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EFFECT OF DIETARY LIPIDS ON LEVELS OF UDP-GLUCURONOSYLTRANSFERASE IN LIVER

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Abstract—Others have shown recently that dietary fish oil protects against acetaminophen-induced liver injury *in vivo*. Fish oil was protective because it increased the clearance of acetaminophen via glucuronidation. This work left unresolved the basis for increased rates of glucuronidation in animals fed fish oil. We therefore have determined how the amount and type of lipid in the diet affect the activity of liver microsomal UDP-glucuronosyltransferase activity. Male Wistar rats were fed a fat-free diet or isocaloric diets containing 5% corn oil, olive oil or fish oil for 4 weeks. The activity of UDP-glucuronosyltransferase was highest in rats fed fish oil and lowest in rats fed the fat-free diet. Treatment with corn oil and olive oil resulted in intermediate levels of activity. Diet-induced differences in amounts of UDP-glucuronosyltransferase were shown by immunoblotting and kinetic measurements. Treatment with fish oil resulted in a 3-fold increase in the amount of UDP-glucuronosyltransferase versus the fat-free diet. Corn oil and olive oil diets caused 2-fold increases in the amount of UDP-glucuronosyltransferase versus the fat-free diet. Fatty acid analysis of microsomal lipids showed that the fat-free diet was associated with decreased levels of arachidonic acid versus the corn oil or olive oil diets. The fish oil diet resulted in increased levels of ω-3 fatty acids versus the other diets.

The microsomal UDP-glucuronosyltransferases are membrane-bound enzymes, which play an important role in detoxifying a wide variety of endogenous and exogenous compounds. Enzyme activity depends on amounts of enzyme and their catalytic states (activity/molecule). The latter depends, in turn, on interactions between these enzymes and the lipid environment. For example, treatment of microsomes with phospholipase A_2 alters the catalytic and allosteric properties of the enzyme [1]. V_{max} varies as much as 700-fold, depending on the lipid used to reconstitute pure enzyme [2]. Changes in dietary lipids can alter the phospholipid composition of the microsomal membrane [3, 4]. This led investigators in the past to explore the role of dietary lipids in regulating the activity of UDP-glucuronosyltransferase [5–7]. Castuma and Brenner [6], for example, demonstrated that a fat-free diet leads to decreased enzyme activity which was attributed to changes in membrane fluidity. Recently, dietary fish oil was shown to protect against acetaminophen-induced liver injury *in vivo* [8]. Fish oil is protective because it increases the clearance of acetaminophen by stimulating rates of glucuronidation. The mechanism by which treatment with fish oil increases glucuronidation is unclear. It was postulated that fish oil-induced changes in the fluidity of the microsomal membrane lead to enhanced rates of glucuronidation by altering the catalytic properties of the enzyme [8]. There is, as mentioned above, considerable experimental support for this idea. But, in addition, there is recent evidence that dietary lipids are important for regulating amounts of xenobiotic metabolizing enzymes in liver [9–13]. Yoo *et al.* [12] for example, demonstrated that treatment with Menhaden fish oil increases activity and levels of P450IIE1. And Dannenberg and Zakim [13] showed that a fat-free diet leads to decreased activity and amounts of UDP-glucuronosyltransferase. We examined in the present work the effects of both the amount and type of dietary lipid in regulating the activity and level of UDP-glucuronosyltransferase. The data presented show that both the amount and type of lipid in the diet are important determinants of enzyme levels and activity.

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MATERIALS AND METHODS

Materials. UDP-glucuronic acid (ammonium salt), *p*-nitrophenol and palmitoyl lysophosphatidylcholine were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade unless otherwise noted.

Animals and diets. Twenty-four male Wistar rats (Charles River, Wilmington, MA) weighing 75–100 g each were divided into four groups of six animals. One group was fed a fat-free test diet prepared by the U.S. Biochemical Corp. (Cleveland, OH). The other groups received isocaloric diets in which 5% corn oil (w/w), 5% olive oil (w/w) or 5% Menhaden fish oil (w/w) replaced sucrose (Table 1). The Menhaden fish oil was contributed by the Zapata Haynie Corp. (Reedville, VA). It was purified to remove free fatty acids, pigments, oxidation products, waxes and metals. Pesticide levels were essentially non-detectable (0 or <0.05 ppm). We also determined whether stringent conditions were required for storing diets containing Menhaden fish oil. Diets with Menhaden fish oil were stored at 22° or under conditions known to prevent oxidation (−20°, under nitrogen, in the presence of antioxidants). These two diets resulted in equal levels of

