0±0.7 and 7 days: 6.4±0.6%; both P<0.005). The improvements in flow-mediated dilation after 7 days remained significant (P<0.05) after accounting for modest changes in plasma total and low-density lipoprotein cholesterol. Endothelium-independent dilation was not affected by fenofibrate or placebo (P>0.05). Intravenous infusion of the antioxidant vitamin C improved brachial flow-mediated dilation at baseline in both groups and during placebo treatment (P<0.05), but not after 2 and 7 days of fenofibrate (P>0.05). Fenofibrate treatment also reduced plasma-oxidized low-density lipoprotein, a systemic marker of oxidative stress, compared with placebo (P<0.05). In vascular endothelial cells sampled from peripheral veins of the subjects, endothelial nitric oxide synthase protein expression was unchanged with placebo and after 2 days of fenofibrate, but was increased after 7 days of fenofibrate (0.54±0.03 versus 2 days: 0.52±0.04 and 7 days: 0.76±0.11 intensity/human umbilical vein endothelial cell control; P<0.05, 7 days). Short-term treatment with fenofibrate improves vascular endothelial function in healthy normolipidemic middle-aged and older adults by reducing oxidative stress and induces an increase in endothelial nitric oxide synthase.

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Key Words: aging • endothelial-dependent dilation • flow-mediated dilation

Cardiovascular disease risk increases progressively with advancing age, resulting, at least in part, from the development of vascular endothelial dysfunction.1,2 Indeed, vascular endothelial function, most commonly assessed by endothelium-dependent dilation (EDD), is impaired in middle-aged and older adults even in the absence of clinical disease.3,4 As such, it is clinically important to identify potential treatment strategies that can improve vascular endothelial function in healthy middle-aged and older adults.

Fenofibrate is a commonly prescribed lipid-lowering agent that can exert pleiotropic effects beyond lipid lowering by activation of peroxisome proliferator-activated receptor α (PPARα).5 In old rats, fenofibrate improves EDD in the microcirculation (ie, small mesenteric arteries).6 However, it is unknown whether fenofibrate treatment can improve EDD in middle-aged and older humans.

Age-related impairments in EDD are a result of increased oxidative stress and decreased bioavailability of the vascular protective vasodilator, nitric oxide (NO).3,7 In old rats, improvements in EDD with fenofibrate are mediated by reductions in oxidative stress,6 but this mechanism has not been established in humans. In cultured endothelial cells8 and the aorta of rats,9 fenofibrate also induces increase in endothelial NO synthase (eNOS), the enzyme that synthesizes NO in the endothelium. However, the effect of fenofibrate on eNOS expression in humans has not been investigated.

We hypothesized that fenofibrate would improve EDD, as measured by brachial artery flow-mediated dilation (FMD), in middle-aged and older adults and that reduced oxidative stress is an important mechanism for these improvements. We also hypothesized that fenofibrate might increase eNOS protein expression in endothelial cells in these subjects. To test these
hypotheses, we performed a prospective, randomized, double-blind study of fenofibrate versus placebo. To minimize the potential influence of marked and sustained reductions in plasma lipids, we studied only normolipidemic adults and used short-term (7 days) administration of fenofibrate with outcomes assessed at an early time point (2 days), as well as end intervention.

Methods

Subjects
Twenty-four healthy middle-aged and older (50–77 years) men and women were enrolled in this study. All subjects had total cholesterol <240 mg/dL, low-density lipoprotein (LDL) cholesterol <160 mg/dL, triglycerides <200 mg/dL, fasting blood glucose <110 mg/dL, resting blood pressure <140/90 mm Hg, body mass index <35 kg/m², and were nonsmokers and otherwise free of clinical diseases as assessed by medical history, physical examination, blood chemistry, and resting and exercise ECG. All subjects were sedentary, defined as no regular exercise (<30 min/d, <2 d/wk) during the previous 2 years. Subjects were not taking medications and refrained from consumption of antioxidants (eg, vitamins C and E) and aspirin within 2 weeks of the start of study involvement. All procedures were approved by the institutional review board of the University of Colorado at Boulder. The nature, benefits, and risks of the study were explained to the volunteers, and their written informed consent was obtained before participation.

