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MODULATION OF THE LEVELS OF CYTOCHROMES P450 IN RAT LIVER AND LUNG BY DIETARY LIPID

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Abstract—This study was undertaken to investigate the effect of dietary lipid on the regulation of several constitutive P450 isozymes. Male Sprague–Dawley rats with body weights of 130–140 g were fed either a 20% corn oil (CO) diet or a fat-free (FF) diet for 4 days following 2 days of fasting. Using liver microsomes, the catalytic activities and immunochemically detectable protein levels of P450s 1A1 and 2, 2A1, 2B1 and 2, 2C11, 2E1, and 3A were determined. The microsomes from rats fed the 20% CO diet exhibited 2-fold higher levels in *N*-nitrosodimethylamine demethylase activity and P450 2E1 protein than those from rats fed the FF diet. The CO group also showed 2.5-fold higher levels in 6β-hydroxylation of testosterone and P450 3A protein than the FF group. In contrast, the CO diet did not affect the immunodetectable level of P450 2C11 protein and its catalytic activities such as benzphetamine demethylase activity and 2α-hydroxylation of testosterone. P450 1A1 was not detectable in either group, but 1A2 was 2.5-fold higher in the CO group than in the FF group. In the liver, the P450 2B1 level was very low in both groups as measured by pentoxyresorufin dealkylase activity and the protein level, whereas 2B2 was 2.5-fold higher in the CO diet group. In lung microsomes from rats fed different amounts of CO, an inverse relationship was observed between the P450 2B1 level and the dietary CO level. The results suggest that the constitutive levels of P450 isozymes are modulated by dietary lipid in a selective manner; the levels of hepatic P450s 1A2, 2B2, 2E1, and 3A were regulated positively but the level of pulmonary P450 2B1 was suppressed by dietary lipid.

Dietary and nutritional factors are known to play important roles in the regulation of the metabolism, clearance, and toxicity of xenobiotics via the modulation of the phase I and phase II enzyme systems. A number of review articles on this subject are available [1–5]. In particular, the effect of dietary lipids on microsomal cytochrome P450 (P450†)-dependent xenobiotic metabolism has been extensively studied and reviewed [4–6]. Recently, we demonstrated that in comparison to a fat-free (FF) diet, a 20% corn oil (CO) diet produces a 2-fold higher level in rat liver microsomal P450 2E1‡ as measured by *N*-nitrosodimethylamine (NDMA) demethylase activity and immunochemically detected P450 2E1 level, whereas the difference in the level of dietary CO does not appear to affect P450 2B1 and 2 [8]. In addition, we showed that both the quantity and the extent of unsaturation of dietary fat affect the hepatic level of P450 2E1 [9]. At moderate fat levels (5 and 20% diet, by weight), rats fed CO and menhaden oil produce higher P450 2E1 enzyme activity than those fed lard and olive oil. Furthermore, rats fed a diet containing linoleic acid as the only lipid source, in which the amount of linoleic acid is equivalent to that in the 20% CO diet, show a 2- to 3-fold higher level in P450 2E1 protein and its catalytic activity than those fed the FF diet, similar to the effect produced by 20% corn oil. The effect of dietary corn oil on P450 2E1 is also reflected in drug metabolism *in vivo* [9]. The rate of enflurane metabolism in rats fed a 25% corn oil diet is 2-fold higher than that in rats fed a 0.5% corn oil diet. These results suggest that the "constitutive" P450 enzyme level can be regulated by dietary lipids.

The effects of dietary lipids on the modulation of other constitutive P450 isozymes have not been characterized. Because testosterone can be oxidized at different positions by different P450 isozymes, its metabolism has been used as a probe to study the change of the composition of P450 isozymes [10, 11]. In the present work, in addition to other known metabolism assays which are highly specific for certain P450 isozymes, the metabolism of testosterone was assayed to study the effect of dietary CO on the regulation of the constitutive levels of several P450 isozymes in rat liver and lung. The results from the metabolism assays were analyzed together with those from Western blot analysis. The effect of dietary CO on the metabolism of an important tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), was also studied.

