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Enalapril reverses high-fat diet-induced alterations in cytochrome P450-mediated eicosanoid metabolism

Katherine N. Theken,1 Yangmei Deng,1 Robert N. Schuck,1 Akinyemi Oni-Orisan,1 Tricia M. Miller,2 M. Alison Kannon,1 Samuel M. Poloyac,1 and Craig R. Lee1

1Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina; and 2Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania

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Enalapril reverses high-fat diet-induced alterations in cytochrome P450-mediated eicosanoid metabolism. Am J Physiol Endocrinol Metab 302: E500–E509, 2012. First published December 20, 2011; doi:10.1152/ajpendo.00370.2011.—Metabolism of arachidonic acid by cytochrome P450 (CYP) to biologically active eicosanoids has been recognized increasingly as an integral mediator in the pathogenesis of cardiovascular and metabolic disease. CYP epoxygenase-derived epoxygenosatrienoic and dihydroxyeicosatrienoic acids (EET and DHET) and CYP ω-hydroxylase-derived 20-hydroxyeicosatetraenoic acid (20-HETE) exhibit divergent effects in the regulation of vascular tone and inflammation; thus, alterations in the functional balance between these parallel pathways in liver and kidney may contribute to the pathogenesis and progression of metabolic syndrome. However, the impact of metabolic dysfunction on CYP-mediated formation of endogenous eicosanoids has not been well characterized. Therefore, we evaluated CYP epoxygenase (EET + DHET) and ω-hydroxylase (20-HETE) metabolic activity in liver and kidney in apoE−/− and wild-type mice fed a high-fat diet, which promoted weight gain and increased plasma insulin levels significantly. Hepatic CYP epoxygenase metabolic activity was significantly suppressed, whereas renal CYP ω-hydroxylase metabolic activity was induced significantly in high-fat diet-fed mice regardless of genotype, resulting in a significantly higher 20-HETE/EET + DHET formation rate ratio in both tissues. Treatment with enalapril, but not metformin or losartan, reversed the suppression of hepatic CYP epoxygenase metabolic activity and induction of renal CYP ω-hydroxylase metabolic activity, thereby restoring the functional balance between the pathways. Collectively, these findings suggest that the kinin-kallikrein system and angiotensin II type 2 receptor are key regulators of hepatic and renal CYP-mediated eicosanoid metabolism in the presence of metabolic syndrome. Future studies delineating the underlying mechanisms and evaluating the therapeutic potential of modulating CYP-derived EETs and 20-HETE in metabolic diseases are warranted.

cytchrome P450 enzymes (CYPs) metabolize arachidonic acid to various biologically active eicosanoids. Olefin epoxidation by CYP2C and CYP2J isoforms produces four epoxyeicosatrienoic acid (EET) regioisomers (5,6-, 8,9-, 11,12-, and 14,15-EET), which possess potent vasodilatory and anti-inflammatory properties (12, 45). The EETs are rapidly hydrolyzed by soluble epoxide hydrolase (sEH, Ephx2) to the corresponding dihydroxyeicosatrienoic acids (DHETs), which are generally less biologically active (12, 45). In contrast, CYP4A and CYP4F isoforms catalyze the ω-hydroxylation of arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE), a vasoconstrictive and proinflammatory eicosanoid (35). Numerous preclinical studies have demonstrated that the CYP epoxygenase and ω-hydroxylation pathways maintain cardiovascular homeostasis following pathological insult (12, 35, 45), and modulating CYP-derived EETs and 20-HETE offers enormous potential as a therapeutic strategy for cardiovascular disease.

The pathophysiology of cardiovascular disease is complex, and multiple risk factors contribute to its development and progression. One risk factor that is becoming increasing prevalent is metabolic syndrome, a prothrombotic, proinflammatory state characterized by the presence of dyslipidemia, insulin resistance, and hypertension (14). The liver and kidney are integrally involved in the pathophysiology of metabolic syndrome via their roles in the regulation of inflammation, insulin sensitivity, and blood pressure. In addition, CYP epoxygenases and ω-hydroxylases are expressed most abundantly, and consequently EETs and 20-HETE are produced at the highest levels in the liver and kidney (38). Because of the divergent effects of the CYP epoxygenase-derived EETs and CYP ω-hydroxylase-derived 20-HETE in the regulation of vascular tone and inflammation, alterations in the functional balance between these parallel pathways in liver and kidney may contribute to the pathogenesis and progression of metabolic syndrome and cardiovascular disease.