Table 1. Composition of diets

Ingredients	% in Diet (w/w)			
	Fat-free	Corn oil	Olive oil	Menhaden oil
Vitamin-free casein	21.0	21.0	21.0	21.0
Sucrose	58.2	47.0	47.0	47.0
Celufil	16.3	22.5	22.5	22.5
Fat	0	5.0	5.0	5.0
USP XXI salt mixture	4.0	4.0	4.0	4.0
Vitamin mix*	0.5	0.5	0.5	0.5
Calculated kcal/g	3.2	3.2	3.2	3.2

Diets were prepared by the United States Biochemical Corp.
Diets were pelleted with the addition of water and proprionic acid.
* U.S. Biochemical Corporation Vitamin Diet Supplement No. 23431.

UDP-glucuronosyltransferase in liver microsomes. Thus, diets were stored at 22° in subsequent experiments. The animals were permitted to eat and drink *ad lib.* and were maintained in a 12-hr light:dark cycle. The animals were killed by cervical dislocation after 4 weeks of treatment with these diets.

Preparation of microsomes. Microsomes were prepared from rat livers as previously described [14]. Samples were stored at −70° and used within 1 month of preparation. Protein concentration was determined using the biuret method [15].

Enzyme assays. UDP-glucuronosyltransferase activity was measured at 30° with *p*-nitrophenol as aglycone by monitoring the change in absorbance at 400 nm. All assays were performed with 50.0 mM Tris (pH 7.5), 1.0 mM $MgCl_2$, 0.05 mM *p*-nitrophenol and UDP-glucuronic acid in a final assay volume of 1.0 mL. In experiments requiring palmitoyl-lysophosphatidylcholine, microsomes were mixed with palmitoyl-lysophosphatidylcholine at 0° and then assayed immediately.

Preparation and characterization of antiserum. The GT_{2P} form of UDP-glucuronosyltransferase, which metabolizes relatively acidic phenols, was purified and delipidated as described in Dannenberg *et al.* [16]. Antiserum was prepared by injecting purified enzyme into rabbits and was used without purification. Antiserum gave a strongly positive reaction on Ouchterlony plates with purified enzyme, and reacted on Western blots with purified GT_{2P}. Preimmune serum did not react with any microsomal proteins on Western blot analysis. The antiserum immunoinhibited pure UDP-glucuronosyltransferase and accurately quantitated the increase in enzyme in response to treatment with 3-methylcholanthrene and phenobarbital.

Immunoblot analysis of microsomes. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed under reducing conditions on 10% polyacrylamide gels by the method of Laemmli [17]. Proteins were transformed from slab gels onto nitrocellulose sheets (Schleicher & Schuell, Keene, NH) by the method of Towbin *et al.* [18]. The nitrocellulose sheet was then incubated with rabbit anti-UDP-glucuronosyltransferase for 3 hr. Subsequently, the nitrocellulose membrane was probed with goat anti-rabbit antibody conjugated to alkaline phosphatase (Promega, Madison, WI). The UDP-glucuronosyltransferases were then detected by the alkaline phosphatase color reaction using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

Densitometry. A computing densitometer (Molecular Dynamics, Sunnyvale, CA) was used to determine the density of bands on Western blots.

Fatty acid analysis. The microsomal fatty acids (as methyl esters) were analyzed by using the Microbial Identification System from Microbial ID, Inc. (Newark, DE) which uses Hewlett–Packard hardware including a 5890A gas chromatograph equipped with a hydrogen flame ionization detector, an automatic injector, a sample controller and sample tray, and an electronic integrator controlled by a minicomputer. The gas chromatograph utilized a fused silica capillary column with methylphenyl silicone (SE54) as the stationary phase. The computer-controlled operating parameters of the instrument were as follows: injector temperature, 250°; detector temperature, 300°; oven temperature, programmed from 170° to 270° at 5° per min. The microsomal fatty acid methyl esters were identified by comparing retention times with known standards and by computer calculation of equivalent chain lengths.

Statistics. Data are expressed as means ± SEM for six animals in each treatment group. Comparisons among groups were made by one-way analysis of variance and Scheffé's test.