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† Abbreviations: P450, cytochrome(s) P450; FF, fat-free; CO, corn oil; NDMA, *N*-nitrosodimethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; keto alcohol, 4-hydroxy-1-(3-pyridyl)-1-butanone; NNAL *N*-oxide, 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanol; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; HCHO, formaldehyde; keto acid, 4-oxo-4(3-pyridyl)butyric acid; and NNK *N*-oxide, 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone.

‡ The nomenclature of P450 isozymes follows the recommendation in Ref. 7.

Table 1. Dietary composition of modified AIN-76A diets

Ingredient	Corn oil (% diet by weight)				
	0	0.5	5	20	35
Corn oil	0.0	0.5	5.0	17.0	25.9
Sucrose	58.3	57.1	47.0	20.0	0.0

All dietary ingredients were obtained from Teklad (Madison, WI) except for corn oil (Mazola Lot No. 28647; a gift from Best Foods, Union, NJ). The values are the amount (g) added to a diet containing the following in grams: casein, 20; DL-methionine, 0.3; corn starch, 18; fiber, 5; AIN-76 mineral mix, 3.5; AIN-76A vitamin mix, 1; and choline bitartrate, 0.2.

MATERIALS AND METHODS

Chemicals. Chemicals were obtained from the following sources: NDMA, Aldrich Chemical Co. (Milwaukee, WI); resorufin and 7-pentoxyresorufin, Pierce Chemical Co. (Rockford, IL); erythromycin, glucose-6-phosphate (disodium salt), glucose-6-phosphate dehydrogenase (Type XV), $NADP^+$, and NADPH, Sigma Chemical Co. (St. Louis, MO); and unlabeled NNK, [5-^{3}H]NNK (sp. act. 2.20 Ci/mmol; purity > 98%), and [^{3}H-*methyl*]NNK (sp. act. 1.06 Ci/mmol; purity > 95%), Chemsyn Science Laboratories (Lenexa, KS). The radiochemicals were purified by using a reverse phase HPLC to obtain purities > 99%. NNK metabolite standards were provided by Dr. Stephen Hecht (American Health Foundation, Valhalla, NY). Benzphetamine·HCl was provided by the Upjohn Co. (Kalamazoo, MI). Testosterone metabolites were obtained as described [10]. Reagents for electrophoresis and Western blotting were obtained from sources described previously [12], and all other chemicals were reagent grade from commercial sources.

Animals, diets, and treatments. Male Sprague–Dawley rats from Taconic, Inc. (Germantown, NY), body weights of 90–100 g, were fed the AIN-76A rodent diet [13, 14] during 4 days of acclimation, followed by 2 days of fasting. Eight rats were then randomly assigned to two groups which were fed a modified AIN-76A diet containing either 0 or 20% CO (Table 1) for 4 days before being killed. Upon killing, individual liver microsomal fractions were prepared by differential centrifugation as described [15]. In other experiments, rats were fed modified AIN-76A diets containing different amounts of CO (Table 1) and lung microsomal fractions were prepared as described [16]. In one experiment, following 4 days of acclimation with the AIN-76A rodent diet [13, 14], rats were fed a modified AIN-76A diet containing either 0.5 or 25% CO for 4 days without fasting, and liver microsomal fractions were prepared [15]. In all experiments, the rats were given their respective diets and water *ad lib.* (except for the 2-day fasting period where applicable), and maintained in individual wire-bottom cages in air-conditioned quarters (22°) with a 12-hr light and 12-hr dark cycle throughout the experiment. The modified diets (Table 1) were formulated based on the AIN-76A diet [13, 14] with modifications as suggested by Chan and Dao [17].

Enzyme assays. Protein content was measured by the method of Lowry *et al.* [18] and microsomal P450 content was measured by the method of Omura and Sato [19]. Microsomal NADPH–P450 reductase activity was assayed at room temperature using cytochrome *c* as an artificial electron acceptor [20, 21]. One unit of reductase activity corresponds to the NADPH-dependent reduction of 1 nmol cytochrome $c \cdot min^{-1} \cdot (mg\ protein)^{-1}$.