Procedures
All measurements were performed at the University of Colorado at Boulder Clinical and Translational Research Center after a 12-hour fast and 24-hour abstention from alcohol and physical activity.

Subject Characteristics and Blood Analyses
For details on the measurement of subject characteristics and blood analyses, please see the online-only Data Supplement.

EDD and Endothelium-Independent Dilation
EDD (brachial FMD), endothelium-independent dilation (EID; response to sublingual glyceryl trinitrate), and shear rate were assessed in the supine position using duplex ultrasonography (Power Vision 6600, Toshiba) with a linear array transducer, as previously described. Further details can be found in the online-only Data Supplement. EID was measured first during saline infusion (control) and then during supraphysiological intravenous infusion of vitamin C as previously described. Further details can be found in the online-only Data Supplement.

Endothelial Cell Protein Expression
Venous endothelial cells collected from an antecubital vein were analyzed for eNOS expression, as described in detail previously by our laboratory. For details of the methodology, see the online-only Data Supplement.

Fenofibrate Administration
In a double-blind parallel group design, subjects were assigned to receive 7 days of fenofibrate (145 mg/d; TriCor, Abbott Laboratories, Abbott Park, IL) or placebo by block randomization. Subjects were instructed to take fenofibrate with their evening meal. Experimental visits (EDD and EID measurements and blood and endothelial cell collection) were conducted the morning before the first dose was taken (baseline) and the morning after the second and seventh doses. Originally, 7 men were randomized to the placebo group, but 2 of these men withdrew before completing the final experimental visits and their data are not included in the analyses.

Data Analysis
Statistical analyses were performed with IBM SPSS (version 20, Armonk, NY). Differences in subject characteristics between groups were assessed by t tests for independent sample comparisons. A 2×3 repeated-measures ANOVA was performed to identify a group (fenofibrate or placebo)×time (baseline, 2 days, and 7 days) interaction for FMD and other variables. In the case of significant interactions, a paired t test for within-group contrast and independent t test for between-group comparisons were performed with Bonferroni correction. The differences in FMD within subjects during saline infusion versus vitamin C infusion on the same day were analyzed by paired t test. Pearson correlation analysis was used to assess bivariate relations between the change in %FMD and the change in variables that could influence FMD. Multiple linear regression was used to determine the effect of fenofibrate on FMD while controlling for potentially confounding variables. Significance was set at P<0.05. Values are mean±SE.

Results

Clinical Characteristics
At baseline, subjects randomized to the fenofibrate or placebo groups did not differ in age, body mass index, waist:hip ratio, blood pressure, lipids, or fasting glucose (Table 1). The response to short-term fenofibrate treatment was not different than the response to placebo for arterial blood pressure, fasting blood glucose, insulin, homeostasis model assessment-insulin resistance, plasma high-density lipoprotein cholesterol, triglycerides, or C-reactive protein (CRP; Table 2). Fenofibrate lowered plasma total cholesterol at 2 and 7 days and LDL cholesterol at 7 days (P<0.05). No changes were observed in the placebo group.

Endothelium-Dependent Dilation
At baseline, brachial FMD did not differ between the fenofibrate and placebo groups (%ΔFMD: P=0.35; mmΔFMD: P=0.17). Brachial FMD improved by ≥20% after 2 days of fenofibrate (P<0.005) and by ≥30% after 7 days of fenofibrate (P<0.001; Figure 1 and Table 3). There was no change in brachial FMD after 2 or 7 days of placebo. Baseline brachial artery diameter, peak shear rate after cuff release, and brachial artery dilation to glyceryl trinitrate (EID) did not differ between groups or within subjects over time (Table 3).