Preclinical studies suggest that CYP expression and metabolic activity are altered in models of metabolic syndrome. Alterations in hepatic and renal CYP expression and metabolic activity have been observed in rodents genetically predisposed to obesity, including obese Zucker rats (47, 49), ob/ob mice (13, 40), and db/db mice (22). Although these models exhibit many of the characteristics of human metabolic syndrome, other metabolic derangements may also be present (3). For example, these obese rodent models appear to have lower renin-angiotensin system activity compared with lean controls (2, 15), whereas the renin-angiotensin system is activated in obese humans (10). Consequently, the alterations in CYP expression and metabolic activity observed in rodents genetically predisposed to obesity may not accurately model the
pathophysiology underlying human metabolic syndrome. In contrast, the phenotype induced by high-fat diet feeding in rodents more closely mimics human metabolic syndrome (4).

Importantly, the effects of a high-fat diet on hepatic and renal CYP epoxygenase and ω-hydroxylase expression and metabolic activity and the functional balance between the pathways have not been rigorously evaluated to date. Moreover, the mechanisms underlying the observed alterations in CYP expression and metabolic activity in response to high-fat diet feeding have not been elucidated. Therefore, we sought to 1) characterize the effect of high-fat diet feeding on the functional balance between CYP epoxygenase and ω-hydroxylase metabolism in liver and kidney and 2) investigate the role of hypercholesterolemia, insulin resistance, and renin-angiotensin system activation in mediating alterations in CYP-mediated eicosanoid metabolism in high-fat diet-fed mice.

MATERIALS AND METHODS

Reagents. All reagents were purchased from Fisher Scientific (Pittsburgh, PA) unless otherwise noted.

Experimental protocol. All experiments were performed in 8- to 10-wk-old male mice (Taconic, Hudson, NY). Mice were randomized to high-fat diet received the RD Western Diet (21% fat, 0.21% cholesterol; Research Diets, New Brunswick, NJ). Standard diet-fed mice received ProLab RMH 3000 rodent chow (5% fat; PMI Nutrition International, Brentwood, MO). Mice had access to food and water ad libitum. The treatment schemes for the effect of high-fat diet over time (experiment 1) and the effects of enalapril, metformin, and losartan treatment (experiments 2–4) are depicted in Fig. 1.

In experiment 1, wild-type (WT) C57BL/6 and apolipoprotein E (apoE)−/− mice were randomized to receive high-fat or standard diet for 2, 4, or 8 wk (n = 4/group), as shown in Fig. 1A. In experiment 2, WT and apoE−/− mice were randomized to high-fat (n = 10/genotype) or standard diet (n = 4/genotype). After 2 wk of the assigned diet, a subset of high-fat diet-fed mice were treated with the angiotensin-converting enzyme (ACE) inhibitor enalapril (30 mg·kg−1·day−1, Sigma-Aldrich, St. Louis, MO) (9) administered in the drinking water for 2 wk (n = 6/genotype), as shown in Fig. 1B. In experiment 3, WT mice were randomized to high-fat (n = 18) or standard diet (n = 23). After 2 wk of the assigned diet, a subset of mice were treated with the insulin sensitizer metformin (30 mg·kg−1·day−1, n = 6/diet; Sigma-Aldrich) (28) or enalapril (30 mg·kg−1·day−1, n = 6/diet) administered in the drinking water for 2 wk, as shown in Fig. 1B. In experiment 4, WT mice were randomized to high fat (n = 12) or standard diet (n = 12). After 2 wk of the assigned diet, a subset of mice were treated with the angiotensin II type 1 (AT1) receptor blocker losartan (25 mg·kg−1·day−1, n = 6/diet; Cayman Chemical, Ann Arbor, MI) (20) administered in the drinking water for 2 wk, as shown in Fig. 1B.