Microsomal demethylase activities using NDMA, benzphetamine, and erythromycin were determined as described previously [22, 23]. In brief, the assay mixture contained (in a total volume of 1 mL) 50 mM Tris·HCl (pH 7.0 at 37°), 10 mM $MgCl_2$, 150 mM KCl, an NADPH-generating system (0.4 mM $NADP^+$, 10 mM glucose-6-phosphate, and 0.4 units glucose-6-phosphate dehydrogenase), microsomes (0.6 to 0.8 mg protein), and 0.2 mM NDMA (or erythromycin) or 1 mM benzphetamine. At the termination of a 20-min incubation, the assay mixture was centrifuged, and 0.7 mL supernatant was used for determination of formaldehyde formed according to Nash [24]. The *O*-dealkylase activities of 7-pentoxyresorufin and 7-ethoxyresorufin were assayed at 10 μM 7-pentoxyresorufin and 5 μM 7-ethoxyresorufin (final concentration) according to Lubet *et al.* [25] using a Perkin–Elmer luminescence spectrometer (model LS-5B; Perkin–Elmer Cetus, Norwalk, CT) with the excitation wavelength set at 522 nm, the emission wavelength set at 586 nm, and the excitation and emission slit widths set at 10 nm.

Testosterone metabolism was assayed according to Sonderfan *et al.* [10] with a slight modification. The microsomal incubations (0.5 mL) contained 0.5 mg protein with the buffer and NADPH-generating system used previously [22]. Testosterone (0.25 mM final concentration) was added in 0.05 mL methanol. The incubations were conducted for 10 min at 37° and terminated by addition of 2 mL methylene chloride. 11β-Hydroxy testosterone (0.75 nmol) was added as an internal standard. After shaking for 5 min, the tubes were centrifuged for 2 min at 2000 rpm. One milliliter of the organic layer was evaporated to dryness and redissolved in 0.1 mL HPLC solvent A (10% aqueous tetrahydrofuran). A 50-μL sample was injected onto a Supelco C_{18} 5 μm column (0.46 × 15 cm) (Supelco, Bellefonte, PA). The following solvent program was used at a flow rate of 1.5 mL/min: the column was eluted with solvent A for the first 22 min; from 22 to 48 min solvent A was mixed with solvent B (5% tetrahydrofuran, 25% water, 70% methanol) in a linear gradient to reach 30% A and 70% B, followed by a linear gradient to reach 100% B from 48 to 52 min; and from 52 to 59 min 100% A was obtained in a linear gradient. The Waters 840 HPLC system included dual model 512 pumps, a model 490E detector set at 254 nm, a model 380 Digital Computer, and a model 712 WISP autoinjector (Waters, Milford, MA). The concentration of each metabolite was determined by comparing its integrated area to that of the corresponding standard compound (2.4 nmol each) that was subjected to a

similar incubation and extraction. The recovery for the standards was > 95%.

NNK metabolism was assayed as described previously [26]. In brief, the incubation mixture consisted of 100 mM sodium phosphate (pH 7.4), 5 mM glucose-6-phosphate, 1.52 units glucose-6-phosphate dehydrogenase, 1 mM $NADP^+$, 1 mM EDTA, 3 mM $MgCl_2$, 10 μM NNK (containing 1 μCi [5-^{3}H]NNK, 1 μCi [^{3}H-*methyl*]NNK, and unlabeled NNK), and 0.1 mg microsomal protein in 400 μL. The reaction mixture was incubated for 30 min at 37° and terminated by the addition of 100 μL each of 25% $ZnSO_4$ and saturated $Ba(OH)_2$. The supernatant was filtered after centrifugation, and 200 μL of the filtrate was coinjected with 5 μL NNK metabolite standards onto a reverse phase HPLC system. The HPLC system consisted of a Waters automated gradient controller, two Waters 6000A pumps, a Waters 710B WISP autoinjector, a Waters 440 UV detector, and a μBondapak C_{18} column (0.39 × 30 cm) (Waters, Milford, MA). It was eluted in a linear gradient of 95% solvent A (0.02 M Tris–HCl buffer, pH 7.0) and 5% solvent B (methanol) to 65% A and 35% B over a 50-min period at a flow rate of 1 mL/min. The radioactive peaks were quantified by a Radiomatic Flo-One/Beta radioactive flow detector (Radiomatic Instrument & Chemical Co., Tampa, FL).