RESULTS

Rats were weighed after 4 weeks of treatment with the different diets (Table 2). The rats fed the fish oil diet weighed 232 ± 4.4 g; in comparison, rats treated with the corn oil diet weighed 269 ± 5.5 g ($P < 0.005$) and rats fed the olive oil diet weighed 265 ± 6.6 g ($P < 0.05$). There was no significant difference in body weight between rats fed the fish oil and fat-free diets (254 ± 7.3 g). Liver weights were unaffected by these different dietary treatments (Table 2). When liver weights were calculated as a percentage of body weight, rats fed the fish oil diet had a higher percentage of liver weight (4.4%) than

Table 2. Effect of diet on body and liver weights

Diet	Body weight (g)	Liver weight (g)	% Liver weight
Fat-free	254 ± 7.3	9.2 ± 0.3	3.6 ± 0.2
Corn oil	269 ± 5.5	9.4 ± 0.3	3.5 ± 0.1
Olive oil	265 ± 6.6	9.1 ± 0.6	3.4 ± 0.2
Fish oil	232 ± 4.4*†	10.1 ± 0.3	4.4 ± 0.2

Values are means ± SEM, N = 6. % Liver weight was calculated as the liver weight divided by the body weight. Comparisons among groups were made by one-way analysis of variance and Scheffe's test.
* Significantly different from corn oil ($P < 0.005$).
† Significantly different from olive oil ($P < 0.05$).

Table 3. Effect of diet on the activity of UDP-glucuronosyltransferase in microsomes

	UDP-glucuronosyltransferase (nmol/mg/min)
Fat-free	0.29 ± 0.03
Corn oil	0.53 ± 0.01
Olive oil	0.55 ± 0.07
Fish oil	0.92 ± 0.08

Microsomes were prepared from male Wistar rats fed a fat-free diet or an isocaloric control diet containing 5% corn oil (w/w), 5% olive oil (w/w) or 5% Menhaden fish oil (w/w) for 4 weeks. UDP-glucuronosyltransferase was measured using 0.05 mM *p*-nitrophenol and 1.0 mM UDP-glucuronic acid. Assay temperature was 30°. Values are means ± SEM, N = 6.

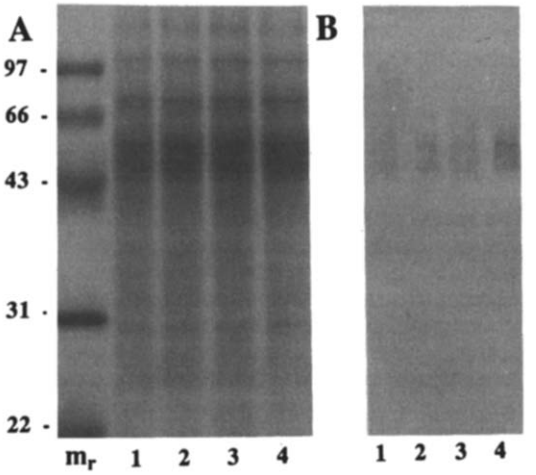

Fig. 1. Diet-induced differences in the amount of UDP-glucuronosyltransferase detected in microsomes by Western blotting. Panel A shows a Coomassie blue-stained SDS–polyacrylamide slab gel of microsomal proteins prepared from rats fed the fat-free (lane 1), corn oil (lane 2), olive oil (lane 3) and fish oil (lane 4) diets for 4 weeks. Migration of molecular mass standards are shown at the left (M_r). Panel B shows a Western blot of microsomal samples identical to those in panel A. Densitometry was performed as described in Materials and Methods: fat-free, 200 arbitary units; corn oil, 355 arbitrary units; olive oil, 439 arbitrary units; and fish oil, 613 arbitrary units.

rats fed the fat-free (3.6%), corn oil (3.5%) or olive oil (3.4%) diets.

The data in Table 3 show the effects of the different diets on the activity of UDP-glucuronosyltransferase. Enzyme activity was highest in microsomes from rats treated with fish oil and lowest in rats fed the fat-free diet. Treatment with olive oil and corn oil led to intermediate levels of enzyme activity.