Mice were euthanized by CO2 inhalation, and liver and kidney tissue were harvested and flash-frozen in liquid nitrogen. Blood was collected via cardiac puncture, and plasma was separated by centrifugation. Tissue and plasma were stored at −80°C pending analysis. Nonfasting plasma insulin concentrations were measured with the Rat/Mouse Insulin ELISA kit (Millipore, Billerica, MA) per the manufacturer’s instructions. Nonfasting plasma glucose and total cholesterol levels were measured by the Animal Clinical Laboratory Core Facility at University of North Carolina at Chapel Hill using a Vitros 350 automated chemical analyzer (Ortho-Clinical Diagnostics, Rochester, NY). All studies were conducted in accordance with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

RNA isolation, reverse transcription, and quantitative RT-PCR. Total RNA was isolated from whole tissue homogenates using the RNeasy Miniprep Kit (Qiagen, Valencia, CA) per the manufacturer’s instructions, as described previously (38). Total RNA was reverse transcribed to cDNA using the ABI High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) with a reaction temperature of 25°C for 10 min and then 95°C for 2 min. Expression of murine Cyp2c29, Cyp2c44, Cyp2j5, Cyp4a12a, Ephx2, Ccl2, and GAPDH were quantified by quantitative RT-PCR using commercially available Taqman Assays on Demand (Applied Biosystems). CYP mRNA levels were normalized to GAPDH and expressed relative to the WT/standard diet controls using the 2−ΔΔCt method (26).

Microsome isolation. Hepatic and renal microsomal fractions were isolated as described previously (38). Briefly, frozen tissue was homogenized in 0.25 M sucrose-10 mM Tris·HCl buffer (pH 7.5) containing protease inhibitors. Homogenates were centrifuged at 4°C at 2,570 g for 20 min and then at 10,300 g for 20 min to remove cellular debris. The supernatants were then centrifuged at 100,000 g at 4°C for 45 min to yield microsomal pellets. The resulting microsomal pellets were resuspended in 50 mM Tris·1 mM DTT·1 mM EDTA buffer (pH 7.5) containing 20% glycerol. Protein concentrations were quantified using the Bio-Rad protein assay (Bio-Rad, Hercules, CA) per the manufacturer’s instructions.

Microsomal incubations. Incubations contained 300 μg of microsomal protein and 50 μM arachidonic acid in a 1-ml volume of 0.12 M potassium phosphate incubation buffer containing 5 mM magnesium chloride, as described previously (33, 38). Reactions were initiated by the addition of 1 mM NADPH and carried out at 37°C for 30 min. Incubations were carried out at saturating concentrations of substrate, and metabolite formation was linear with respect to incubation time and microsomal protein, as determined from preliminary incubations. In the presence of these saturating substrate concentrations, formation rates reflect the amount of metabolically active protein (33) and are significantly correlated with CYP mRNA and protein levels (38). The reactions were stopped by placing the samples on ice, and 12.5 ng of 20-HETE-d6 was added as an internal standard. Because of high metabolite formation, liver incubations were diluted 10-fold in incubation buffer prior to the addition of internal standard. Metabolites were extracted with diethyl ether, evaporated to dryness under nitrogen gas, and reconstituted in 80% methanol in deionized water for analysis.

Tissue extraction. Tissue concentrations of EETs, DHETs, and 20-HETE in liver and kidney were quantified as described previously (33). Briefly, frozen tissue was homogenized in 0.12 M potassium phosphate buffer containing 0.113 mM butylated hydroxytoluene and centrifuged at 4°C at 10,000 g for 30 min to remove cellular debris.
The supernatant was retained, and 12.5 mg of 20-HETE-d6 was added as an internal standard. Samples were loaded onto Oasis HLB (30 mg) SPE cartridges (Waters, Milford, MA) that were conditioned and equilibrated with 1 ml of methanol and 1 ml of water, respectively. Columns were washed with three 1-ml volumes of 5% methanol and were eluted with 100% methanol. Extracts were evaporated to dryness under nitrogen gas and reconstituted in 80% methanol in deionized water for analysis.