Western blot analysis. Purified P450 2E1 and polyclonal antibodies against P450 2E1 were prepared as described previously [12]. Antibodies against P450s 1A1 and 2, 2A1, 2B1 and 2, 2C11, and 3A were prepared and characterized [27]. Western blot analysis of liver microsomal proteins using the above antibodies was performed by a modification of the method of Guengerich *et al.* [28] as described previously [12]. Intensities of immunostained bands were measured using a Shimadzu Dual-wavelength Thin-layer Chromato Scanner (model CS-930; Shimadzu Corp., Kyoto, Japan).

RESULTS

Effects of dietary corn oil on hepatic microsomal monooxygenase activities. Rats were fed either a 20% CO or a FF diet for 4 days following 2 days of fasting. There was no difference in food intake and apparent growth as determined by the weight gain of the rats (data not shown). Liver microsomes were prepared and measured for monooxygenase activities using various substrates which are known to be highly specific for certain P450 isozymes [27, 29]. Microsomes from rats fed the 20% CO diet exhibited significantly higher demethylase activities with NDMA and erythromycin than those from rats fed the FF diet (Table 2), reflecting higher levels or catalytic activities of P450s 2E1 and 3A, respectively. However, the 20% CO diet did not affect dealkylase activities with benzphetamine, pentoxyresorufin, and ethoxyresorufin which are selective for P450s 2C11, 2B1, and 1A1 and 2, respectively.

Effects of dietary corn oil on hepatic microsomal testosterone metabolism. Testosterone is preferentially hydroxylated at positions 2α, 6β, 7α, and 16β by P450s 2C11, 3A (and 2C13), 2A1, and 2B1 and 2, respectively. In the present work, testosterone

Table 2. Effects of dietary corn oil on hepatic microsomal monooxygenase activities

Diet	Total P450 (nmol/mg)	Reductase (nmol/min/mg)	NDMA demethylase (nmol/min/mg)	Benzphetamine demethylase (nmol/min/mg)	Erythromycin demethylase (nmol/min/mg)	Pentoxyresorufin dealkylase (pmol/min/mg)	Ethoxyresorufin dealkylase (pmol/min/mg)
20% Corn oil	0.82 ± 0.07	332 ± 36	1.76 ± 0.24	6.9 ± 0.5	0.28 ± 0.05	5.6 ± 0.9	59.4 ± 12.1
Fat-free	0.41 ± 0.08*	255 ± 33†	0.81 ± 0.19*	5.7 ± 1.3	0.08 ± 0.04*	4.9 ± 1.1	45.8 ± 9.5

Values are means ± SD from four rats.
* Significantly different ($P < 0.01$) by the Student's *t*-test.
† Significantly different ($P < 0.05$) by the Student's *t*-test.

Table 3. Effects of dietary corn oil on hepatic microsomal testosterone metabolism

Diet	Testosterone metabolites (pmol/min/mg protein)				
	7α-OH T	6β-OH T	16α-OH T	2α-OH T	2β-OH T
20% Corn oil	253 ± 75	911 ± 175	2107 ± 337	1364 ± 181	110 ± 14
Fat-free	188 ± 60	338 ± 79*	2202 ± 399	1291 ± 248	58 ± 12*

Values are means ± SD from four rats. The peak for 7α-OH T coeluted with that for 15β-OH T.