Table 1. Group Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fenofibrate</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (m/f)</td>
<td>12 (7/5)</td>
<td>10 (5/5)</td>
</tr>
<tr>
<td>Age, y</td>
<td>63±2</td>
<td>63±2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24±1</td>
<td>27±1</td>
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<tr>
<td>Waist:hip ratio</td>
<td>0.8±0.02</td>
<td>0.88±0.03</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>119±3</td>
<td>122±3</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76±3</td>
<td>77±1</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>207±10</td>
<td>205±9</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>128±9</td>
<td>126±5</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>57±3</td>
<td>58±8</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>108±10</td>
<td>105±11</td>
</tr>
<tr>
<td>FBG, mg/dL</td>
<td>94±2</td>
<td>92±3</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; m, male; f, female. Data are mean±SE.
Oxidative Stress

Intravenous infusion of the antioxidant vitamin C improved brachial FMD at baseline for both groups by $\approx 25\%$ ($P<0.05$, Figure 2 and Tables S1 and S2, available in the online-only Data Supplement). After 2 and 7 days of fenofibrate, brachial FMD was no longer changed in response to vitamin C ($P>0.05$; Figure 2A and Table S1). In contrast, the improvement in brachial FMD seen at baseline was maintained in the placebo group after 2 and 7 days ($P<0.05$; Figure 2B and Table S2). Baseline brachial artery diameter and peak shear rate were not affected by vitamin C in either group at any time point (Tables S1 and S2).

Oxidized LDL (oxLDL), a circulating (systemic) marker of oxidative stress, was not different between groups at baseline (Table 2). There was no change in oxLDL after 2 days of fenofibrate or placebo (Table 2). After 7 days of fenofibrate, oxLDL decreased by 21\% ($P<0.005$; Table 2), which was significantly greater than the slight decrease observed with placebo (8\%, ANOVA interaction $P<0.05$).

Endothelial Cell eNOS

Endothelial cell expression of eNOS protein did not differ between groups at baseline (Figure 3). eNOS did not change after 2 days of fenofibrate, but increased by 41\% after 7 days of treatment ($P<0.05$). There was no change in eNOS after 2 or 7 days of placebo.

Table 2. Clinical Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fenofibrate</th>
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<th></th>
<th>Placebo</th>
<th></th>
<th></th>
<th>RM ANOVA</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Day 2</td>
<td>Day 7</td>
<td></td>
<td>Day 2</td>
<td>Day 7</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>117±5</td>
<td>116±5</td>
<td>113±4</td>
<td>112±3</td>
<td>112±4</td>
<td>113±3</td>
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<tr>
<td>DBP, mm Hg</td>
<td>72±3</td>
<td>69±3</td>
<td>68±3</td>
<td>69±2</td>
<td>69±2</td>
<td>70±2</td>
<td>0.13</td>
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<tr>
<td>Total cholesterol, mg/dL</td>
<td>192±9</td>
<td>183±9*</td>
<td>167±6*</td>
<td>184±11</td>
<td>189±10</td>
<td>187±9</td>
<td>0.001</td>
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<tr>
<td>LDL-C, mg/dL</td>
<td>119±8</td>
<td>117±8</td>
<td>103±5*</td>
<td>115±7</td>
<td>120±7</td>
<td>118±6</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>52±3</td>
<td>51±4</td>
<td>50±4</td>
<td>47±7</td>
<td>49±6</td>
<td>49±5</td>
<td>0.58</td>
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<tr>
<td>Triglycerides, mg/dL</td>
<td>108±15</td>
<td>75±8</td>
<td>71±11</td>
<td>106±12</td>
<td>99±11</td>
<td>101±16</td>
<td>0.12</td>
</tr>
<tr>
<td>FBG, mg/dL</td>
<td>92±1</td>
<td>92±2</td>
<td>92±2</td>
<td>98±3</td>
<td>97±3</td>
<td>96±3</td>
<td>0.46</td>
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<td>Insulin, mU/L</td>
<td>10±1</td>
<td>10±1</td>
<td>11±2</td>
<td>11±2</td>
<td>12±1</td>
<td>11±1</td>
<td>0.28</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.3±0.1</td>
<td>2.2±0.1</td>
<td>2.4±0.2</td>
<td>2.8±0.5</td>
<td>2.9±0.4</td>
<td>2.7±0.3</td>
<td>0.44</td>
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<tr>
<td>oxLDL, mg/dL</td>
<td>56±5</td>
<td>53±5</td>
<td>45±3*</td>
<td>58±3</td>
<td>55±4</td>
<td>53±3*</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.8±0.2</td>
<td>0.8±0.2</td>
<td>0.7±0.2</td>
<td>1.0±0.2</td>
<td>1.3±0.5</td>
<td>1.0±0.2</td>
<td>0.60</td>
</tr>
</tbody>
</table>