**Ultraperformance liquid chromatography-tandem mass spectrometry.** Arachidonic acid metabolites (14,15-EET, 11,12-EET, 8,9-EET, 14,15-DHET, 11,12-DHET, 8,9-DHET, 5,6-DHET, and 20-HETE) in microsomal incubations and tissue extracts were quantified by ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), as described previously (30, 38). Briefly, analytes were separated on a UPLC BEH C-18, 1.7 μm (2.1 × 100 mm) reversed-phase column (Waters). Mass spectrometric analysis was performed with a TSQ Quantum Ultra (Thermo Fisher Scientific, San Jose, CA) triple-quadrupole mass spectrometer coupled with heated electrospray ionization operated in negative selective reaction monitoring mode. Analytical data was acquired and analyzed using Xcaliber software version 2.0.6 (ThermoFinnigan, San Jose, CA). Metabolite concentrations were calculated from a standard curve and expressed as formation rates (pmol·mg protein⁻¹·min⁻¹) or relative to tissue weight (pmol/g tissue).

**Statistical analysis.** All data are expressed as means ± SE. The sum formation rate of all EET and DHET regioisomers was calculated by extrapolation of all CYP epoxygenase and ω-hydroxylase pathways was assessed by calculating the ratio of 20-HETE formation to EET + DHET formation (20-HETE/EET + DHET). Because the data were not normally distributed, mRNA levels were transformed to ranks, and metabolite formation rates, clinical chemistry values, and metabolite concentrations were log-transformed prior to statistical analysis. To evaluate the effect of high-fat diet on CYP expression and metabolic activity over time, data from WT/standard diet-fed mice at each time point were pooled to create a single control group, and data were analyzed by one-way ANOVA followed by post hoc Dunnett’s test for comparison with the pooled control group. To determine the effect of enalapril, metformin, and losartan treatment, functional balance was shifted in favor of the CYP DHET formation (20-HETE/EET) and hepatic and renal CYP mRNA levels were significantly higher in apoE−/− mice fed a standard diet, but high-fat diet feeding resulted in significantly higher plasma insulin levels in both genotypes. No significant differences in plasma glucose levels were observed among the diet and genotype groups.

High-fat diet feeding differentially altered CYP epoxygenase and ω-hydroxylase expression and metabolic activity in liver and kidney in both WT and apoE−/− mice (Fig. 2). However, no significant differences were observed between WT and apoE−/− mice fed a standard diet.

In liver, high-fat diet significantly suppressed EET + DHET formation at 4 and 8 wk in both WT and apoE−/− mice relative to the WT/standard diet control group (Fig. 2A). At 2 wk, EET + DHET formation appeared to be lower in WT mice, but this difference was not statistically significant (P = 0.204). Hepatic Cyp2c29 and Cyp2c44 mRNA levels were significantly lower in high-fat diet-fed mice (data not shown), and mRNA levels for each CYP epoxygenase examined significantly correlated with EET + DHET formation (Cyp2c29: rs = 0.68; Cyp2c44: rs = 0.56; Cyp2j5: rs = 0.53; P < 0.001 for all). No significant differences in hepatic Ephx2 mRNA levels were observed across either diet or genotype (data not shown). In contrast, no significant differences in hepatic 20-HETE formation (Fig. 2B) or Cyp4a12a mRNA levels (data not shown) were observed in response to high-fat diet. The 20-HETE/EET + DHET formation rate ratio was significantly greater in high-fat diet-fed mice at 4 and 8 wk, suggesting that the functional balance was shifted in favor of the CYP ω-hydroxylase pathway secondary to suppression of EET + DHET formation (Fig. 2C).

In contrast to what was observed in liver, no significant differences in renal EET + DHET formation (Fig. 2D) or Cyp2c44 and Cyp2j5 mRNA levels (data not shown) were

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**Table 1. Weight and plasma clinical chemistry in WT and apoE−/− mice fed a standard or high-fat diet**

