0026-895X/98/030504-10$3.00/0

MOLECULAR PHARMACOLOGY, **54:**504–513 (1998).

Nutritional Status Modulates Rat Liver Cytochrome P450 Arachidonic Acid Metabolism

WEI QU, RICHARD A. RIPPE, JIXIANG MA, PAULA SCARBOROUGH, CHRISTINE BIAGINI, FRED T. FIEDOREK, GREGORY S. TRAVLOS, CAROL PARKER, and DARRYL C. ZELDIN

Laboratories of Pulmonary Pathobiology (W.Q., J.M., P.S., D.C.Z.), Experimental Pathology (G.S.T.), Signal Transduction (C.B.), and Structural Biology (C.P.), National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, and the Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599 (F.T.F., R.A.R.)

Received December 17, 1997; Accepted May 14, 1998

This paper is available online at http://www.molpharm.org

ABSTRACT

Alterations in nutritional status affect hepatic cytochrome P450 levels. Since cytochromes P450 participate in the metabolism of arachidonic acid, we hypothesized that changes in liver P450 arachidonic acid metabolism occur during fasting and refeeding. Male Fisher 344 rats were either fed, fasted 48 hr (F48), fasted 48 hr and then refed 6 hr (F48/R6), or fasted 48 hr and then refed 24 hr (F48/R24). F48 rats had reduced body weight, increased plasma β-hydroxybutyrate, and reduced plasma insulin compared with the other groups. Although there was no significant change in total liver P450 content, there was a significant 20%, 48%, and 24% reduction in total hepatic microsomal arachidonic acid metabolism in F48, F48/R6, and F48/R24 rats, respectively, compared with fed rats. Epoxygenase activity decreased by 28%, 51%, and 26% in F48, F48/R6, and F48/R24 rats, respectively. In contrast, ω-1 hydroxylase activity increased by 126% in F48 rats compared with fed rats. Immunoblotting revealed that levels of CYP2C11 protein were markedly reduced, whereas levels of CYP2E1 protein were markedly increased in the F48 and F48/R6 groups. In contrast, levels of CYP1A1, CYP1A2, CYP2B1, CYP2J3, CYP4A1, and CYP4A3 were unchanged with fasting/refeeding. Northern blots revealed that levels of CYP2C11 mRNAs were decreased, whereas CYP2E1 mRNAs were increased in F48 and F48/R6 rats. Recombinant CYP2C11 metabolized arachidonic acid primarily to epoxides with preference for the 14(*S*),15(*R*)-, 11(*R*),12(*S*)-, and 8(*S*),9(*R*)- epoxyeicosatrienoic acid enantiomers. We conclude that (1) nutritional status affects hepatic microsomal arachidonic acid metabolism, (2) reduced epoxygenase activity in F48 and F48/R6 rats is accompanied by decreased levels of CYP2C11, (3) increased ω-1 hydroxylase activity is accompanied by augmented levels of CYP2E1, and (4) the effects of fasting on CYP2C11 and CYP2E1 expression occur at the pretranslational level.

In addition to cyclooxygenases and lipoxygenases, P450 monooxygenases metabolize AA to compounds that play important functional roles in the regulation of fundamental cellular processes (Capdevila *et al*., 1992a, 1995). Three types of eicosanoid products are formed: (1) 5,6-, 8,9-, 11,12-, and 14,15-EETs; (2) midchain *cis-trans*-conjugated dienols, or 5-, 8-, 9-, 11-, 12-, and 15-HETEs; and (3) ω-terminal alcohols of AA (16-, 17-, 18-, 19-, and 20-HETEs) (Capdevila *et al*., 1992a, 1995). The EETs are hydrated by epoxide hydrolases to DHETs (Zeldin *et al*., 1993, 1996). Studies using purified and/or recombinant enzymes have demonstrated that multiple P450s can metabolize AA and that the products depend largely on the particular P450 enzyme involved in catalysis. For example, members of the CYP2B and CYP2C subfamilies are primarily AA epoxygenases (Capdevila *et al*., 1990a; Rifkind *et al*., 1995); members of the CYP1A, CYP2E, and CYP4A subfamilies are principally ω-terminal hydroxylases (Capdevila *et al*., 1990a; Laethem *et al*., 1993; Nishimoto *et al*., 1993; Rifkind *et al*., 1995); and members of the CYP2J subfamily are both epoxygenases and ω-terminal hydroxylases (Wu *et al*., 1997). The epoxygenase and hydroxylase reactions are both regioselective and enantioselective, and the reaction asymmetry is P450 enzyme specific (Capdevila *et al*., 1990a).

Compared with other organs, the liver has the highest total P450 content and contains the highest levels of individual P450 enzymes involved in the metabolism of fatty acids (Gonzalez and Lee, 1996). Liver microsomal fractions actively metabolize AA to EETs as the principle reaction products and liver contains on the order of 0.5–1 μg of EET/g of wet tissue (Karara *et al*., 1989; Capdevila *et al*., 1990a; Rifkind *et al*., 1995; Zeldin *et al*., 1996). Within the liver,

This work was supported in part National Institutes of Health Grant R29-AA10459 (R.A.R.).

ABBREVIATIONS: P450, cytochrome P450; CYPOR, NADPH-cytochrome P450 oxidoreductase; AA, arachidonic acid; EET, *cis*-epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; DHET, dihydroxyeicosatrienoic acid; HPLC, high performance liquid chromatography; GC/MS, gas chromatography/mass spectrometry; PFB, pentafluorobenzyl; SDS, sodium dodecyl sulfate.

these eicosanoids have been shown to play important physiological roles. For example, the EETs activate phosphorylase *a* and increase cytosolic Ca^{2+} concentration in isolated rat hepatocytes and increase Ca^{2+} uptake, binding, and release in rat liver microsomes (Yoshida *et al*., 1990; Kutsky *et al*., 1983). Furthermore, it has been proposed that the EETs are involved in vasopressin-stimulated glycogenolysis in the liver (Kutsky *et al*., 1983). Liver EETs may also be secreted into the circulation and have effects in extrahepatic tissues (Karara *et al*., 1992; Zeldin *et al*., 1996). For example, EETs have been shown to stimulate glucagon and insulin release from isolated pancreatic islets suggesting that epoxygenase

TABLE 1

Effect of fasting-refeeding on physiological parameters and P450 content in rats

Male Fisher 344 rats were either fed, fasted 48 hr (F48), fasted 48 hr/refed 6 hr (F48/R6), or fasted 48 hr/refed 24 hr (F48/R24) as described in Experimental Procedures. Values are mean ± standard error from 7 rats in each group.

Treatment	Body weight		Plasma			Urine ketone		Liver P450
	Prefast	Postfast	Glucose	β-Hydroxybutyrate	Insulin	Fasted	Refed	
	g		*mg/dl*	*mg/dl*	*ng/ml*	*units*		*nmol/mg protein*
Fed	314 ± 5	312 ± 5	166 ± 5	4.9 ± 0.3	2.6 ± 0.2	0		0.58 ± 0.07
F48	315 ± 11	282 ± 11	148 ± 13	21.5 ± 1.0[a]	1.2 ± 0.3[a]	2.6 ± 0.1[a]		0.52 ± 0.06
F48/R6	313 ± 13	284 ± 12	210 ± 18[a]	3.7 ± 0.2	2.5 ± 0.3	2.6 ± 0.1[a]	1.3 ± 0.1[b]	0.53 ± 0.06
F48/R24	312 ± 8	286 ± 9	171 ± 5	3.3 ± 0.5	2.2 ± 0.3	2.6 ± 0.1[a]	1.1 ± 0.2[b]	0.53 ± 0.07

[a] $p < 0.05$ for comparison with fed in the same column by ANOVA.
[b] $p < 0.05$ for comparison with fasted in the same row by Student's *t* test.