RM indicates repeated measures; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment-insulin resistance; oxLDL, oxidized LDL; CRP, C-reactive protein. Data are mean±SE.

* $P<0.01$ vs baseline within group.

Table 3. Brachial Artery Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fenofibrate</th>
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<th></th>
<th>Placebo</th>
<th></th>
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<th>RM ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 2</td>
<td>Day 7</td>
<td></td>
<td>Day 2</td>
<td>Day 7</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>BA diameter, mm</td>
<td>3.65±0.23</td>
<td>3.66±0.23</td>
<td>3.65±0.23</td>
<td>3.74±0.18</td>
<td>3.66±0.18</td>
<td>3.66±0.17</td>
<td>0.28</td>
</tr>
<tr>
<td>FMD, mm</td>
<td>0.17±0.02</td>
<td>0.21±0.02*</td>
<td>0.23±0.02*</td>
<td>0.22±0.02</td>
<td>0.22±0.02</td>
<td>0.22±0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>GTN, % change</td>
<td>23±2</td>
<td>24±2</td>
<td>23±2</td>
<td>27±1</td>
<td>27±2</td>
<td>26±2</td>
<td>0.77</td>
</tr>
<tr>
<td>GTN, mm change</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>0.41</td>
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<tr>
<td>Peak SR, 1/s</td>
<td>904±101</td>
<td>979±138</td>
<td>955±123</td>
<td>981±115</td>
<td>969±150</td>
<td>977±95</td>
<td>0.28</td>
</tr>
</tbody>
</table>

RM indicates repeated measures; BA, brachial artery; FMD, flow-mediated dilation; GTN, dilation to glyceryl trinitrate; SR, shear rate. Data are mean±SE.

* $P<0.005$ vs baseline within group.
Potential Correlates of EDD

After 2 days, the change in brachial FMD in response to fenofibrate was related to only the change in total cholesterol ($r = -0.62; P < 0.05$), and accounting for changes in total cholesterol rendered the effect on brachial FMD nonsignificant ($P = 0.22$). After 7 days, the change in brachial FMD in response to fenofibrate was related to only the change in systolic blood pressure ($r = -0.59; P < 0.05$) and diastolic blood pressure ($r = -0.71; P < 0.05$). However, multiple linear regression analysis revealed that fenofibrate treatment significantly affected the change in brachial FMD ($P < 0.05$), whereas systolic and diastolic blood pressure did not ($P > 0.05$).

The potential impact of baseline brachial FMD, although not significant between groups, was also addressed with subgroup comparisons. In a subset of subjects matched for baseline FMD (fenofibrate: 5.65%, placebo: 5.60%; $n = 9$ per group), the change in brachial FMD over time was still significantly greater for the fenofibrate, compared with the placebo, treated group (repeated-measures ANOVA interaction, $P < 0.01$).

Discussion

We demonstrate for the first time that fenofibrate improves EDD in healthy normolipidemic middle-aged and older men and women and that the improvements are mediated by reduced oxidative stress. We also show for the first time in humans that fenofibrate induces increased endothelial cell expression of eNOS protein. These results indicate that fenofibrate may be an effective strategy to treat vascular endothelial dysfunction even in middle-aged and older adults with normal plasma lipids.