* Significantly different ($P < 0.01$) by the Student's *t*-test.

metabolism was assayed to study the effect of dietary CO on the modulation of the constitutive levels of certain P450 isozymes. The CO group showed 170 and 90% higher ($P < 0.01$) testosterone 6β- and 2β-hydroxylase activities, respectively, than the FF group, suggesting increases of P450s 3A and/or 2C13, but no difference in the formation of 2α-hydroxy testosterone, suggesting that there was no change in 2C11 (Table 3). Although our HPLC system could not separate 7α- from 15β-hydroxy testosterone, no difference in these metabolites was observed between the two dietary groups. The level of 16β-hydroxylation (due to P450 2B1 and 2) was too low to be detected, which confirms the result of pentoxyresorufin dealkylase activity. This result suggests that there is a very low level of P450 2B1 and 2 in hepatic microsomes in both dietary groups and that the activity of 16α-hydroxylation of testosterone was mostly due to the presence of 2C11.

Effects of dietary corn oil on hepatic microsomal P450 isozymes. Hepatic microsomal proteins were immunoblotted using antibodies against several P450 isozymes (Fig. 1), and the intensities of the protein bands were measured using a densitometer. In addition to a 2-fold higher level in P450 2E1 which confirmed previous results [8, 9], rats fed a 20% CO diet showed 2.5-fold higher levels in P450s 1A2 and 3A than those fed a FF diet (Fig. 1 and Table 4). The level of P450 1A1 was not detectable in either group (Fig. 1). The levels of P450s 2C11 and 2A1 (result not shown) were not different between the two dietary groups. P450 2B1 (M_r 52k) was nearly not detectable in both groups, whereas the level of 2B2 (M_r 52.5k) in the 20% CO group was 2.5-fold higher than that in the FF group (Fig. 2 and Table 4). The identity of a band in the lower molecular weight region in control (uninduced) microsomes (lane C) and sample microsomes (lanes 1–4) is not known. The results of immunoblotting were in agreement with those of the enzyme catalytic activities.

Effects of dietary corn oil on hepatic microsomal enzymes in a nonfasted model. The principal feeding protocol employed in the present study was a fasted–refed model according to Wade *et al.* [30]. We adopted this model in order to observe the effect of experimental diets within a short period. It may be questioned whether such a model causes an unstable baseline for a short-term study, which could result in the misinterpretation of data. In particular, P450s 2E1 and 3A are inducible by fasting [23, 31, 32].

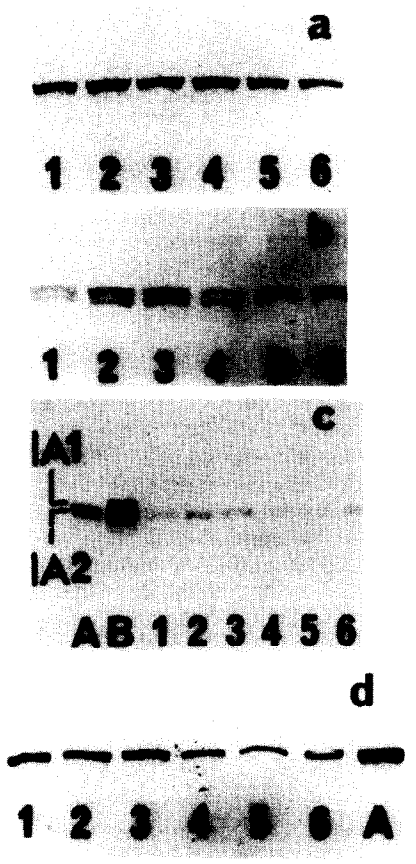

Fig. 1. Western blot analyses of rat liver microsomes using polyclonal antibodies against (a) P450 2C11, (b) P450 3A, (c) P450 1A1 and 2, and (d) P450 2E1. Lanes 1–3 and 4–6 represent individual microsomal samples from 20% CO and FF groups, respectively, with the following protein concentrations: (a) 6.6 μg/lane, (b) 33 μg/lane, (c) 16.5 μg/lane, and (d) 3.3 μg/lane. Lanes A and B (1.7 μg protein/lane) in panel (c) represent safrole- and 3-methylcholanthrene-induced microsomes, respectively. Lane A (2.7 μg protein/lane) in panel (d) represents acetone-induced microsomes.