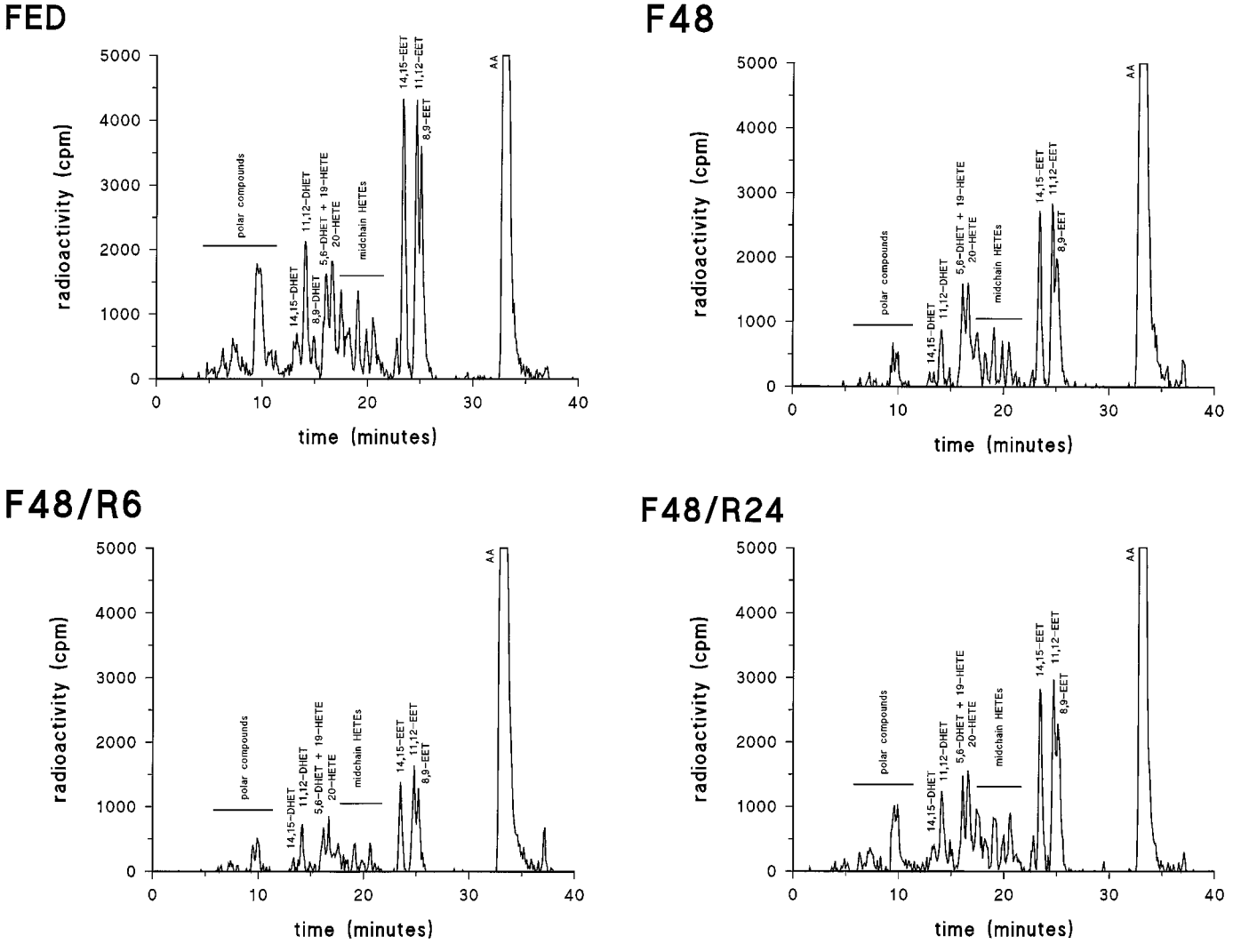

Fig. 1. Effect of the animal nutritional state on the microsomal AA metabolism. Rat liver microsomal fractions (1 mg of protein/ml) prepared from fed, F48, F48/R6, or F48/R24 rats were incubated with [1-^{14}C]AA in the presence of an NADPH-regenerating system at 37° for 30 min. The ethyl ether-soluble products were resolved by reverse-phase HPLC, and the eluent radioactivity was monitored with an on-line radioactive flow detector as described in Experimental Procedures. Metabolites were identified based on coelution with authentic standards and by GC/MS analysis. The chromatograms shown are representative of incubations with microsomes prepared from 28 different animals (seven rats in each of four groups).

metabolites of AA may be involved in the regulation of glucose homeostasis (Falck *et al*., 1983). Thus, liver P450-derived eicosanoids may modulate the use and storage of glucose via both autocrine and paracrine mechanisms.

Previous studies have demonstrated that nutritional status modulates liver microsomal P450 composition and that certain nutritional states are associated with altered hepatic metabolism of drugs, carcinogens, steroid hormones, and fatty acids (Imaoka *et al*., 1990; Johansson *et al*., 1990; Orellana *et al*., 1992; Yoo *et al*., 1992; Zannikos *et al*., 1994). For example, starvation has been reported to increase the hepatic content of CYP2E1 and correspondingly increase the metabolic activity of hepatic microsomes toward aniline and *N*-nitrosodimethylamine (Imaoka *et al*., 1990; Johansson *et al*., 1990). Similarly, starvation increased liver CYP1A1 and CYP4A2 resulting in increased benzo[*a*]pyrene and lauric acid hydroxylation (Imaoka *et al*., 1990; Orellana *et al*., 1992). In contrast, fasting decreased hepatic CYP2C11 protein levels resulting in reduced testosterone 2α- and 16α-hydroxylation activities (Imaoka *et al*., 1990). Rats fed a high lipid diet (20% corn oil) exhibited increased *N*-nitrosodimethylamine and erythromycin demethylase activities reflecting higher hepatic CYP2E1 and CYP3A2 levels but had no change in hepatic CYP2C11 levels or activity (Yoo *et al*., 1992). In contrast, there was no change in P450 levels or activity in rats fed an obesity-producing energy-dense diet (Zannikos *et al*., 1994). Diabetes induced by streptozotocin or alloxan was associated with complex alterations in hepatic and extrahepatic P450 enzyme levels and activity; several P450 forms were increased (e.g., CYP2A1, CYP2B1, CYP2C7, CYP2E1, CYP4A1, and CYP4A2), whereas others were decreased (e.g., CYP2C11, CYP2C13, and CYP3A2) (Thummel and Schenkman, 1990; Shimojo *et al*., 1993). Similar changes in P450 content and activity occurred in the spontaneously diabetic (BB/Wor) rat (Favreau and Schenkman, 1988). Treatment of diabetic rats with insulin reversed the alterations in P450 levels in nonhypophysectomized animals only, suggesting that insulin may act indirectly through normalization of a pituitary hormone-mediated process (Shimojo *et al*., 1993; Thummel and Schenkman, 1990). In that regard, a role for growth hormone and thyroid hormone in modulating P450 expression in diabetic animals has been proposed by several investigators (Thummel and Schenkman, 1990; Donahue *et al*., 1991; Imaoka *et al*., 1993).

Little is known about the effects of nutritional status on hepatic AA availability and metabolism. Fasting has been reported to increase AA accumulation in hepatic neutral lipid and phospholipid pools (Larsson-Backstrom *et al*., 1990). In contrast, the relative amount of AA was significantly reduced in polymorphonuclear leukocytes from rats with streptozotocin-induced diabetes compared with control rats (Nakagawa and Ishii, 1996). Liver microsomes prepared from chronic protein-energy malnourished rats metabolized AA with a higher turnover rate than microsomes from fed and chronic malnourished/refed animals (Orellana *et al*., 1990). Furthermore, chronic malnutrition caused alterations in the regiospecificity of AA oxygenation by rat liver microsomal P450 (Orellana *et al*., 1990). Finally, Pfister *et al*. (1991) showed that aortas from rabbits fed a cholesterol-rich diet metabolized AA to EETs, whereas aortas from control rabbits did not, suggesting that dietary cholesterol alters AA metabolism via the P450 epoxygenase pathway.

We hypothesized that alterations in hepatic P450 enzyme levels that occur during fasting and refeeding lead to changes in the liver microsomal metabolism of AA to eicosanoids that modulate physiological events involved in glucose homeostasis. Hence, the purpose of this initial study was to examine changes in liver P450 and P450-mediated metabolism of AA in an acute fasting-refeeding paradigm. We used biochemical, molecular, and immunological studies to demonstrate that fasting and refeeding cause a marked decrease in the hepatic content of an active AA epoxygenase (CYP2C11) and a corresponding increase in the hepatic content of an active AA ω-1 hydroxylase (CYP2E1), resulting in altered liver microsomal AA metabolism. We further demonstrate that the

TABLE 2

Product profile from liver microsomal incubations with AA as a function of nutritional status

Rat liver microsomes were incubated with [1-^{14}C]AA as described in Experimental Procedures. Values are mean ± standard error from 7 rats in each group. Turnover values are reported as nanomoles of product per milligram of microsomal protein per minute. Distribution values are reported as percentage of total metabolism.

Treatment		EET + DHET	HETEs	Polar	Total
Fed	Turnover	0.72 ± 0.04	0.37 ± 0.02	0.35 ± 0.06	1.44 ± 0.09
	Distribution	50.7 ± 2.4	26.0 ± 1.2	23.3 ± 3.5	100
F48	Turnover	0.52 ± 0.03[a]	0.33 ± 0.02	0.30 ± 0.05	1.15 ± 0.09[a]
	Distribution	45.7 ± 1.8	29.6 ± 2.2	24.7 ± 3.7	100
F48/R6	Turnover	0.35 ± 0.02[a]	0.25 ± 0.02[a]	0.15 ± 0.04[a]	0.75 ± 0.07[a]
	Distribution	48.2 ± 2.3	33.3 ± 2.6	18.5 ± 4.2	100
F48/R24	Turnover	0.53 ± 0.05[a]	0.31 ± 0.03	0.26 ± 0.06	1.10 ± 0.13[a]
	Distribution	49.2 ± 2.2	28.8 ± 2.1	21.9 ± 4.1	100

[a] $p < 0.05$ for comparison with fed in the same column by ANOVA.

TABLE 3

Regiochemical composition of EETs produced during liver microsomal incubations with AA as a function of nutritional status

Rat liver microsomes were incubated with [1-^{14}C]AA as described in Experimental Procedures. Values are mean ± standard error from 7 rats in each group. Concentration values are reported as nanomoles of product per milligram of microsomal protein per minute. Data represent the sum of EETs and their hydration products (DHETs). For 5,6-EET, data represent the sum of 5,6-EET, 5,6-DHET, and the δ-lactone of 5,6-DHET.