Effect of dietary treatment on the amount of UDP-glucuronosyltransferase. The possibility that diet-induced differences in enzyme activity reflected differences in enzyme concentration was evaluated by Western blotting. Figure 1A shows a Coomassie blue-stained SDS–polyacrylamide slab gel of microsomal proteins from rats fed the different diets. Similar staining intensities were seen for most bands. An immunoblot of this gel with antiserum to UDP-glucuronosyltransferase is shown in Fig. 1B. A large zone of staining exists because our antiserum detects multiple UDP-glucuronosyltransferases in this molecular weight range. This result was not unexpected since even monoclonal antibodies cross-react with multiple isoforms of UDP-glucuronosyltransferase in this molecular weight range due to sequence homology [19]. The quantity of protein in microsomes immunoreactive with anti-UDP-glucuronosyltransferase was quantified by densitometry. The fish oil diet led to a 300% increase in the amount of UDP-glucuronosyltransferase versus the fat-free diet. Treatment with diets containing olive oil and corn oil led to approximately a 200% increase in enzyme levels versus the fat-free diet.

Since our antiserum is not specific for *p*-nitrophenol conjugating isoforms of UDP-glucuronosyltransferase, we also measured the amount of UDP-glucuronosyltransferase(s) conjugating *p*-nitrophenol, using a kinetic method. Microsomes were treated with a 10-fold excess (mol exogenous phospholipid/mol endogenous phospholipid) of palmitoyl-lysophosphatidylcholine, which is an excellent activator of pure enzyme [2] as well as enzyme in microsomes [20]. Addition of palmitoyl-lysophosphatidylcholine displaces endogenous

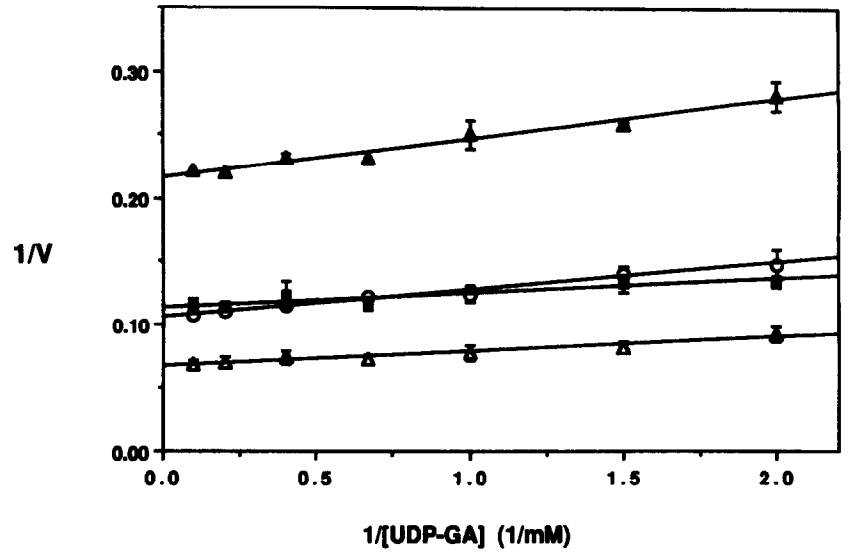

Fig. 2. Regulation of levels of UDP-glucuronosyltransferase activity by the amount and type of lipid in the diet. Microsomes from rats fed the fat-free (▲), olive oil (■), corn oil (○) or fish oil (△) diets for 4 weeks were treated at 0° with palmitoyl-lysophosphatidylcholine at a detergent/protein ratio (w/w) of 1/5 prior to measuring enzyme activity at 30°. Activities are expressed as nanomoles per milligram per minute. The assay mixture contained 1.0 mM $MgCl_2$, 0.05 mM *p*-nitrophenol, 50.0 mM Tris–HCl (pH 7.5) and concentrations of UDP-glucuronic acid which varied from 0.5 to 10.0 mM. Values are means ± SEM, N = 6.

microsomal lipids from the annular layer of UDP-glucuronosyltransferase, thereby creating similar if not identical environments for enzyme in different microsomal preparations. This allows us to measure activities for enzyme in a constant lipid environment in animals fed different diets. The data in Fig. 2 are double-reciprocal plots of the activity of UDP-glucuronosyltransferase as a function of the concentration of UDP-glucuronic acid in microsomes treated with a maximally activating concentration of palmitoyl-lysophosphatidylcholine. Activity at V_{max} was about 3-fold higher in rats fed the fish oil versus the fat-free diet. Corn oil and olive oil diets caused 2-fold increases in the activity of UDP-glucuronosyltransferase versus the fat-free diet. These differences in activities for maximally activated UDP-glucuronosyltransferase in rats fed different diets are essentially identical to the data on amounts of enzymes in the Western blot (Fig. 1). Thus, the data show that changes in amounts of enzyme(s) lead to diet-dependent changes in the activities of *p*-nitrophenol conjugating forms of UDP-glucuronosyltransferase.