Endothelium-Dependent Dilation

The improvements in brachial FMD in this study were specific to the vascular endothelium, because there were no changes in EID with fenofibrate treatment. Fenofibrate improves EDD in individuals with type 2 diabetes mellitus$^{14,15}$ and hyperlipidemia,$^{16-19}$ and we now extend these findings to show that fenofibrate improves endothelial function in healthy normolipidemic middle-aged and older adults. Our results here in conduit arteries of middle-aged and older humans are also consistent with earlier observations that fenofibrate improves EDD in mesenteric resistance arteries of old rats.$^6$

Oxidative Stress

Beyond its lipid-lowering influence, fenofibrate is believed to exert pleiotropic effects on other potentially adverse factors in patients with hyperlipidemia. Here, we demonstrate that fenofibrate lowers plasma oxLDL, a marker of systemic oxidative stress, in normolipidemic middle-aged and older adults, in agreement with a previous report in patients with hypertriglyceridemia.$^{20}$ We extend these findings by showing that vitamin C improves brachial FMD at baseline, but not after fenofibrate treatment. This indicates that oxidative stress impaired EDD in our subjects at baseline and that fenofibrate improved EDD by reducing the tonic suppression of endothelial function by oxidative stress. The present results also translationally extend previous observations that administration of antioxidants improves EDD assessed ex vivo in vehicletreated old rats, but not in old rats receiving fenofibrate.$^6$
Endothelial NO Synthase

eNOS is the key enzyme that produces NO in the vascular endothelium in healthy adults.21 In the present study, we report for the first time that endothelial cell expression of eNOS protein is increased in humans with fenofibrate treatment. This finding is consistent with previous data showing that fenofibrate increases eNOS protein expression in cultured endothelial cells8,22 and the aorta of rodents.9 In contrast, eNOS protein expression was reported not to change and NO was reported not to contribute to the improvements in dilation in response to acetylcholine in the small mesenteric arteries of old rats with fenofibrate treatment.6 It is possible that fenofibrate may influence eNOS and the NO contribution to pharmacologically-induced dilation of small resistance arteries differently than in conduit arteries of humans in response to flow (present study) or in aorta or cell culture.8,22 We were unable to measure the NO contribution to dilation using the brachial FMD model, and thus cannot determine whether the increased eNOS expression with fenofibrate had functional benefit.

Potential Modulating Factors

For this study, we were interested in the pleiotropic effects of fenofibrate and sought to minimize or account for the potential influence of changes in clinical characteristics that could influence EDD. In particular, we attempted to avoid the effects of marked, long-term reductions in plasma lipids by studying only normolipidemic individuals treated with fenofibrate for a short period. Although total and LDL cholesterol decreased modestly with treatment, the improvements in brachial FMD at 7 days were not related to plasma lipids. Our results suggest that improvements in endothelial function after 1 week of fenofibrate were independent of changes in lipids, but we cannot completely discount an influence of the latter on brachial FMD because it may have been masked by the variability associated with plasma lipid measurements, particularly triglycerides. It is also possible that the relation between changes in vascular function and plasma lipids with fenofibrate treatment differs in normolipidemic adults and patients with hyperlipidemia. However, in patients with hypertriglyceridemia or diabetes mellitus, improvements in EDD with fenofibrate have been reported to be both related to19,17 and independent of19 reductions in lipids. Moreover, in patients with normal plasma lipids, the reduction in cardiovascular disease events with fibrate (gemfibrozil) treatment is only weakly related to changes in lipids.23 Thus, our results after 7 days of fenofibrate administration are consistent with the range of observations in previous studies, but provide potential support for the nonlipid-lowering effects of fenofibrate in mediating its vascular health-promoting influence in humans.