Treatment	14,15-EET	11,12-EET	8,9-EET	5,6-EET	Total EETs
Fed	0.222 ± 0.013	0.257 ± 0.018	0.172 ± 0.009	0.078 ± 0.008	0.729 ± 0.043[a]
F48	0.168 ± 0.013[a]	0.201 ± 0.016[a]	0.129 ± 0.011[a]	0.029 ± 0.004[a]	0.525 ± 0.037[a]
F48/R6	0.114 ± 0.009[a]	0.140 ± 0.009[a]	0.086 ± 0.008[a]	0.014 ± 0.002[a]	0.354 ± 0.026[a]
F48/R24	0.170 ± 0.015[a]	0.192 ± 0.022	0.123 ± 0.012[a]	0.049 ± 0.007[a]	0.534 ± 0.054[a]

[a] $p < 0.05$ for comparison with fed in the same column by ANOVA.

nutritionally triggered changes in CYP2C11 and CYP2E1 expression occur at the pretranslational level.

Experimental Procedures

Materials. [α-^{32}P]dATP and [1-^{14}C]AA were purchased from DuPont-New England Nuclear (Boston, MA). Triphenylphosphine, α-bromo-2,3,4,5,6-pentafluorotoluene, *N*,*N*-diisopropyl-ethylamine, and *N*,*N*-dimethylformamide were purchased from Aldrich Chemical (Milwaukee, WI). Unlabeled AA was purchased from Nu-Chek-Prep (Elysian, MN). HETE standards were purchased from Cayman Chemical (Ann Arbor, MI). All other chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise specified.

Experimental animals. Male Fisher 344 rats (12–16 weeks) were either fed NIH 31 rodent chow (Agway, St. Mary, OH) *ad libitum*, fasted 48 hr (F48), fasted 48 hr and then refed for 6 hr before death (F48/R6), or fasted 48 hr and then refed for 24 hr before death (F48/R24). All animals were allowed to drink water *ad libitum* and housed individually in suspended, metabolic cages to control coprophagy. Oral intake, urine output, and body weight were carefully monitored during fasting and refeeding. Animals were killed by lethal CO_2 inhalation and exsanguinated by cardiac puncture. Livers were immediately perfused with ice-cold phosphate-buffered saline, frozen in liquid nitrogen, and stored at −80° until use. All animal studies were conducted in accordance with principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the National Institute of Environmental Health Sciences Committee on Animal Care and Use.

Plasma insulin, glucose, and β-hydroxybutyrate and urine ketone determinations. Plasma insulin was quantified using a double antibody radioimmunoassay kit (Linco, St. Charles, MO) according to the manufacturer's instructions using purified rat insulin as a standard. Plasma concentrations of glucose and β-hydroxybutyrate were determined on a Monarch 2000 chemistry analyzer (Instrumentation Laboratory, Lexington, MA). Glucose concentrations were measured by the hexokinase method using reagents obtained from the instrument manufacturer. β-Hydroxybutyrate concentrations were measured with an enzymatic method using reagents obtained from Sigma Diagnostics (St. Louis, MO). Urine ketones were measured using Multistix 10 SG urinalysis reagent strips (Bayer, Elkhart, IN).

Incubations of rat liver microsomes with AA. Microsomal fractions were prepared from frozen rat livers by differential centrifugation at 4° as described previously (Zeldin *et al*., 1993); resuspended in 50 mM Tris-Cl, pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, and 20% (v/v) glycerol; and used within 48 hr of preparation. Reaction mixtures containing 0.05 M Tris-Cl buffer, pH 7.5, 0.15 M KCl, 0.01 M $MgCl_2$, 8 mM sodium isocitrate, 0.5 IU/ml isocitrate dehydrogenase, 1.0 mg/ml microsomal protein, and [1-^{14}C]AA (25–55 μCi/μmol; 50 μM, final concentration) were stirred constantly at 37°. After temperature equilibration, NADPH (1 mM, final concentration) was added to initiate the reaction. At 30-min intervals, aliquots were withdrawn, and the reaction products were extracted into ethyl ether, dried under a nitrogen stream, analyzed by reverse-phase HPLC, and quantified by on-line liquid scintillation counting using a Radiomatic Flo-One β-detector (Radiomatic Instruments, Tampa, FL) as described previously (Capdevila *et al*., 1990b). Metabolites were identified by comparing their reverse- and normal-phase HPLC properties with those of authentic standards (Capdevila *et al*., 1990b). Epoxidation at the 5,6-olefin was determined as the sum of the following metabolites: (1) 5,6-EET, (2) 5,6-DHET, and (3) δ-lactone of 5,6-DHET. The 5,6-DHET was resolved from ω-terminal alcohols of arachidonic acid by reverse-phase HPLC on a 5-μm Microsorb C_{18} column (4.6 × 250 mm; Rainin Instruments, Woburn, MA) using the following solvent program: $CH_3CO_2H/H_2O/CH_3CN$ (0.1:49.95:49.95) isocratic conditions for 30 min and then a linear solvent gradient from $CH_3CO_2H/H_2O/CH_3CN$ (0.1:49.95:49.95) to $CH_3CO_2H/H_2O/CH_3CN$ (0.1:24.95:74.95) over 70 min and a linear solvent gradient from $CH_3CO_2H/H_2O/CH_3CN$ (0.1:24.95:74.95) to CH_3CO_2H/CH_3CN (0.1:99.9) over 25 min at 1 ml/min.

Incubations of recombinant CYP2C11 with AA. Coexpression of CYP2C11 and rabbit CYPOR in *Sf9* insect cells was accomplished using the baculovirus expression vector pAcUW31 (Clontech, Palto Alto, CA) as previously described for other P450s (Wu *et al*., 1997) (Biagini C, Celier C, and Philpot RM, Structure-function relationship analyses of cytochrome P450 2C11 coexpressed with NADPH-P450 oxidoreductase in insect cells, manuscript in preparation). Briefly, *Sf9* insect cells, grown in monolayer cultures at 27° in Grace's complete media (InVitrogen, San Diego, CA), were infected with a high titer CYP2C11/CYPOR baculovirus stock (moi = 10) in the presence of δ-aminolevulinic acid and iron citrate (100 μM each). Cells coexpressing recombinant CYP2C11 and CYPOR were harvested 72 hr after infections, washed twice with phosphate-buffered saline, and used to prepare microsomal fractions by differential centrifugation at 4° (Zeldin *et al*., 1993). Reaction mixtures containing 0.05 M Tris-Cl buffer, pH 7.5, 0.15 M KCl, 0.01 M $MgCl_2$, 8 mM sodium isocitrate, 0.5 IU/ml isocitrate dehydrogenase, 100 nM CYP2C11, and [1-^{14}C]AA (55 μCi/μmol; 40 μM, final concentration) were stirred constantly at 37°. After temperature equilibration, NADPH (1 mM, final concentration) was added to initiate the reaction. At 5-min intervals, aliquots were withdrawn and the reaction products were extracted into ethyl ether, dried under a nitrogen stream, analyzed by reverse-phase HPLC, and quantified by on-line liquid scintillation counting as described previously (Capdevila *et al*., 1990b). Metabolites were identified by coelution with authentic EET and HETE standards on reverse- and normal-phase HPLC. As before, epoxidation at the 5,6-olefin was determined as the sum of 5,6-EET, 5,6-DHET, and the δ-lactone of 5,6-DHET. Control experiments demonstrated negligible metabolism of AA by microsomes prepared from uninfected *Sf9* cells. For chiral analysis, the EETs were collected from the HPLC eluent, derivatized to corresponding EET-PFB or EET-methyl esters, purified by normal-phase HPLC, resolved into the corresponding antipodes by chiral-phase HPLC, and quantified by liquid scintillation counting as described previously (Capdevila *et al*., 1990b; Wu *et al*., 1997).

Quantification of endogenous EETs in rat liver. Methods used to quantify EETs present in rat liver have been described elsewhere (Karara *et al*., 1989). Briefly, frozen liver tissues (0.3 g each) were homogenized in 15 ml of phosphate-buffered saline containing 5–10 mg of triphenylphosphine. The homogenate was extracted twice, under acidic conditions, with 2 volumes of chloroform/

TABLE 4

Regiochemical composition of HETEs produced during liver microsomal incubations with AA as a function of nutritional status

Rat liver microsomes were incubated with [1-^{14}C]AA as described in Experimental Procedures. Values are mean ± standard error from 7 rats in each group. Concentration values are reported as nanomoles of product per milligram mg of microsomal protein per minute.

Treatment	19-HETE	20-HETE	Other HETE	Total HETEs
Fed	0.031 ± 0.003	0.090 ± 0.004	0.250 ± 0.017	0.370 ± 0.02
F48	0.070 ± 0.008[a]	0.076 ± 0.010	0.189 ± 0.012[a]	0.330 ± 0.02
F48/R6	0.048 ± 0.004[a]	0.063 ± 0.007[a]	0.135 ± 0.016[a]	0.250 ± 0.02[a]
F48/R24	0.038 ± 0.006[a]	0.077 ± 0.007	0.193 ± 0.021[a]	0.310 ± 0.03

[a] $p < 0.05$ for comparison with fed in the same column by ANOVA.

methanol (2:1) and once again with an equal volume of chloroform. The combined organic phases were evaporated in tubes containing mixtures of [1-^{14}C]8,9-, 11,12-, and 14,15-EET (56 μCi/μmol, 30 ng each) internal standards. To recover phospholipid-bound EETs, saponification was followed by silica column purification. The eluent, containing a mixture of radiolabeled internal standards and total endogenous EETs, was resolved into individual regioisomers by HPLC. For analysis, aliquots of individual EET-PFB esters were dissolved in dodecane and analyzed by GC/MS on a VG TRIO-1 quadrupole mass spectrometer (Fisons/VG; Altrincham, Manchester, UK) operating under negative-ion chemical ionization conditions (source temperature, 100°; ionization potential, 75 eV; filament current, 500 μA) at unit mass resolution and using methane as a bath gas. Quantifications were made by selected ion monitoring of m/z 319 (loss of PFB from endogenous EET-PFB) and m/z 321 (loss of PFB from [1-^{14}C]EET-PFB internal standard). The EET-PFB/[1-^{14}C]EET-PFB ratios were calculated from the integrated values of the corresponding ion current intensities.