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<td>Weight (g)</td>
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<td>WT/standard diet</td>
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<td>3.6 ± 0.2</td>
<td>5.0 ± 0.5</td>
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<td>WT/high-fat diet</td>
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<td>10.3 ± 0.2*</td>
<td>11.0 ± 1.5*</td>
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<td>apoE−/−/standard diet</td>
<td>3.7 ± 0.7</td>
<td>4.3 ± 0.2</td>
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<td>apoE−/−/high-fat diet</td>
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<td>4.7 ± 0.2</td>
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Values are means ± SE. WT, wild-type; apoE, apolipoprotein E. *P < 0.05 compared with WT/standard diet. †All values were below the limit of quantification.
observed in high-fat diet-fed mice. Similarly, no significant differences in renal Ephpx2 mRNA levels were observed (data not shown). However, renal 20-HETE formation was markedly induced after 2, 4, and 8 wk of high-fat diet in both WT and apoE<sup>-/-</sup> mice, relative to the WT/standard diet group (Fig. 2B), and was significantly correlated with Cyp4a12a mRNA levels (r<sub>S</sub> = 0.60, P < 0.001). This induction of renal CYP ω-hydroxylase metabolic activity resulted in a significantly higher renal 20-HETE/EET DHET formation rate ratio in high-fat diet-fed mice in both genotype groups (Fig. 2F).

**Experiment 2:** effect of enalapril administration on high-fat diet-induced alterations in CYP epoxygenase and ω-hydroxylase metabolic activity. Consistent with the results of experiment 1, high-fat diet significantly suppressed CYP epoxygenase metabolic activity in liver (Fig. 3A). Hepatic Cyp2c29 mRNA levels also tended to be lower in high-fat diet-fed mice, but this difference was not statistically significant (WT: P = 0.129 vs. standard diet; apoE<sup>-/-</sup>: P = 0.139 vs. standard diet; Fig. 3C). Enalapril administration reversed these effects in both WT and apoE<sup>-/-</sup> mice. Similarly, the high-fat diet-induced elevation in the 20-HETE/EET + DHET formation rate ratio in liver was reversed by enalapril treatment (Fig. 3E). No significant differences in hepatic CYP ω-hydroxylase metabolic activity (Fig. 3B) or Cyp4a12a mRNA levels (Fig. 3D) were observed in high-fat diet-compared with standard diet-fed mice. Enalapril decreased Cyp4a12a mRNA levels significantly (P = 0.014 vs. high-fat diet) in apoE<sup>-/-</sup> but not WT mice.

In kidney, 20-HETE formation (Fig. 4B), Cyp4a12a mRNA levels (Fig. 4D), and the 20-HETE/EET + DHET formation rate ratio (Fig. 4E) were significantly higher in high-fat diet-compared with standard diet-fed mice, regardless of genotype, and these effects were reversed by enalapril treatment. No significant differences in renal EET + DHET formation (Fig. 4A) or Cyp2j5 mRNA levels (Fig. 4C) were observed in high-fat diet-fed mice. Enalapril appeared to modestly increase renal EET + DHET formation in WT (P = 0.072 vs. high-fat diet) and apoE<sup>-/-</sup> (P = 0.009 vs. high-fat diet) mice; however, no significant differences in Cyp2j5 mRNA levels were observed.

Consistent with prior studies demonstrating that ACE inhibitors have insulin-sensitizing effects (34), enalapril significantly lowered plasma insulin levels in high-fat diet-fed WT mice (high-fat diet/no treatment: 2.95 ± 0.82 ng/ml; high-fat diet/enalapril: 0.72 ± 0.08 ng/ml; P = 0.022). A similar trend was observed in apoE<sup>-/-</sup> mice, but this difference was not statistically significant (high-fat diet/no treatment: 1.85 ± 0.27 ng/ml; high-fat diet/enalapril: 0.90 ± 0.07 ng/ml; P = 0.260).

**Experiment 3:** effect of enalapril and metformin administration on high-fat diet-induced alterations in CYP epoxygenase and ω-hydroxylase metabolic activity. To discern whether the effect of enalapril on high-fat diet-induced alterations in CYP epoxygenase and ω-hydroxylase metabolic activity was related to its insulin-sensitizing effects, high-fat diet-fed mice were administered metformin, an insulin-sensitizing agent with a mechanism of action independent of the renin-angiotensin system. Compared with untreated high-fat diet-fed mice (3.14 ± 0.60 ng/ml), both enalapril (1.53 ± 0.30 ng/ml, P = 0.036) and metformin (1.40 ± 0.16 ng/ml, P = 0.028) lowered plasma insulin levels significantly. As observed in the two previous experiments, high-fat diet suppressed hepatic CYP epoxygenase (Fig. 5A) and induced renal CYP ω-hydroxylase metabolic activity significantly (Fig. 5D), and enalapril administration reversed these effects. In contrast, metformin treat-
ment had no effect on high-fat diet-induced changes in hepatic EET + DHET or renal 20-HETE formation. Hepatic CYP epoxygenase activity was modestly higher in standard diet-fed mice treated with metformin compared with untreated mice (Fig. 5A). Enalapril modestly increased hepatic 20-HETE formation in high-fat diet-fed mice (Fig. 5B), but no significant differences in renal EET + DHET formation (Fig. 5C) were observed across diet or treatment groups.