With regard to arterial blood pressure, the effect of fenofibrate is inconsistent, with reports of no effect25 or decreases in diastolic blood pressure25,26 in disease populations, and even evidence for elevations in young healthy subjects.27 Although blood pressure was not significantly reduced with fenofibrate in the present study, there was a relation between changes in systolic and diastolic blood pressures and improvements in brachial FMD after 7 days of fenofibrate among individual subjects. However, results of our regression analysis indicate that the improvements in brachial FMD were independent of changes in blood pressure.

The actions of fenofibrate are thought to be mediated primarily via activation of PPARα, a nuclear transcription factor that increases fatty acid oxidation and can suppress oxidative stress and inflammatory signaling.5 Assessment of PPARα activity requires a reporter assay or a measure of DNA binding that cannot be performed with the limited number of cells available from endothelial biopsy in human subjects. However, 7 days of fenofibrate treatment in rodents are sufficient to increase PPARα activity in cardiac and liver tissue28,29 and studies using PPARα knockout mice demonstrate that the vascular and oxidative stress-related effects of fenofibrate are PPARα dependent.30–32 In contrast, a recent study found that a brief (25-minute) ex vivo incubation with PPARα agonist GW7647 improved EDD in mesenteric arteries from adults <60 years of age with cardiovascular risk factors, but not in older adults and postulated that the latter might be because of a reduction in PPARα receptor expression at older ages.33 Thus, PPARα activation may have contributed to the improvements in EDD, reductions in oxidative stress, and increases in eNOS expression in the middle-aged and older adults treated with fenofibrate in the present study, but this remains to be proven. The beneficial effects of fenofibrate also may have been the result of PPARα-mediated reductions in inflammatory signaling. Fenofibrate treatment did not reduce CRP, but CRP is a circulating inflammatory marker that does not necessarily reflect vascular inflammatory status. Thus, it is possible that reduced vascular inflammatory signaling by PPARα activation contributed to the improvements in endothelial function observed. PPARα protein expression decreases with aging in a number of tissues,34–36 and targeting this pathway with medications such as the fibrates could be important in preventing/treating many age-related dysfunctions.

Lipid-Lowering Medications in Normolipidemic Individuals

The use of lipid-lowering medications in normolipidemic individuals is controversial. The use of these agents in healthy normolipidemic adults is supported by the observations that optimal LDL cholesterol is lower than current guidelines37 and, thus, lipid-lowering agents could be used to attain optimal LDL cholesterol targets and because medications such as statins and fibrates have pleiotropic benefits beyond lipid lowering. For example, in Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), rosuvastatin decreased cardiovascular events in normolipidemic individuals with high CRP.38 However, the potential adverse effects of statins and fibrates argue that their use may be limited to specific populations with demonstrated benefit, such as normolipidemic adults with high CRP or possibly of older age, in addition to patients with clinical dyslipidemia.

Limitations

There are a few limitations of the present study. We only assessed conduit artery function and can only speculate on whether improvements might also occur in resistance arteries.
However, fenofibrate does improve EDD in mesenteric arteries of old rats. In addition, we assessed eNOS in endothelial cells obtained from venous sampling because the noninvasive vascular measurements and short-term intervention period did not support repeated placements of arterial catheters over the 7-day treatment period. We have previously demonstrated that endothelial cell samples obtained from peripheral veins and arteries of the same subjects show good agreement for eNOS protein (r=0.81). Lastly, we performed a short-term fenofibrate treatment and do not know the effect of long-term fenofibrate treatment in this subject population.

Perspectives

Here, we demonstrate for the first time that short-term treatment with fenofibrate improves vascular endothelial function in healthy normolipidemic middle-aged and older adults. The improvements in EDD were mediated, at least in part, by reduced vascular oxidative stress and are concomitant with relatively sizeable reductions in plasma oxLDL, a marker of systemic oxidative stress. After 7 days of fenofibrate, the relatively sizeable reductions in plasma oxLDL, a marker by reduced vascular oxidative stress and are concomitant with improvements in EDD are not related to changes in plasma lipids, but are associated with increases in eNOS protein expression. Thus, fenofibrate has pleiotropic effects beyond improving age-related endothelial dysfunction in rat resistance arteries.