Protein immunoblotting. Antibodies to rat CYP1A1/CYP1A2, CYP2B1, CYP2E1, and CYP4A1/CYP4A3 were purchased from Gentest (Woburn, MA) and used according to the manufacturer's instructions. Polyclonal IgG raised in New Zealand White rabbits against recombinant CYP2C11 and purified by ammonium sulfate precipitation/DEAE-cellulose chromatography was a gift from Dr. Jorge Capdevila (Vanderbilt University, Nashville, TN). Polyclonal anti-human CYP2J2 IgG, which cross-reacts with rat CYP2J3 (Wu *et al.*, 1997), was raised in New Zealand White rabbits against the purified, recombinant CYP2J2 protein and affinity purified as described previously (Wu *et al.*, 1997). For immunoblotting, microsomal proteins were resolved by electrophoresis in SDS-10% (w/v) polyacrylamide gels (80 × 80 × 1 mm) (Novex, San Diego, CA) and transferred electrophoretically to nitrocellulose membranes. Membranes were immunoblotted using the specific primary antibodies, goat anti-rabbit IgG conjugated to horseradish peroxidase (BioRad, Richmond, CA), and the ECL Western Blotting Detection System (Amersham Life Sciences, Buckinghamshire, England) as described previously (Wu *et al.*, 1997). Autoradiographs were scanned using an LKB Ultrascan XL Enhanced Laser Densitometer (Pharmacia, Piscataway, NJ).

Isolation of total RNA and Northern analysis. Liver total RNA was prepared using TRIreagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. Total RNA (20 μg) was denatured and electrophoresed in 1.2% agarose gels containing 0.2 M formaldehyde. After capillary-pressure transfer to GeneScreen Plus nylon membranes (DuPont-New England Nuclear), the blots were hybridized with either a 1.856-kb CYP2C11 cDNA probe or a 1.625-kb rat CYP2E1 cDNA probe, both obtained from Dr. Jorge Capdevila. The cDNA fragments were gel purified using a Qiaex Gel Extraction Kit (Qiagen, Chatsworth, CA) and labeled with [α-^{32}P]dATP using the Megaprime DNA labeling system (Amersham). Hybridizations were

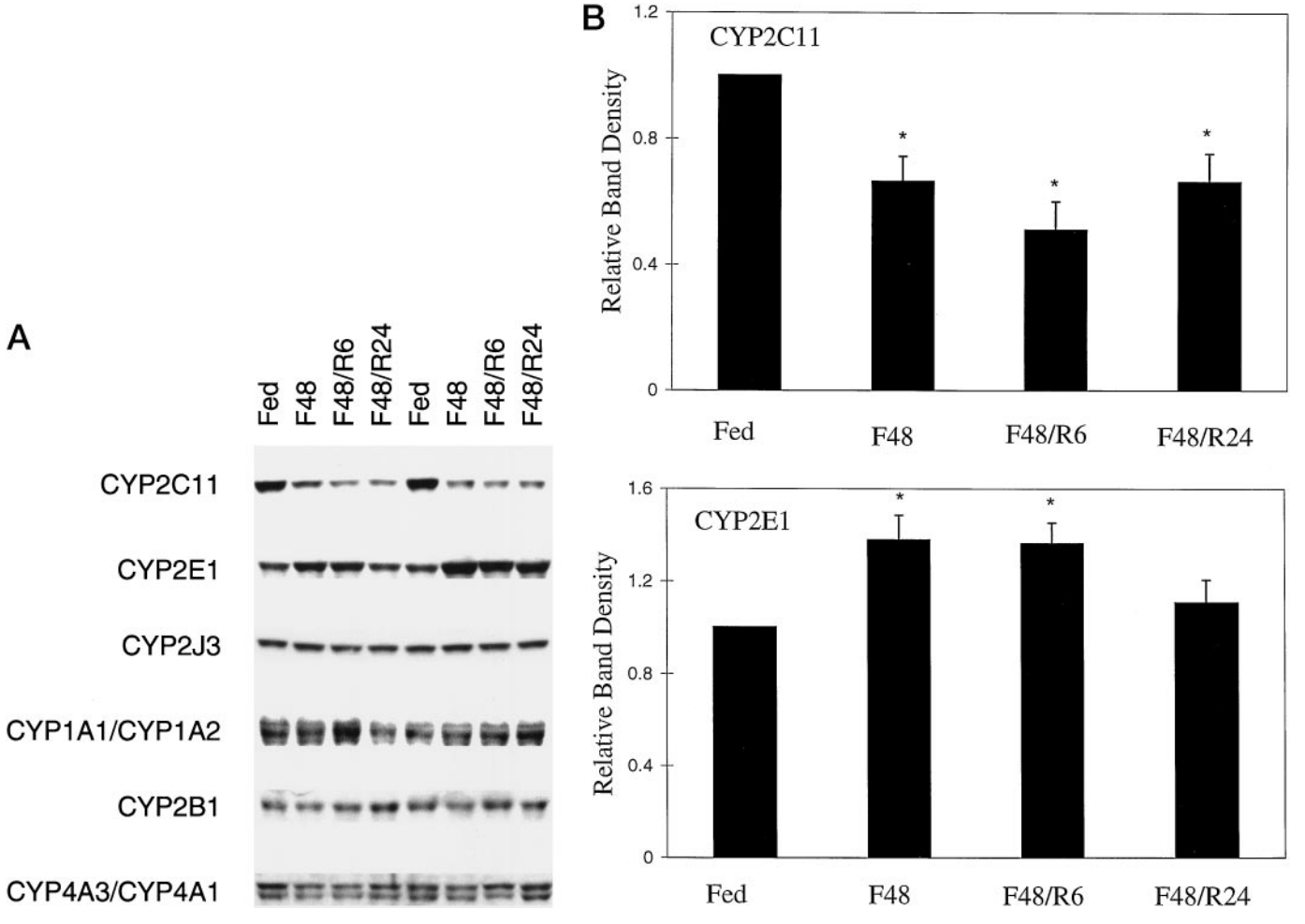

Fig. 2. Levels of P450 proteins in liver microsomes during fasting and refeeding. A, Liver microsomal fractions (20–40 μg/lane) prepared from fed, F48, F48/R6, and F48/R24 rats were electrophoresed, transferred to nitrocellulose, and immunoblotted with antibodies to CYP2C11, CYP2E1, CYP2J3, CYP1A1/CYP1A2, CYP2B1, and CYP4A1/CYP4A3 as described in Experimental Procedures. The protein immunoblots are representative of experiments with 24 different animals (six rats in each of four groups). B, Protein immunoblots for CYP2C11 and CYP2E1 proteins were analyzed by scanning densitometry. Values are the mean ± standard error (six animals in each group) and are normalized to values in fed rats. *, $p < 0.05$ for comparison among fed, F48, F48/R6, and F48/R24 groups by analysis of variance.

performed at 42° in 50% formamide containing 1 M NaCl, 1% (w/v) SDS, 10% (w/v) dextran sulfate, and 0.1 mg/ml heat-denatured salmon sperm DNA. After autoradiography, the radiolabeled probes were removed by boiling, and the blots were rehybridized with a 1.2-kb *Bgl*I fragment of the rat β-actin cDNA (from the plasmid LK280 originally obtained from Dr. Laurence Kedes, University of Southern California, Los Angeles, CA). Autoradiographs were scanned, and relative P450 mRNA levels were determined by normalization to the β-actin signal. Control studies demonstrated that conditions were linear with respect to RNA levels. RNA loading was also assessed by comparing the densities of the 28S and 18S rRNA bands on ethidium bromide-stained gels by scanning densitometry.

Other methods. P450 content of microsomal fractions was determined spectrally according to the method of Omura and Sato (1964) using a Schimadzu UV-3000 dual-wavelength/double beam spectrophotometer (*S*himadzu Scientific Instruments, Columbia, MD). [1-^{14}C]EET internal standards were synthesized from [1-^{14}C]AA (55–57 μCi/μmol) by nonselective epoxidation and purified by reverse-phase HPLC (Capdevila *et al*., 1990b). Methylations were performed using an ethereal solution of diazomethane. PFB esters were formed by reaction with pentafluorobenzyl bromide as described previously (Karara *et al*., 1989). Protein determinations were performed using the BioRad Protein Assay Kit.

Statistical analysis. All values are expressed as mean ± standard error. Data were analyzed by analysis of variance using Systat software (Systat, Evanston, IL). When F values indicated that a significant difference was present, Tukey's HSD test for multiple comparisons was used. Values were considered significantly different at $p < 0.05$.