We, therefore, carried out a feeding experiment which did not involve fasting. The results from the nonfasted model were essentially the same as those obtained in the aforementioned fasted–refed model

Table 4. Densitometric measurement of immunoblotted hepatic microsomal proteins

Diet	P450 isozymes (arbitrary unit)				
	1A2	2B2	2C11	2E1	3A
20% Corn oil	0.95 ± 0.30	1.46 ± 0.73	2.5 ± 0.3	4.6 ± 0.6	12.3 ± 3.0
Fat-free	0.40 ± 0.23*	0.59 ± 0.25	2.2 ± 0.1	2.1 ± 0.5†	5.1 ± 0.9*

Values are arbitrary units based on the peak area and expressed as means ± SD from three rats.
* Significantly different ($P < 0.05$) by the Student's *t*-test.
† Significantly different ($P < 0.01$) by the Student's *t*-test.

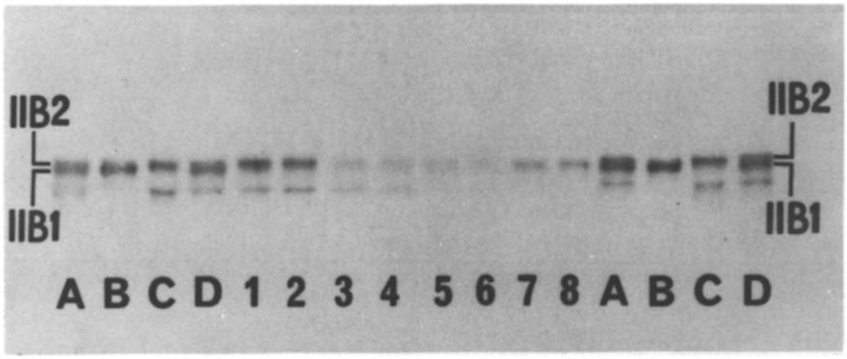

Fig. 2. Western blot analyses of rat liver and lung microsomes using polyclonal antibodies against P450 2B1 and 2. Lanes A and D (8.6 μg protein/lane) represent a 1:1 mixture of phenobarbital-induced and control microsomes; lane B (0.67 μg/lane), phenobarbital-induced microsomes; and lane C (16.5 μg/lane), control (uninduced) microsomes. Lanes 1–4 and 5–8 represent individual liver and lung samples, respectively (16.5 μg protein/lane): lanes 1, 2, 5, and 6, samples from the 20% CO group; and lanes 3, 4, 7, and 8, samples from the FF group.

Table 5. Effects of dietary corn oil on hepatic microsomal enzymes in a nonfasting model

Diet	P450 (nmol/mg)	Reductase (nmol/min/mg)	NDMA demethylase (nmol/min/mg)	P450 2E1 (arbitrary unit)
25% Corn oil	0.96 ± 0.10	321 ± 40	1.92 ± 0.25	2.62 ± 0.67
0.5% Corn oil	0.65 ± 0.18*	313 ± 42	0.88 ± 0.19†	0.85 ± 0.27†

Values are means ± SD from four rats. Values for P450 2E1 are arbitrary units based on the peak area.
* Significantly different ($P < 0.05$) by the Student's *t*-test.
† Significantly different ($P < 0.01$) by the Student's *t*-test.

as determined by total P450 content, reductase acticity, NDMA demethylase activity, and P450 2E1 protein level (Table 5). The result suggests that the procedure involving a 2-day fasting did not disturb the regulation of P450 2E1 at a later date.

Effects of dietary corn oil on lung microsomal enzymes. Lung microsomes were prepared from rats fed a FF, 0.5% CO, or 20% CO diet, and assayed for pentoxyresorufin dealkylase activity. The dealkylase activities of the 0.5 and 20% CO groups were 60 and 5%, respectively, of the FF group, showing an inverse relationship ($r = -0.913$) between the amount of CO in the diet and the enzyme activity (Expt. 1, Table 6). Immunoblotting analysis showed a 2.2-fold higher level in P450 2B1 in the FF group than in the 20% CO group (Fig. 2 and Table 6). In Expts. 2 and 3, rats fed higher levels of CO in the diet showed lower enzyme activities. The enzyme activities were similar between rats fed a 5% CO and those fed laboratory chow. The reductase activity did not appear to contribute to the differences in the enzyme activities among different diets (Expts. 1 and 3, Table 6).