To determine whether these alterations in CYP epoxygenase and ω-hydroxylase metabolic activity reflected CYP-derived eicosanoid levels in vivo, we quantified EET + DHET and 20-HETE tissue concentrations in liver and kidney. Consistent with the microsomal incubation studies, renal 20-HETE concentrations were significantly higher in high-fat diet-fed mice treated with metformin compared with untreated mice (Fig. 5A). Enalapril modestly increased hepatic 20-HETE formation in high-fat diet-fed mice (Fig. 5B), but no significant differences in renal EET + DHET formation (Fig. 5C) were observed across diet or treatment groups.

Experiment 4: effect of losartan administration on high-fat diet-induced alterations in CYP epoxygenase and ω-hydroxylase metabolic activity. To determine whether the effects of enalapril on high-fat diet-induced alterations in CYP-mediated eicosanoid metabolism were mediated by suppression of angiotensin II signaling through the AT1 receptor, high-fat diet-fed mice were administered losartan, an AT1 receptor blocker. Consistent with experiments 1–3, high-fat diet significantly suppressed hepatic CYP epoxygenase and induced renal CYP ω-hydroxylase metabolic activity (Fig. 7). However, treatment increased the 20-HETE/EET + DHET concentration ratio significantly in both liver and kidney compared with standard diet-fed mice (Fig. 6). Enalapril treatment reversed the high-fat diet-induced increase in the renal 20-HETE/EET + DHET concentration ratio. A similar trend was observed in liver, but this result was not statistically significant (P = 0.103 vs. high-fat diet/no treatment). Metformin treatment did not significantly alter the 20-HETE/EET + DHET concentration ratio in either liver (P = 0.632 vs. high-fat diet/no treatment) or kidney (P = 0.956 vs. high-fat diet/no treatment).
with losartan did not reverse these high-fat diet-induced alterations in CYP-mediated eicosanoid metabolism.

**DISCUSSION**

CYP expression and metabolic activity is altered in rodent models of metabolic syndrome, but the underlying mechanisms remain poorly understood. To our knowledge, this is the first study to demonstrate that high-fat diet feeding shifts the functional balance between the CYP epoxygenase and ω-hydroxylase pathways in liver and kidney in favor of proinflammatory, vasoconstrictive 20-HETE formation. Moreover, enalapril, but not metformin or losartan, reverses high-fat diet-induced suppression of hepatic CYP epoxygenase metabolic activity and induction of renal CYP ω-hydroxylase metabolic activity and restores the functional balance between the pathways. Collectively, these findings suggest that the kinin-kallikrein system and AT2 receptor are key regulators of CYP-mediated eicosanoid metabolism in the presence of metabolic syndrome.

High-fat diet feeding lowered hepatic CYP epoxygenase activity and *Cyp2c29* and *Cyp2c44* expression relative to standard diet controls, consistent with a prior study showing that hepatic Cyp2c protein was suppressed by high-fat diet (44). In kidney, we observed a marked induction of 20-HETE formation in microsomal incubations and tissue concentrations in vivo but no differences in EET / DHET formation or tissue concentrations in high-fat diet-fed mice. In contrast, suppression of renal tubular CYP2C and CYP4A expression and metabolic activity has been observed in rats fed a high-fat diet relative to lean controls (39). These conflicting results may be due to species differences in the regulation of renal CYP expression. In both liver and kidney, the 20-HETE/EET / DHET ratios in both the microsomal incubations and direct tissue extractions were significantly greater in high-fat diet-fed mice relative to controls, suggesting that the functional balance between the pathways had been shifted toward the CYP ω-hydroxylase pathway secondary to changes in CYP expression.