Results

Effect of nutritional status on physiological parameters and liver P450 content. Table 1 summarizes the effects of fasting/refeeding on body weight, plasma glucose, plasma β-hydroxybutyrate, plasma insulin, urine ketones, and liver total P450 content. Base-line (i.e., prefasting) body weights were nearly identical in the four animal groups; however, postfasting body weights tended to be lower (by

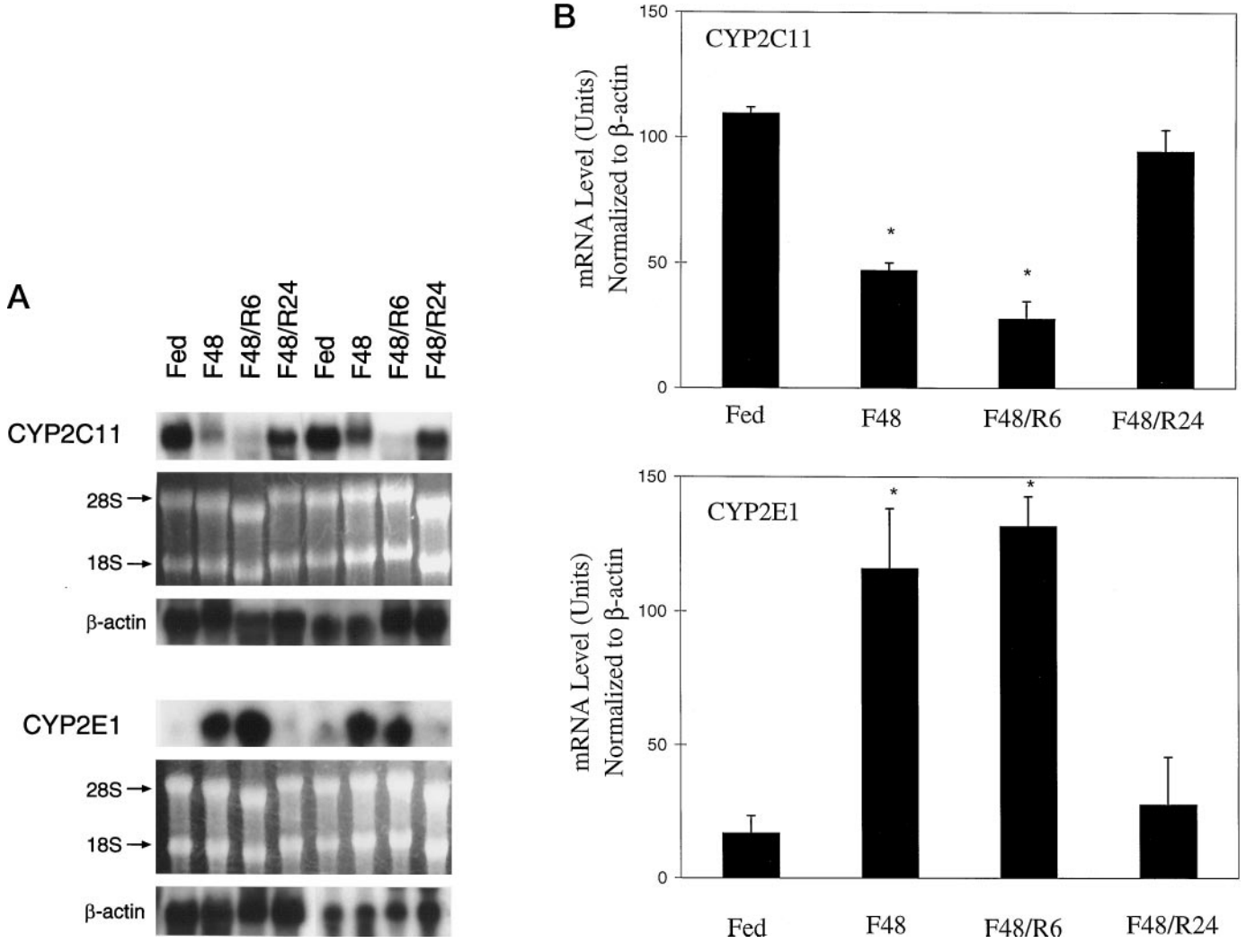

Fig. 3. Effect of fasting and refeeding on CYP2C11 and CYP2E1 mRNA levels. A, Total RNA (20 μg) isolated from fed, F48, F48/R6, and F48/R24 rat livers was denatured, electrophoresed in 1.2% agarose gels containing 0.2 M formaldehyde, transferred to nylon membrane, and blot hybridized with radiolabeled CYP2C11 and CYP2E1 cDNA probes as described in Experimental Procedures. After autoradiography, the radiolabeled probes were removed by boiling, and the blots were rehybridized with a rat β-actin cDNA fragment. The Northern blots are representative of experiments with 12 different animals (three rats in each of four groups). *Top*, autoradiograph of blot after 24-hr exposure. *Middle*, ethidium bromide-stained membrane after transfer. *Bottom*, autoradiograph of stripped/β-actin reprobed blot after 24-hr exposure. B, Autoradiographs were scanned and relative P450 mRNA levels were determined by normalization to the β-actin signal. Values are the mean ± standard error (three animals in each group). *, $p < 0.05$ for comparison among fed, F48, F48/R6, and F48/R24 groups by analysis of variance.

~10%) in F48, F48/R6, and F48/R24 animals. Plasma glucose was unaffected in F48 animals, increased 26% in F48/R6 animals, and then returned to normal in F48/R24 animals. Plasma insulin levels were decreased by >50% in F48 animals compared with fed animals but normalized in refed animals. Plasma β-hydroxybutyrate, a measure of the ketotic state, was increased >400% in F48 animals but returned to normal in refed animals. Similarly, urine ketones increased significantly with fasting in the F48, F48/R6, and F48/R24 groups and partially normalized with refeeding in the F48/R6 and F48/R24 animals. Fasting and refeeding had no significant effect on total liver P450 content.

Effect of the animal nutritional state on the microsomal AA metabolism. To examine changes in liver P450 metabolism of AA during fasting and refeeding, liver microsomal fractions prepared from either fed, F48, F48/R6, or F48/R24 rats were incubated with [1-^{14}C]AA in the presence of NADPH, and the organic soluble metabolites were resolved by reverse-phase HPLC. As shown in Fig. 1 and Table 2, liver microsomes prepared from fed rats metabolized AA (catalytic turnover, 1.44 ± 0.09 nmol of product/mg of microsomal protein/min) to a variety of products, including EETs, DHET, HETEs, and more polar compounds. None of these metabolites were formed in the absence of NADPH suggesting that the products were P450 derived (data not shown). Interestingly, microsomes prepared from F48, F48/R6, and F48/R24 animals metabolized AA at significantly lower rates (catalytic turnover, 1.15 ± 0.09, 0.75 ± 0.07, and 1.10 ± 0.13 nmol of product/mg of microsomal protein/min, respectively) (Fig. 1 and Table 2). Thus, there was a significant 20%, 48%, and 24% reduction in total hepatic microsomal AA metabolism in F48, F48/R6, and F48/R24 rats, respectively, compared with fed rats. A more detailed analysis of the product profiles revealed that epoxygenase activity (EETs plus DHETs) decreased by 28%, 51%, and 26%, whereas hydroxylase activity (midchain HETEs plus ω-terminal alcohols of AA) decreased by only 11%, 32%, and 16% in F48, F48/R6, and F48/R24 rats, respectively, compared with fed rats (Table 2). Thus, there seemed to be a disproportionate reduction in liver AA epoxygenase activity with fasting and refeeding. Regiochemical analysis of the epoxygenase products showed that the reduction in epoxygenase activity with fasting and refeeding was regioselective. Thus, 14,15-, 11,12-, and 8,9-EETs were reduced by 22–25% in F48 rats and 46–50% in F48/R6 rats compared with fed rats (Table 3). In contrast, epoxidation at the 5,6-olefin decreased by 63% and 82% in F48 and F48/R6 rats, respectively, compared with fed rats (Table 3). Regiochemical analysis of hydroxylase products demonstrated that while 20-HETE (ω-hydroxylase activity) and midchain HETE formation decreased with fasting and refeeding, 19-HETE formation (ω-1 hydroxylase activity) increased by 126%, 55%, and 23% in F48, F48/R6, and F48/R24 rats, respectively, compared with fed rats (Table 4). In summary, fasting-refeeding caused a significant decrease in the ability of liver microsomal fractions to catalyze AA epoxidation, midchain hydroxylation, and ω-hydroxylation and a concomitant increase in the ability of liver microsomal fractions to catalyze ω-1 hydroxylation.