Acknowledgment

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Sources of Funding

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Disclosures

None.

References

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Novelty and Significance

What Is New?
- This is the first study to demonstrate a beneficial effect of fenofibrate on vascular endothelial function in healthy, normolipidemic middle-aged and older adults.
- For the first time, a direct connection is established between improved vascular function with fenofibrate treatment and reduced oxidative stress in humans.
- This is the first study to collect vascular endothelial cells and assess changes in protein expression after fenofibrate treatment. We were able to show for the first time that fenofibrate induces an increase in the protein expression of endothelial NO synthase in humans.

What Is Relevant?
- Older individuals have a greater risk for cardiovascular diseases, at least in part, as a result of reduced vascular endothelial function. Thus, it is important to identify potential treatment strategies to improve vascular endothelial function in older adults.
- This study demonstrates that fenofibrate and strategies that target related signaling pathways may hold promise for the treatment of age-related vascular dysfunction and prevention of cardiovascular disease.

Summary
- Short-term fenofibrate treatment improves vascular endothelial function in healthy, normolipidemic middle-aged and older adults. These improvements are independent of changes in lipids and are a result of reduced oxidative stress. Protein expression of endothelial NO synthase, the key enzyme synthesizing NO in the endothelium, increased in endothelial cells collected from middle-aged and older adults after fenofibrate treatment.
Fenofibrate Improves Vascular Endothelial Function by Reducing Oxidative Stress While Increasing Endothelial Nitric Oxide Synthase in Healthy Normolipidemic Older Adults
Ashley E. Walker, Rachelle E. Kaplon, Sara Marian S. Lucking, Molly J. Russell-Nowlan, Robert H. Eckel and Douglas R. Seals

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Fenofibrate Improves Vascular Endothelial Function by Reducing Oxidative Stress While Increasing eNOS in Healthy Normolipidemic Older Adults

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Supplemental Methods.

**Subject Characteristics.** Waist and hip circumferences and BMI were measured by anthropometry. Arterial blood pressure was measured in triplicate over the brachial artery during seated (for baseline subject characteristics) or supine (for experimental visits) rest using a semi-automated device (Dinamap Pro 100, GE Health Care, Waukesha, WI).

**Blood Analyses.** Fasting plasma metabolic factors were determined by the Clinical and Translational Research Center core laboratory using standard assays. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: \([\text{fasting glucose (mg/dL)} \times \text{fasting insulin (}\mu \text{U/L})]/405\). Serum concentrations of oxidized LDL (oxLDL) were measured by ELISA (Alpco Diagnostics, Salem, NH) and C-reactive protein (CRP) was measured using a high-sensitivity Chemistry Immuno Analyzer (AU400e, Olympus America, Center Valley, PA).

**EDD and endothelium-independent dilation.** Briefly, an ultrasound probe was clamped 3-6 cm proximal to the antecubital crease. After obtaining a baseline image, reactive hyperemia was produced by inflation of a blood pressure cuff placed on the upper forearm distal to the antecubital fossa for 5 min at 250 mmHg followed by a rapid deflation. Pulsed doppler signals were recorded at an angle of insonation of 68 degrees with a sample volume the entire width of the artery as previously described. Time-averaged peak velocity was obtained from recording the first 10 velocity envelopes. Brachial artery peak hyperemic shear rate was calculated as 8 times (due to wide sample volume) the peak velocity immediately following 5 minutes of forearm occlusion, divided by occlusion diameter. Flow-mediated dilation (FMD) responses were expressed as relative (%) and absolute (mm) change from baseline diameter. FMD was measured first during saline infusion (control) and then during supraphysiological intravenous infusion of vitamin C as previously described. 0.06 g/kg fat-free mass of vitamin C was infused for 20 minutes, immediately followed by FMD measurements during a 0.02 g/kg fat-free mass vitamin C maintenance drip infusion. Endothelium-independent dilation was assessed by measurement of maximal brachial artery dilation in response to sublingual glyceryl trinitrate (GTN, 0.4 mg). GTN was only administered to subjects with resting blood pressure sufficient to safely tolerate the vasodilation, thus only 11 individuals in the fenofibrate group and 7 individuals in the placebo group received GTN.