Effects of dietary corn oil on hepatic and lung microsomal NNK metabolism. Since P450 2B1 has been suggested to be involved in NNK metabolism

Table 6. Effects of dietary corn oil on lung microsomal enzymes

Expt.	Diet	Reductase (nmol/min/mg)	Pentoxyresorufin dealkylase (pmol/min/mg)	P450 2B1 (arbitrary unit)
1	20% Corn oil	68	1.9	0.55
	0.5% Corn oil	64	21.5	ND
	Fat-free	59	36.2	1.21
2	35% Corn oil	ND	4.8 ± 4.0	ND
	5% Corn oil	ND	21.1 ± 5.4*	ND
3	20% Corn oil	103 ± 7	1.9 ± 2.4	ND
	5% Corn oil	ND	15.2 ± 7.2†	ND
	Lab. chow	103 ± 6	15.5 ± 8.6†	ND

Values are an average of two pooled samples from four rats in Expt. 1 and mean ± SD from four rats in Expts. 2 and 3. ND = not determined.
* Significantly different ($P < 0.01$) by the Student's *t*-test.
† Significantly different ($P < 0.05$) by the Student's *t*-test.

Table 7. Effects of dietary corn oil on microsomal NNK metabolism

Diet	HCHO	Keto alcohol	Keto acid	NNK *N*-oxide	NNAL *N*-oxide	NNAL
Lung						
35% Corn oil	18.3 ± 2.1	18.1 ± 2.3	<0.2	8.2 ± 1.1	<0.2	11.2 ± 0.7
5% Corn oil	20.8 ± 3.2	20.0 ± 4.3	<0.2	10.5 ± 2.3	<0.2	14.1 ± 2.9
20% Corn oil	18.3 ± 2.6	18.5 ± 2.3	<0.2	8.7 ± 0.7	<0.2	13.0 ± 3.8
5% Corn oil	21.5 ± 2.5	20.9 ± 3.1	<0.2	10.9 ± 1.2	<0.2	11.9 ± 0.8
Lab. chow	19.4 ± 2.3	19.8 ± 2.7	<0.2	10.5 ± 2.3	<0.2	12.3 ± 1.3
Liver						
20% Corn oil	5.12 ± 0.57	5.94 ± 0.59	1.16 ± 0.33	1.58 ± 0.13	0.55 ± 0.13	157.4 ± 46.0
Fat-free	4.80 ± 0.37	4.47 ± 0.65*	1.18 ± 0.06	1.49 ± 0.17	<0.2	108.7 ± 38.1

Values are means ± SD from four rats and expressed as pmol/min/mg protein.
* Significantly different ($P < 0.05$) by the Student's *t*-test.

[33], the presently observed high level of this enzyme in the lung microsomes from the low-fat and fat-free diet groups may be expected to result in high activity in the metabolism of NNK. However, lung microsomes from rats fed different amounts of dietary CO did not show any differences in the profiles of NNK metabolism (Table 7). On the other hand, liver microsomes from rats fed a 20% CO diet produced significantly higher ($P < 0.05$) 4-hydroxy-1-(3-pyridyl)-1-butanone (keto alcohol) formation than those from rats fed a FF diet (Table 7). The 20% CO group also showed an apparently higher level of 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanol (NNAL *N*-oxide) formation than the FF group. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) formation was 30% lower in the FF group than in the 20% CO group, but the difference was not significant ($P > 0.05$). No differences were observed in the formation of formaldehyde (HCHO), 4-oxo-4(3-pyridyl)butyric acid (keto acid), and 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone (NNK *N*-oxide) between the two groups.