Alterations in the hepatic content of P450 enzymes during fasting and refeeding. The observed changes in P450-dependent AA metabolism suggested that the levels of more than one hepatic P450 enzyme may be altered with fasting and refeeding. We therefore used specific polyclonal antibodies and immunoblotting to investigate protein levels of eight different P450 enzymes reported to metabolize AA in rat liver. Western analysis showed that levels of CYP2C11, a major male-specific rat liver P450, were significantly decreased in F48, F48/R6, and F48/R24 animals compared with fed animals (Fig. 2A). Densitometric analysis based on data from 24 rats (6 animals in each group) demonstrated that the amount of immunoreactive CYP2C11 protein in F48 and F48/R6 rats was ~35–50% lower than that in fed animals (Fig. 2B). In contrast, levels of CYP2E1 protein were significantly increased in F48 and F48/R6 rats compared with fed animals (Fig. 2A). Densitometry

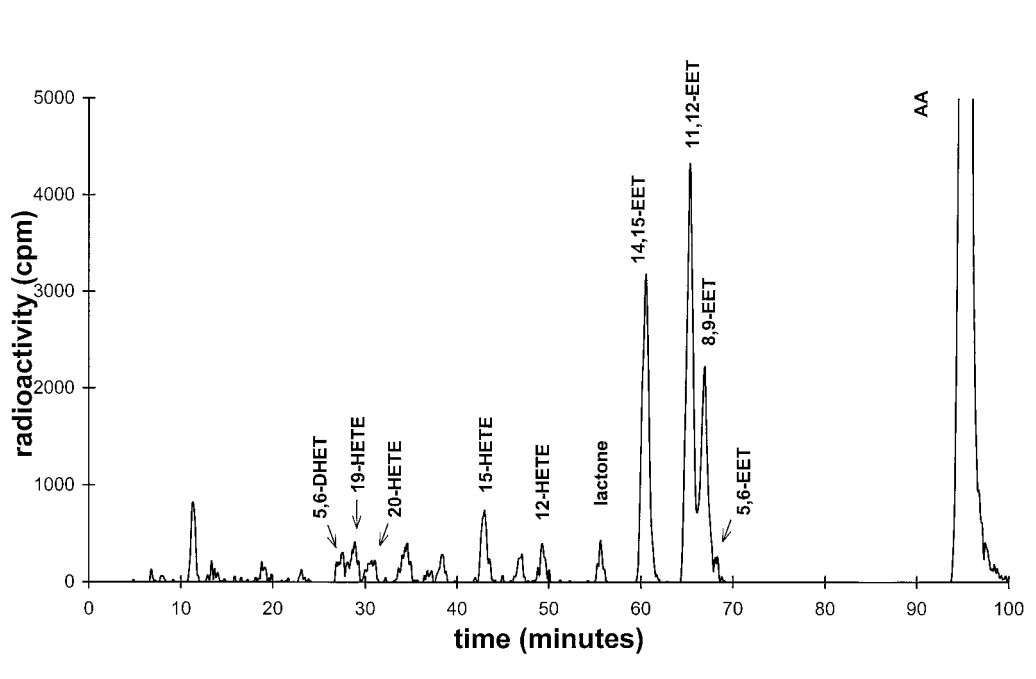

Fig. 4. Reverse-phase HPLC chromatogram of the organic soluble metabolites generated during incubation of recombinant CYP2C11 with [1-^{14}C]AA. Microsomal fractions prepared from *Sf9* cells infected with CYP2C11-CYPOR baculovirus were incubated with [1-^{14}C]AA (40 μM) in the presence of NADPH and an NADPH-regenerating system. After 5 min, the reaction products were extracted and resolved by reverse-phase HPLC as described in Experimental Procedures. Metabolites were identified based on coelution with authentic standards on reverse- and normal-phase HPLC. The chromatogram shown is representative of six incubations.

based on data from 24 rats revealed that the amount of immunoreactive CYP2E1 protein in F48 and F48/R6 animals was ~40% higher than that in fed animals (Fig. 2B). Levels of other rat liver P450s, including CYP2J3, CYP1A1/CYP1A2, CYP2B1, and CYP4A1/CYP4A3, were unchanged with fasting and refeeding (Fig. 2A).

CYP2C11 and CYP2E1 regulation during fasting and refeeding. To investigate whether the observed changes in CYP2C11 and CYP2E1 proteins with fasting and refeeding were associated with changes in the corresponding mRNAs, we performed Northern blot analysis of total rat liver RNA using the respective cDNA probes. As shown in Fig. 3A, CYP2C11 mRNAs were markedly reduced in F48 and F48/R6 animals and returned to fed levels in the F48/R24 animals. Densitometry normalized to the β-actin signal and based on data from 12 rats (3 animals in each group) revealed that CYP2C11 mRNAs were reduced ~60–75% in F48 and F48/R6 animals compared with fed animals (Fig. 3B). In contrast, CYP2E1 mRNAs were markedly increased in F48 and F48/R6 animals and returned to fed levels in the F48/R24 animals (Fig. 3A). Densitometry normalized to the β-actin signal and based on data from 12 rats showed that CYP2E1 mRNAs increased ~600–700% in F48 and F48/R6 animals compared with fed animals (Fig. 3B). The changes in CYP2C11 and CYP2E1 mRNAs with fasting and refeeding were not due to differences in the amount of RNA applied to each lane as assessed by ethidium bromide staining of the gels and nylon membranes. Taken together, these data show that the nutritionally induced changes in CYP2C11 and CYP2E1 occur at a pretranslational level.

Metabolism of AA by recombinant CYP2C11. The significant reduction in epoxidation of AA by rat liver microsomes (Fig. 1 and Table 2), together with the decreased expression of CYP2C11 during fasting/refeeding (Fig. 2), suggested that CYP2C11 is one of the major constitutive AA epoxygenases present in rat liver. To confirm the AA epoxygenase activity of CYP2C11, we coexpressed CYP2C11 protein with CYPOR in *Sf9* insect cells using the baculovirus expression system (Wu *et al*., 1997) (Biagini C, Celier C, and Philpot RM, Structure-function relationship analyses of cytochrome P450 2C11 coexpressed with NADPH-P450 oxidoreductase in insect cells, manuscript in preparation) and examined the ability of recombinant CYP2C11 to metabolize AA *in vitro*. Microsomes prepared from CYP2C11-CYPOR baculovirus infected Sf9 cells catalyzed the NADPH-dependent metabolism of AA to EETs, DHETs, and HETEs (catalytic turnover, 20.0 ± 1.7 nmol of product/nmol of P450/min) (Fig. 4). The major products formed were the EETs and their hydration products, the DHETs (66% of the total). Midchain HETEs and ω-terminal alcohols of AA were formed in significantly lower amounts (Fig. 4). Regiochemical analysis of the CYP2C11-derived EETs showed a preference for epoxidation at the 11,12-olefin (Table 5). Epoxidation at the 14,15-, 8,9-, and 5,6-olefins occurred less often. Importantly, the regiochemical distribution of recombinant CYP2C11 epoxygenase products was remarkably similar to that of fed rat liver microsomes (Table 6), suggesting that CYP2C11 is one of the predominant AA epoxygenases present in rat liver. Stereochemical analysis of CYP2C11-derived EETs revealed a preference for 14(*S*),15(*R*)-, 11(*R*),12(*S*)-, and 8(*S*),9(*R*)-EETs (optical purities, 70%, 77%, and 58%, respectively) (Table 5).

Measurement of total endogenous EETs in rat liver during fasting and refeeding. We used a combination of HPLC and GC/MS techniques to examine the effect of fasting and refeeding on total endogenous rat liver EETs (free plus phospholipid-bound). As shown in Table 7, fasting and refeeding had no significant effect on total liver EETs or on the regiochemical distribution of liver EETs. This suggests that the changes in liver microsomal epoxygenase activity that occur with 48 hr of fasting and 6–24 hr of refeeding are not associated with changes in total liver EET content.

Discussion

The hepatic P450 monooxygenase system has long been thought to function primarily in the metabolism of xenobiotics including drugs and carcinogens (Imaoka *et al*., 1990; Shimojo *et al*., 1993; Gonzalez and Lee, 1996). Over the past 15 years, there has been an increased awareness that these liver heme-thiolate proteins also may be involved in the metabolism of endogenous substrates such as AA (Capdevila *et al*., 1992a, 1995). Recent advances in the cDNA cloning and heterologous expression of several different liver P450s have permitted detailed enzymatic studies that show that AA metabolism is both regioselective and stereoselective and that the product profile is P450 isoform dependent (Capdevila *et al*., 1990a; Rifkind *et al*., 1995; Wu *et al*., 1997). Furthermore, the documentation of EETs and HETEs as endogenous constituents of rat and human liver (Karara *et al*., 1989; Omata *et al*., 1992a; Carroll *et al*., 1996; Zeldin *et al*., 1996), together with the demonstration that these P450-derived eicosanoids have numerous biological effects within the liver and in extrahepatic tissues (Falck *et al*., 1983; Kutsky *et al*., 1983; Yoshida *et al*., 1990; Capdevila *et al*., 1992a, 1995; Carroll *et al*., 1996), suggest that this enzyme system may play an integral role in the control of fundamental cellular processes. For example, the EETs are thought to be involved in vasopressin-stimulated glycogenolysis in the liver and have been shown to stimulate glucagon and insulin release from isolated pancreatic islets, suggesting that epoxygenase metabolites of AA may be involved in the regulation of glucose homeostasis (Yoshida *et al*., 1990; Falck *et al*., 1983). Herein, we provide biochemical, molecular, and immunological data to demonstrate that acute changes in nutritional status alter this enzyme system by causing changes in at least two key hepatic P450 enzymes involved in the metabolism of AA.

A number of factors are known to regulate P450-dependent

TABLE 5

Regioselective and enantioselective composition of EETs produced by recombinant CYP2C11

The regiochemical and stereochemical composition of EETs formed by incubations of recombinant CYP2C11 with [1-^{14}C]AA were quantified by HPLC and liquid scintillation as described in Experimental Procedures. Values shown were averages of at least five different experiments with standard errors of <5%. The lability of the 5,6-EET precluded chiral analysis.