**Endothelial cell protein expression.** Two sterile J wires (Daig Corp, Minnetonka, Minn) were advanced into an antecubital vein (~4 cm beyond the tip of the catheter) and retracted through an 18-gauge catheter. The wires were then transferred to a dissociation buffer solution and cells were recovered after a washing and centrifugation protocol. Collected cells were fixed with 3.7% formaldehyde and plated on poly-L-lysine–coated slides (Sigma Chemical, St. Louis, Mo) and then frozen at –80°C until analysis. Cells were rehydrated and nonspecific binding sites were blocked with 5% donkey serum (Jackson Immunoresearch, West Grove, Pa). Cells were incubated with monoclonal antibodies for endothelial nitric oxide synthase (eNOS; Transduction Laboratories, San Jose, Calif., USA). Cells were then incubated with an Alexaflour 555 fluorescent secondary antibody (Invitrogen Corp, Carlsbad, Calif). For analysis, slides were viewed with a fluorescence microscope (Eclipse 600, Nikon, Melville, NY), and cell images were captured digitally by a Photometrics CoolSNAPfx digital camera (Roper...
Scientific, Inc, Tucson, Ariz). Endothelial cells were identified by staining for von Willebrand factor and nuclear integrity was confirmed with DAPI (4',6'-diamidino-2-phenylindole hydrochloride). Once endothelial cells with intact nuclei were identified, they were analyzed with Metamorph Software (Universal Imaging Corp, Downingtown, Pa). Values for eNOS are reported as a ratio of endothelial cell to human umbilical vein endothelial cell average pixel intensity. The technician was blinded to the identity of the subject and the experimental condition during the staining and analysis procedures. Endothelial cells were not successfully collected for all subjects at all time points; therefore, the data presented are for 9 subjects in the fenofibrate group and 7 subjects in the placebo group.
Supplemental References


### Supplemental Results

#### Table S1: Brachial artery characteristics in response to vitamin C in fenofibrate treatment group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th></th>
<th></th>
<th>Day 2</th>
<th></th>
<th></th>
<th>Day 7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Vitamin C</td>
<td>Saline</td>
<td>Vitamin C</td>
<td>Saline</td>
<td>Vitamin C</td>
<td>Saline</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>BA diameter, mm</td>
<td>3.65 ± 0.23</td>
<td>3.65 ± 0.24</td>
<td>3.66 ± 0.23</td>
<td>3.65 ± 0.24</td>
<td>3.65 ± 0.23</td>
<td>3.67 ± 0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD, mm</td>
<td>0.17 ± 0.02</td>
<td>0.23 ± 0.03 *</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak SR, 1/s</td>
<td>904 ± 101</td>
<td>902 ± 127</td>
<td>979 ± 138</td>
<td>964 ± 127</td>
<td>955 ± 123</td>
<td>898 ± 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SE. BA, brachial artery; FMD, flow-mediated dilation; SR, shear rate. *P<0.05 vs. saline within day.
**Table S2: Brachial artery characteristics in response to vitamin C in placebo treatment group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Day 2</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Vitamin C</td>
<td>Saline</td>
</tr>
<tr>
<td>BA diameter, mm</td>
<td>3.74 ± 0.18</td>
<td>3.65 ± 0.18</td>
<td>3.66 ± 0.18</td>
</tr>
<tr>
<td>FMD, mm</td>
<td>0.22 ± 0.02</td>
<td>0.25 ± 0.03 *</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>Peak SR, 1/s</td>
<td>981 ± 115</td>
<td>954 ± 92</td>
<td>969 ± 150</td>
</tr>
</tbody>
</table>

Data are mean±SE. BA, brachial artery; FMD, flow-mediated dilation; SR, shear rate. *P<0.05 vs. saline within day.