DISCUSSION

We have reported previously that liver microsomes from rats fed a 20% CO diet have 2-fold higher levels of immunodetectable P450 2E1 and NDMA demethylase activity than those from rats fed a FF diet [8, 9]. The present work demonstrated that in addition to P450 2E1, feeding the 20% CO diet to rats affected the hepatic levels of other P450 isozymes. The 20% CO group showed a significantly higher ($P < 0.05$) level of P450 3A than the FF group as indicated by the protein level and its catalytic activities such as erythromycin demethylation and testosterone 6β-hydroxylation. However, the male-specific P450 2C11 was not affected by dietary CO as reflected in the protein level and its catalytic activities such as benzphetamine demethylation and testosterone 2α-hydroxylation. Although the presently employed HPLC method for the analysis of testosterone metabolites did not separate 7α-from 15β-hydroxylation, the immunoblot analysis did not show a difference in P450 2A1 levels between the 20% CO and the FF groups (result not shown).

In control (uninduced) rats, the hepatic P450 1A2 is present at a higher level than P450 1A1 [34]. Our present results confirmed that the level of P450 1A1 was too low to detect in both dietary groups, whereas P450 1A2 was present at a 2.5-higher level in the 20% CO group than in the FF group. For hepatic

P450 2B genes, 2B2 is constitutively expressed, whereas 2B1 is not [35]. Our results showed that barely detectable amounts of P450 2B1 were present in the liver of the two dietary groups, whereas substantial amounts of 2B2 were present with a 2.5-fold higher level in the CO group than in the FF group. Conversely, in rat lung, P450 2B1 but not 2B2 is constitutively expressed [35]. Our present immunoblotting result confirmed that P450 2B1 is present but 2B2 is not detectable in rat lung. However, in contrast to the effect observed in the liver, the 20% CO diet produced a lower level in P450 2B1 than did the FF diet and this result was also reflected in its catalytic activity showing an inverse relationship between the enzyme activity and the level of dietary CO. The lower levels in P450 2B1 and its activity in groups with higher levels of dietary CO did not appear to be due to the effect of NADPH–P450 reductase. Additional experiments confirmed the inverse relationship between the P450 2B1 activity and the level of dietary CO (Expts. 2 and 3, Table 6). The enzyme activity of lung microsomes from rats fed laboratory chow was similar to that from rats fed 5% dietary CO.

NNK is a potent tobacco-specific carcinogen inducing tumors in the lung, nasal cavity, and liver of rats [36], and its metabolism in relation to P450 isozymes is of great interest. Devereux *et al.* [33] reported that antibodies against rabbit P450 2 (orthologous to rat P450 2B1) inhibited DNA methylation by rat lung microsomes by 83%, suggesting a key role of P450 2B1 in the metabolic activation of NNK. Our present data for NNK metabolism by rat lung microsomes showed a similar metabolism profile to that by mouse lung microsomes. The difference in the P450 2B1 content of lung microsomes between the 20% CO and FF groups was not displayed in NNK metabolism. This result suggests that P450 2B1 is not the major enzyme for pulmonary NNK metabolism [26]. Furthermore, liver microsomes from the 20% CO group showed higher levels of keto alcohol and NNAL *N*-oxide than those from the FF group. However, the effect of such differences on NNK bioactivation remains to be studied.

We have reported previously that dietary fat/carbohydrate ratios and unsaturated fatty acids (e.g. linoleic acid) are mainly responsible for a 2-fold higher level of hepatic P450 2E1 in rats fed a 20% CO diet than in those fed a FF diet [9]. In the present study, in comparison to the FF diet we have observed that the 20% CO diet can affect other P450 isozymes such as P450 3A. Induction of P450s 2E1 and 3A by fasting for about 4 days was also reported previously [23]. We demonstrated that a high fat diet and fasting result in the synthesis of ketone bodies which may play an important role in the observed induction [9, 37]. Although induction of P450 2E1 by growth hormone was suggested, the role of this hormone in the regulation of P450 2E1 is not clear [38]. Since the conditions of a high fat diet and fasting elicit gluconeogenesis, hormones and other regulatory factors associated with this pathway may also affect the level of certain P450 isozymes. Further investigation is required to demonstrate this issue.

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