Regioisomer	Distribution	Enantioselectivity	
		R,S	*S,R*
	% of total	*%*	
14,15-EET	31.5	30	70
11,12-EET	36.0	77	23
8,9-EET	22.8	42	58
5,6-EET	9.7	ND	ND

ND, not determined.

metabolism of AA in hepatic and extrahepatic tissues, including xenobiotics, developmental factors, hormonal stimuli, salt intake, physiological conditions such as pregnancy, and pathological conditions such as hypertension (Karara *et al*., 1989; Capdevila *et al*., 1990a, 1992b; Omata *et al*., 1992a, 1992b). In each case, the oxidation of AA is augmented, resulting in an increase in either epoxygenase metabolites (e.g., phenobarbital, angiotensin II, dietary salt, pregnancy, hypertension) or ω/ω-1 hydroxylase metabolites (e.g., clofibrate, parathyroid hormone, deoxycorticosterone acetate, epidermal growth factor). In contrast, we demonstrate here that fasting and refeeding cause an acute decrease in the overall rate of liver microsomal AA metabolism. Our findings differ from those of Orellana *et al*. (1992), who observed higher AA turnover rates in liver microsomes prepared from chronic protein-energy malnourished rats. Regiochemical analysis of the products revealed that although AA epoxidation, midchain hydroxylation, and ω-hydroxylation were significantly decreased with fasting and refeeding, the ability of liver microsomes to catalyze ω-1 hydroxylation was markedly increased. Orellana *et al*. (1992) observed similar qualitative differences in the regiospecificity of AA oxygenation in chronically malnourished rats. Interestingly, we observed a disproportionate reduction in epoxidation at the 5,6-olefin, resulting in a >80% decrease in the formation of 5,6-EET and its more stable metabolites. This finding is particularly relevant because 5,6-EET is the only epoxygenase product that stimulates insulin release from isolated pancreatic islets (Falck *et al*., 1983). Orellana *et al*. (1992) did not observe changes in EET regiochemistry with chronic protein-energy malnutrition, but these investigators did not evaluate epoxidation at the 5,6-olefin.

The observed changes in liver microsomal AA metabolism with fasting-refeeding were accompanied by alterations in the levels of two hepatic P450 monooxygenases, CYP2C11 and CYP2E1; the levels of six other rat liver P450s known to catalyze AA oxygenation remained unchanged. Work in several laboratories has demonstrated that purified and/or recombinant CYP2E1 is active in the ω-terminal hydroxylation of AA producing 19-HETE as the major reaction product (Laethem *et al*., 1993; Rifkind *et al*., 1995). Hence, the increase in liver CYP2E1 expression in the F48 and F48/R6 animals could account for the corresponding increase in 19-HETE formation in liver microsomes prepared from these rats. A partially purified preparation of rat liver CYP2C11 has been shown to metabolize AA to EETs, midchain HETEs, and ω-terminal alcohols (Capdevila *et al*., 1990a). Although the relatively high degree of sequence homology among the different members of the rat CYP2C subfamily has made the isolation of this hemoprotein free of contaminant CYP2C isoforms difficult, the recent heterologous coexpression of the CYP2C11 and CYPOR cDNAs in insect cells (Biagini C, Celier C, and Philpot RM, Structure-function relationship analyses of cytochrome P450 2C11 coexpressed with NADPH-P450 oxidoreductase in insect cells, manuscript in preparation) afforded the opportunity to assess AA monooxygenase activity of the recombinant protein. Microsomes prepared from *Sf9* insect cells coexpressing CYP2C11 and CYPOR were highly active in the metabolism of AA to EETs as the principle products, thus confirming the AA epoxygenase activity of this rat liver hemoprotein. The regiochemical and stereochemical distribution of epoxide metabolites formed by recombinant CYP2C11 is different than that previously reported for the partially purified protein preparation (Capdevila *et al*., 1990a). In particular, recombinant CYP2C11 favors epoxidation at the 11,12-olefin and produces mainly 14(*S*),15(*R*)- and 11(*R*),12(*S*)-EET. These differences are probably caused by the presence of other CYP2C isoforms in the partially purified preparation. Importantly, the regiochemical and stereochemical distribution of epoxygenase products produced by recombinant CYP2C11 is remarkably similar to that produced by rat liver microsomes, providing indirect evidence that CYP2C11 is one of the predominant AA epoxygenases present in rat liver. These data support our recent finding that polyclonal antibodies raised against liver CYP2C11 selectively inhibit rat liver microsomal epoxygenase activity, whereas antibodies to several other P450s do not (Capdevila *et al*., 1995). Interestingly, the decrease in microsomal 5,6-EET production (63–82%) with fasting-refeeding was greater than the corresponding decrease in CYP2C11 protein levels (35–50%), suggesting that CYP2C11 may not be the only P450 isoform that biosynthesizes this eicosanoid in rat liver.

Northern analysis revealed that the nutritionally induced decrease in CYP2C11 protein was accompanied by a corresponding decrease in CYP2C11 mRNA. These data demonstrate that nutritional down-regulation of CYP2C11 occurs at the pretranslational level. In this regard, Legraverend *et al*. (1992) have recently shown that the regulation of CYP2C11 by the sexually dimorphic pattern of growth hormone secretion occurs at the levels of transcriptional initiation both *in vivo* and in primary hepatocyte cultures. The up-regulation of CYP2E1 protein was associated with an increase in CYP2E1 mRNA, demonstrating that regulation of CYP2E1 by nutritional factors also occurs at the pretranslational level. Johansson *et al*. (1990) have shown that the transcription of the CYP2E1 gene is activated by starvation. In contrast, Song *et al*. (1987) have shown that induction of CYP2E1 during experimental diabetes is primarily due to RNA stabilization. Further work will be necessary to better define the molecular mechanisms underlying the regulation

TABLE 6

Comparison of the regiochemical properties of fed rat liver microsomes and recombinant CYP2C11

The regioisomers of EETs formed by incubations of rat liver microsomes (n = 7) or recombinant CYP2C11 (n = 6) with AA were quantified by reverse-phase HPLC and liquid scintillation as described in Experimental Procedures. Data represents the sum of EETs and their hydration products (DHETs). Epoxidation at the 5,6-olefin was determined as the sum of 5,6-EET, 5,6-DHET, and the δ-lactone of 5,6-DHET. All values are expressed as a percentage of the total epoxygenase products.

	14,15-EET	11,12-EET	8,9-EET	5,6-EET
		%		
Fed rat liver	30.5	35.3	23.5	10.7
CYP2C11	31.5	36.0	22.8	9.7

TABLE 7

Regiochemical composition of endogenous rat liver EETs during fasting and refeeding

EET regioisomers were extracted, purified, and quantified from rat liver as described in Experimental Procedures. Values are mean ± standard error from 3 rats in each group. Concentration values are reported as nanograms of EET/gram of liver. The lability of 5,6-EET precluded the determination of endogenous levels of this regioisomer.

Treatment	14,15-EET	11,12-EET	8,9-EET	Total EETs
Fed	313 ± 8	124 ± 19	270 ± 29	706 ± 53
F48	373 ± 42	175 ± 33	270 ± 61	818 ± 134
F48/R6	343 ± 23	108 ± 15	277 ± 29	728 ± 40
F48/R24	322 ± 15	132 ± 18	257 ± 41	710 ± 71

of CYP2C11 and CYP2E1 in different nutritional states.

Forty-eight hours of fasting and 6–24 hr of refeeding had no significant effect on total liver EET content or on the regiochemical distribution of total liver EETs despite clear-cut changes in liver microsomal epoxygenase activity. The GC/MS methods used in the current work measure both free EETs and phospholipid-bound EETs. Because the majority of total liver EETs (~85%) are stored bound to the *sn*-2 position of cellular phospholipids (Karara *et al*., 1989), rapid alterations in free EET production by liver microsomal epoxygenase or epoxygenases may not necessarily be reflected by corresponding changes in total tissue EET levels. We cannot rule out the possibility that fasting-refeeding causes alterations in the relative proportion of free and bound EETs within the liver. In addition, once formed by liver microsomal epoxygenase or epoxygenases, EETs can undergo hydration to DHETs by cytosolic and microsomal epoxide hydrolases, conjugation to glutathione, or further oxidation by cytochrome P450 or other lipid-metabolizing enzymes (Capdevila *et al*., 1992a, 1995; Zeldin *et al*., 1993, 1996). Thus, multiple factors, including EET production by epoxygenase or epoxygenases, EET metabolism by phase I and/or phase II enzymes, and EET lysolipid EET acylation, may influence total tissue EET levels.

In summary, we conclude that (1) nutritional status affects hepatic microsomal AA metabolism, (2) reduced epoxygenase activity with fasting-refeeding is accompanied by decreased levels of CYP2C11, (3) increased ω-1 hydroxylase activity with fasting-refeeding is accompanied by augmented levels of CYP2E1, and (4) the effects of fasting on CYP2C11 and CYP2E1 expression occur at the pretranslational level. We speculate that nutritional alterations in hepatic P450-mediated metabolism of AA may contribute to physiological events involved in glucose homeostasis.