High-fat diet feeding induces multiple metabolic derangements, including dyslipidemia, insulin resistance, activation of the renin-angiotensin system, and suppression of the kinin-kallikrein system (4, 10, 24), which may contribute to the alterations in CYP-mediated eicosanoid metabolism that we observed. To investigate the effect of dyslipidemia and inflam-
mation, we evaluated CYP epoxygenase and ω-hydroxylase expression and metabolic activity in WT and apoE−/− mice. apoE−/− mice have impaired cholesterol clearance and develop profound hyperlipidemia, which promotes the development of atherosclerotic lesions and vascular inflammation similar to what is observed in humans with atherosclerotic disease (29). Although plasma total cholesterol and hepatic inflammatory gene expression was substantially higher in apoE−/− mice, no significant differences in CYP epoxygenase or ω-hydroxylase activity in liver or kidney were observed between WT and apoE−/− mice, and suppression of hepatic EET DHET formation and induction of renal 20-HETE formation was observed in both genotypes in response to high-fat diet feeding. Thus, the observed changes in CYP epoxygenase and ω-hydroxylase metabolic activity were driven by the high-fat diet rather than by genotype and were independent of plasma cholesterol levels.

Interestingly, enalapril treatment reversed the effects of high-fat diet feeding on CYP-mediated eicosanoid metabolism in both apoE−/− and WT mice. In addition to inhibiting the formation of angiotensin II and the degradation of bradykinin, ACE inhibitors have also improved insulin sensitivity in animal models (34) and prevented the development of diabetes in humans (1). Consistent with these reports, enalapril lowered plasma insulin levels in high-fat diet-fed mice. Insulin modulates CYP expression in vitro (42), and CYP expression and metabolic activity are altered in models of diabetes (22, 37). Consequently, we administered metformin, an insulin-sensitizing agent, to determine whether insulin resistance was driving the changes in CYP-mediated eicosanoid metabolism observed in high-fat diet-fed mice. Although metformin treatment normalized plasma insulin levels, it did not reverse the alterations in CYP expression and metabolic activity, suggesting that the effect of enalapril was mediated by inhibition of ACE rather than by improving insulin sensitivity. However, administration of the AT1 receptor antagonist losartan also did not reverse the observed suppression of hepatic CYP epoxygenase or induction of renal CYP ω-hydroxylase metabolic activity. Collectively, these data demonstrate that high-fat diet-induced alterations in renal and hepatic CYP-mediated eicosanoid metabo-

Fig. 5. Effect of enalapril and metformin treatment on hepatic total CYP epoxygenase (EET + DHET) metabolic activity (A), hepatic CYP ω-hydroxylase (20-HETE) metabolic activity (B), renal total CYP epoxygenase (EET + DHET) metabolic activity (C), and renal CYP ω-hydroxylase (20-HETE) metabolic activity (D) (standard diet: no treatment, n = 11; metformin: n = 6; enalapril: n = 6; high-fat diet: n = 6/group). *P < 0.05 vs. no treatment/standard diet; #P < 0.05 vs. no treatment/high-fat diet.

Fig. 6. Effect of enalapril and metformin treatment on the 20-HETE/EET + DHET concentration ratio in liver (A) and kidney (B) (n = 4–6/group). *P < 0.05 vs. no treatment/standard diet; #P < 0.05 vs. no treatment/high-fat diet.
lism are not mediated via angiotensin II signaling through the
AT1 receptor and suggest that enalapril reverses these effects
via inhibition of bradykinin degradation and suppression of
angiotensin II signaling through the AT2 receptor.