Diet-induced changes in the fatty acid composition of the microsomal membrane. An analysis of microsomal fatty acid composition was performed (Table 4). The fat-free diet was associated with decreased levels of arachidonic acid versus microsomes prepared from rats fed the corn oil or olive oil diets. Treatment with the fish oil diet resulted in increased levels of ω-3 fatty acids versus the other diets.

DISCUSSION

Previous investigators concluded that diet-induced changes in the fatty acid composition of the microsomal membrane are responsible for the observed changes in the activity of UDP-glucuronosyltransferase via changes in the lipid-dependent function of the enzyme [5–7]. We recently showed, however, that the amount of lipid in the diet is a determinant of the amount of UDP-glucuronosyltransferase in microsomes [13]. The current experiments demonstrated that the type of lipid in the diet has specific effects on the amounts of UDP-glucuronosyltransferase in microsomes. These results are important because they may be the basis for the recent observation that dietary fish oil protected against acetaminophen-induced liver injury by enhancing rates of glucuronidation of this agent [8]. The current data also have general significance for understanding the regulation of xenobiotic metabolizing enzymes. Yoo *et al.* [12] recently showed that dietary treatment with fish oil leads to greater amounts of P450 than diets containing either corn oil or olive oil. It appears, therefore, that the levels of UDP-glucuronosyltransferase and P450 in liver microsomes are co-regulated by dietary lipids. Although the idea that dietary lipids can induce levels of xenobiotic metabolizing enzymes is novel, it is well known that these enzymes are co-induced by classical inducers such as phenobarbital and 3-methylcholanthrene [21–23].

The level of an enzyme is determined by transcriptional and translational rates in addition to the stability of mRNA and protein. The basis for dietary lipid-induced changes in the amount of UDP-glucuronosyltransferase remains to be explained. It is already known, however, that animals fed a diet high in linoleic acid exhibit higher RNA efflux from

Table 4. Fatty acid composition of microsomal membranes from livers of rats fed the experimental diets for 4 weeks

Fatty acid	Percent total fatty acids* Diets			
	Fat-free	Corn oil	Olive oil	Menhaden oil
14:0	0.3	0.2	0.3	0.4
16:0	19.1	19.1	18.0	21.3
16:1 *cis* 9	2.8	0.7	0.8	3.6
17:0	—	0.4	0.3	0.5
17:1 *iso*	0.2	0.2	0.2	0.2
18:0	20.8	21.8	23.0	20.4
18:1 *cis* 9	11.5	4.0	9.3	11.3
18:1 *cis* 11	3.6	2.4	3.0	4.3
18:2 *cis* 9, 12	3.3	8.9	3.7	3.5
20:4 *cis* (ω-6)	11.4	24.7	22.7	7.2
20:1 *cis* 14 (ω-6)	1.3	2.6	2.4	0.6
22:6 (ω-3)	1.8	1.8	2.5	6.8

Microsomal fatty acid composition was determined by gas–liquid chromatography as described in Materials and Methods.

* Minor components make for 100%.

isolated nuclei than animals fed a diet low in linoleic acid [24]. One possibility suggested by our data is that n-3 and n-6 fatty acids affect some signal such as RNA efflux involved in the *de novo* synthesis of xenobiotic metabolizing enzymes and that n-3 fatty acids are more potent than n-6 fatty acids. In comparison to the fat-free diet, the corn oil and olive oil diets did not increase the concentration of n-3 fatty acids in microsomes, but they led to a large increase in the concentration of n-6 fatty acids and to intermediate levels of UDP-glucuronosyltransferase. Treatment with dietary fish oil resulted in the highest levels of both n-3 fatty acids and UDP-glucuronosyltransferase. The fat-free diet resulted in both the lowest levels of n-3 and n-6 fatty acids and UDP-glucuronosyltransferase. Future experiments will be directed to understanding the mechanism(s) underlying the regulation of levels of UDP-glucuronosyltransferase by dietary lipids.

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