Acknowledgments

We thank Dr. Jorge Capdevila for providing the CYP2C11 cDNA, CYP2E1 cDNA, and polyclonal anti-CYP2C11 IgG and Dr. Laurence Kedes for providing the rat β-actin cDNA. We are grateful to the Molecular Biology Core of the Center for Gastrointestinal Biology and Disease (National Institutes of Health Grant P30-DK34987) for their technical assistance and to Drs. Richard Philpot and Joyce Goldstein for providing helpful comments during preparation of the manuscript.

References

Capdevila JH, Falck JR, Dishman E, and Karara A (1990b) Cytochrome P450 arachidonate epoxygenase. *Methods Enzymol* **187:**385–394.

Capdevila JH, Falck JR, and Estabrook RW (1992a) Cytochrome P450 and the arachidonic acid cascade. *FASEB J* **6:**731–736.

Capdevila JH, Karara A, Waxman DJ, Martin MV, Falck JR, and Guengerich FP (1990a) Cytochrome P450 enzyme-specific control of the regio- and enantiofacial selectivity of microsomal arachidonic acid epoxygenase. *J Biol Chem* **265:**10865–10871.

Capdevila JH, Wei S, Yan J, Karara A, Jacobson HR, Falck JR, Guengerich FP, and Dubois RN (1992b) Cytochrome P450 arachidonic acid epoxygenase: regulatory control of the renal epoxygenase by dietary salt loading. *J Biol Chem* **267:**21720–21726.

Capdevila JH, Zeldin DC, Makita K, Karara A, Falck JR (1995) Cytochrome P450 and the metabolism of arachidonic acid and oxygenated eicosanoids, in *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 2nd ed. (Ortiz de Montellano PR, ed). pp 443–471, Plenum Press, New York.

Carroll MA, Balazy M, Margiotta P, Huang,DD, Falck JR, and McGiff JC (1996) Cytochrome P450-dependent HETEs: profile of biological activity and stimulation by vasoactive peptides. *Am J Physiol* **271:**R863–R869.

Donahue BS, Skottner-Lundin A, Morgan ET (1991) Growth hormone-dependent and independent regulation of cytochrome P450 isozyme expression in streptozotocin-diabetic rats. *Endocrinology* **128:**2065–2076.

Falck JR, Manna S, Moltz J, Chacos N, and Capdevila J (1983) Epoxyeicosatrienoic acids stimulate glucagon and insulin release from isolated pancreatic islets. *Biochem Biophys Res Commun* **114:**743–749.

Favreau LV and Schenkman JB (1988) Cytochrome P450 alterations in the BB/Wor spontaneously diabetic rat. *Biochem Pharmacol* **37:**3505–3509.

Gonzalez FJ and Lee YH (1996) Constitutive expression of hepatic cytochrome P450 genes. *FASEB J* **10:**1112–1117.

Imaoka S, Nakamura M, Ishizaki T, Shimojo N, Ohishi N, Fujii S, and Funae Y (1993) Regulation of renal cytochrome P450s by thyroid hormone in diabetic rats. *Biochem Pharmacol* **46:**2197–2200.

Imaoka S, Terano Y, and Funae Y (1990) Changes in the amount of cytochrome P450s in rat hepatic microsomes with starvation. *Arch Biochem Biophys* **278:**168–178.

Johansson I, Lindros KO, Eriksson H, and Ingelman-Sundberg M (1990) Transcriptional control of CYP2E1 in the perivenous liver region and during starvation. *Biochem Biophys Res Commun* **173:**331–338.

Karara A, Dishman E, Blair I, Falck JR, and Capdevila JH (1989) Endogenous epoxyeicosatrienoic acids: cytochrome P450 controlled stereoselectivity of the hepatic arachidonic acid epoxygenase. *J Biol Chem* **264:**19822–19827.

Karara A, Wei S, Spady D, Swift L, Capdevila JH, and Falck JR (1992) Arachidonic acid epoxygenase: structural characterization and quantification of epoxyeicosatrienoates in plasma. *Biochem Biophys Res Commun* **182:**1320–1325.

Kutsky P, Falck JR, Weiss GB, Manna S, Chacos N, and Capdevila J (1983) Effects of newly reported arachidonic acid metabolites on microsomal Ca^{2+} binding, uptake, and release. *Prostaglandins* **26:**13–21.

Laethem RM, Balazy M, Falck JR, Laethem CL, and Koop DR (1993) Formation of 19(*S*)-, 19(*R*)-, and 18(*R*)-hydroxyeicosatetraenoic acids by alcohol-inducible cytochrome P450 2E1. *J Biol Chem* **268:**12912–12918.

Larsson-Backstrom C, Arrhenius E, Sagge K, Lindmark L, Paprocki J, and Svensson L (1990) Sequential changes in lipid metabolism and the fatty acid profile in liver lipids during fasting and sepsis. *Circ Shock* **30:**331–347.

Legraverend C, Mode A, Westin S, Strom A, Eguchi H, Zaphiropoulos PG, and Gustafsson JA (1992) Transcriptional regulation of rat P450 2C subfamily members by the sexually dimorphic pattern of growth hormone secretion. *Mol Endocrinol* **6:**259–266.

Nakagawa Y and Ishii E (1996) Changes in arachidonic acid metabolism and the aggregation of polymorphonuclear leukocytes in rats with streptozotocin-induced diabetes. *Biochim Biophys Acta* **1315:**145–51.

Nishimoto M, Clark JE, and Masters BSS (1993) Cytochrome P450 4A4: expression in *Escherichia coli*, purification, and characterization of catalytic properties. *Biochemistry* **32:**8863–8870.

Omura T and Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* **239:**2370–2385.

Omata K, Abraham NG, Escalante B, and Schwartzman ML (1992a) Age-related changes in renal cytochrome P450 arachidonic acid metabolism in spontaneously hypertensive rats. *Am J Physiol* **262:**F8–F16.

Omata K, Abraham NG, and Schwartzman ML (1992b) Renal cytochrome P450-arachidonic acid metabolism: localization and hormonal regulation in SHR. *Am J Physiol* **262:**F591–F599.

Orellana M, Fuentes O, Rosenbluth H, Lara M, and Valdes E (1992) Modulation of rat liver peroxisomal and microsomal fatty acid oxidation by starvation. *FEBS Lett* **310:**193–196.

Orellana M, Valdes E, Capdevila J, and Gil L (1989) Nutritionally triggered alterations in the regiospecificity of arachidonic acid oxygenation by rat liver microsomal cytochrome P450. *Arch Biochem Biophys* **274:**251–258.

Pfister SL, Falck JR, and Campbell WB (1991) Enhanced synthesis of epoxyeicosatrienoic acids by cholesterol-fed rabbit aorta. *Am J Physiol* **261:**H843–H852.

Rifkind AB, Lee C, Chang TKH, and Waxman DJ (1995) Arachidonic acid metabolism by human cytochrome P450s 2C8, 2C9, 2E1, and 1A2: regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. *Arch Biochem Biophys* **320:**380–389.

Shimojo N, Ishizaki T, Imaoka S, Funae Y, Fujii S, and Okuda K (1993) Changes in amounts of cytochrome P450 isozymes and levels of catalytic activities in hepatic and renal microsomes of rats with streptozocin-induced diabetes. *Biochem Pharmacol* **46:**621–627.

Song BJ, Matsunaga T, Hardwick JP, Park SS, Veech RL, Yang CS, Gelboin HV, and Gonzalez FJ (1987) Stabilization of cytochrome P450j messenger ribonucleic acid in the diabetic rat. *Mol Endocrinol* **1:**542–547.

Thummel KE and Schenkman JB (1990) Effects of testosterone and growth hormone treatment on hepatic microsomal P450 expression in the diabetic rat. *Mol Pharmacol* **37:** 119–129.

Wu S, Chen W, Murphy E, Gabel S, Tomer KB, Foley J, Steenbergen C, Falck JR, Moomaw CR, and Zeldin DC (1997) Molecular cloning, expression, and functional significance of a cytochrome P450 highly expressed in rat heart myocytes. *J Biol Chem* **272:**12551–12559.

Yoo JSH, Smith TJ, Ning SM, Lee MJ, Thomas PE, and Yang CS (1992) Modulation of the levels of cytochromes P450 in rat liver and lung by dietary lipid. *Biochem Pharmacol* **43:**2535–2542.

Yoshida S, Hirai A, Tamura Y, and Yoshida S (1990) Possible involvement of arachidonic acid metabolites of cytochrome P450 monooxygenase pathway in vasopressin-stimulated glycogenolysis in isolated rat hepatocytes. *Arch Biochem Biophys* **280:**346–351.

Zannikos PN, Bandyopadhyay AM, Robertson LW, and Blouin RA (1994) Expression of the CYP3A and CYP2C11 enzymes in a nutritionally obese rodent model: response to phenobarbital treatment. *Int J Obesity* **18:**369–374.

Zeldin DC, Kobayashi J, Falck JR, Winder BS, Hammock BD, Snapper JR and Capdevila JH (1993) Regio- and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. *J Biol Chem* **268:**6402–6407.

Zeldin DC, Moomaw CR, Jesse N, Tomer KB, Beetham J, Hammock BD, and Wu S (1996) Biochemical characterization of the human liver cytochrome P450 arachidonic acid epoxygenase pathway. *Arch Biochem Biophys* **329:**87–96.

Send reprint requests to: Dr. Darryl C. Zeldin, Laboratory of Pulmonary Pathobiology, NIH/NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709.