Several prior studies have demonstrated that angiotensin
II alters CYP-mediated eicosanoid metabolism. For example,
renal and vascular CYP2C and EET formation are
suppressed in angiotensin II-infused rats (48) and double
transgenic rats that overexpress the human renin and ang-
iotensinogen genes (19). In contrast, angiotensin II stimu-
lates 20-HETE release in isolated perfused kidneys (5), and
Ren-2 transgenic rats, which have elevated renal and plasma
angiotensin II levels, exhibit significantly higher renal 20-
HETE formation relative to wild-type controls (7). Consis-
tent with these preclinical studies, humans with renovascular
disease, a condition characterized by activation of the
renin-angiotensin system, tended to have lower plasma EET
levels and had significantly higher plasma 20-HETE levels
compared with healthy controls (31). Although suppression
of EET and induction of 20-HETE biosynthesis have been
ascribed primarily to angiotensin II signaling through the
AT1 receptor, AT2 receptor activation has been shown to
promote 20-HETE biosynthesis in rat renal microvessels (8)
and EET biosynthesis in rabbit afferent arterioles and hu-
man dermal fibroblasts (21, 36). Several studies have also
demonstrated that bradykinin stimulates the production and
release of EETs in multiple vascular beds (16, 35, 41). In
addition, chronic treatment with ACE inhibitors, but not an
angiotensin AT1 receptor blocker, directly altered renal
CYP-mediated eicosanoid metabolism via a bradykinin B2
receptor-dependent mechanism (18). Although these and our
studies collectively demonstrate that the kinin-kiniklein
system and renin-angiotensin system are key regulators of
CYP-mediated eicosanoid metabolism, further studies re-
main necessary to delineate the relative contribution of B2
and AT2 receptor signaling to the regulation of renal and
hepatic CYP-mediated eicosanoid metabolism in the pres-
ence of metabolic syndrome.

Potentiation of the CYP epoxygenase pathway via en-
hanced EET biosynthesis or inhibition of EET hydrolysis
has been shown to lower blood pressure in angiotensin
II-dependent models of hypertension (23) and attenuate
acute and chronic inflammation (11, 46). A series of studies
have demonstrated that EETs also activate the phosphati-
dylinositol 3-kinase/Akt pathway (12), a key component of
insulin signaling, and potentiation of EETs enhances insulin
signaling and improves insulin sensitivity (27, 43). Thus,
prolonged suppression of hepatic CYP epoxygenase meta-
bolism in the presence of the metabolic syndrome
would be hypothesized to promote inflammation and hepatic
insulin resistance. In contrast, 20-HETE is a potent vaso-
constrictor (35) and promotes inflammation via activation of
nuclear factor-κB (17); although renal inflammatory gene
expression was not upregulated in this model, prolonged
induction of renal CYP ω-hydroxylase metabolic activity
would be hypothesized to promote hypertension and renal
injury. Indeed, prior studies have demonstrated that inhibi-
tion of 20-HETE formation has antihypertensive effects and
attenuates renal inflammation and injury (6, 7). A recent
study demonstrated that elevations in plasma 20-HETE
levels were associated with shortened bleeding time in mice
(25), suggesting that induction of 20-HETE formation may
also contribute to the prothrombotic state observed in met-
abolic syndrome patients. Collectively, our findings suggest
that a shift in the functional balance between the CYP
epoxygenase and ω-hydroxylase pathways in favor of 20-
HETE formation may be a key contributor to the patholog-
ic consequences of a high-fat diet and the metabolic
syndrome and that increasing EETs and/or decreasing 20-
HETE may have therapeutic utility to abrogate these effects.
Future studies are necessary to test this hypothesis. It also is
important to note that our experiments were performed
exclusively in male mice. Renal Cyp4a12a expression is
regulated by androgens, and male mice exhibit markedly
higher renal Cyp4a12a expression than female mice (32).
Thus, future studies are necessary to evaluate the effects of
sex hormones on high-fat diet-induced alterations in CYP
epoxygenase and ω-hydroxylase metabolic activity.

In conclusion, induction of the metabolic syndrome by
high-fat diet feeding suppressed hepatic CYP epoxygenase and
induced renal CYP ω-hydroxylase expression and metabolic
activity, thereby shifting the functional balance between the
pathways in favor of 20-HETE formation. Treatment with
enalapril, but not metformin or losartan, reversed this effect,
suggesting that the kinin-kiniklein system and AT2 receptor
are key mediators of high-fat diet-induced alterations in CYP-
mediated eicosanoid metabolism. Further studies are necessary
to delineate the mechanisms underlying these alterations, elu-
cidate the pathophysiological significance, and ultimately de-
terminate the therapeutic potential of modulating the CYP ep-
oxygenase and ω-hydroxylase pathways in metabolic diseases.

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