Effect of Dietary Fat on Rat Liver Microsomal Enzymes and Lipid Composition

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Summary Dietary fat is known to influence the activities of various membrane-bound enzymes. The impact of various commonly consumed vegetable fats of India, viz., groundnut, coconut, safflower or mustard oil with varied fatty acid composition on the activities of liver microsomal membrane-bound enzymes and microsomal lipid composition was studied. In a feeding experiment, male weanling rats were fed these oils at 20 g per 100 g of the diet for 4 months. Liver microsomal cytochrome P-450 content was found to be significantly higher in the safflower oil-fed group and lowest in the coconut- and mustard oil-fed groups. The activity of UDP-glucuronyltransferase was highest in the mustard oil- and safflower oil-fed groups and lowest in the coconut oil-fed group and intermediary in the groundnut oil-fed group. The activity of Mg^{2+} -ATPase was markedly lower in the safflower oil-fed group than in the rest of the groups. Na^+, K^+ -ATPase activity was distinctly higher in the mustard oil-fed group, while Mg^{2+} -ATPase activity was markedly higher in the groundnut oil-fed group. Further, the liver microsomes of safflower oil and mustard oil-fed groups showed higher amounts of cholesterol and phospholipids than the other two groups. However, the safflower oil group showed a higher cholesterol to phospholipid molar ratio than the other groups. Changes in cholesterol to phospholipid molar ratio as well as in fatty acid composition of the microsomal membranes could be responsible for the differences in the activities of the enzymes observed in the different groups.

Key Words: membrane-bound enzymes, cytochrome P-450, UDP-glucu-

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Dietary fat is known to alter the activities of various membrane-bound enzymes of plasma membranes [1-4] as well as those of intracellular membranes such as mitochondrial and microsomal membranes [5-7] by altering their lipid milieu. Liver microsomes play an important role in drug-metabolizing enzymes. The cytochrome P-450-dependent, mixed-function oxidase system of liver microsomes is involved in the phase I reaction of a wide range of xenobiotics and of endogenous steroids such as cholesterol and steroid hormones [8]. Further, it is well documented that several nutritional factors modulate the activity of the mixed-function oxidase system. These mixed-function oxidases are membrane bound [9-11] and exhibit functional requirement for phosphatidylcholine, which is a structural lipid component of biomembranes. Phospholipids that contain mainly essential fatty acids are required for optimal activity of mixed-function oxidases [12, 13]. Therefore, it is possible that variations in the fatty acid profiles of different dietary fats would alter the fatty acid composition of the membrane phospholipids, which might in turn modify the activity of mixed-function oxidases [14, 15]. The scientific interest in this area has led to the emergence of several reports on the effect of the quality and quantity of dietary fat on the mixed-function oxidase system [16, 17].

Further studies in this area have shown that dietary fat can also affect the activity of a phase II reaction enzyme, UDP-glucuronyltransferase (EC 2.4.1.17). However, there are no reports on the impact of various commonly consumed vegetable fats of India on the drug-metabolizing enzymes and other membrane-bound enzymes of liver microsomes. Therefore, in the present study an attempt was made to assess the influence of different dietary fats with varied fatty acid compositions on the activities of some of the membrane-bound enzymes and on the lipid microenvironment of the membrane.

MATERIALS AND METHODS

Weanling male CFY rats were distributed into 4 groups of 8 animals each and were fed groundnut, coconut, safflower or mustard oil at 20 g per 100 g of diet for 4 months. The composition of the experimental diet was based on AIN-76 [18, 19] and is shown in Table 1. Fatty acid composition of the dietary fats is given in Table 2. Coconut oil is rich in saturated fatty acids, safflower oil has a high amount of linoleic acid (18:2), mustard oil has a high content of erucic acid (22:1), and groundnut oil has a fairly good proportion of oleic (18:1) and linoleic (18:2) acids. At the end of experimental feeding, the animals were killed by cervical dislocation. Livers were dissected out and liver microsomes were prepared by differential centrifugation as described earlier [20]. Protein was determined by the method of Lowry *et al.* [21] using crystalline serum albumin as standard.

Table 1. Diet composition.

Ingredient	Diet composition (g/100 g diet)
Casein	20.00
DL-Methionine	0.30
Cellulose	5.00
Oil	20.00
Mineral mixture	4.00*
Vitamin mixture	0.10**
Choline chloride	0.20
Starch	50.40

Diet was prepared as per AIN standards for nutrients. *USP XVII. **AIN Standard.

Table 2. Fatty acid composition of oils.*

Fatty acids	Coconut oil	Mustard oil	Groundnut oil	Safflower oil
8:0	4.4	—	—	—
10:0	6.8	—	—	—
12:0	36.7	—	—	—
14:0	26.5	—	—	0.1
16:0	10.5	2.2	12.3	2.9
18:0	3.1	1.3	4.2	2.1
18:1	9.0	10.4	38.6	15.6
18:2	3.0	15.3	38.1	79.4
18:3	—	14.3	2.2	—
20:0	—	—	1.8	—
20:1	—	3.0	—	—
22:0	—	—	2.8	—
22:1	—	53.4	—	—

*Expressed as % distribution of fatty acids. —, not detectable.

Cytochrome P-450 was measured according to the method of Omura and Sato [22], which is based on the principle that the reduced form of cytochrome P-450 readily combines with carbon monoxide to form a complex having an absorption maximum at 450 nm when read against its reduced form not treated with carbon monoxide. The difference in absorption at 450 nm from that at 490 nm was used for calculating the cytochrome P-450 content.

UDP-glucuronyltransferase activity was assayed according to the method of Woodcock and Wood [23]. The assay was performed in a medium containing 0.1 M phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 0.2 mM *p*-nitrophenol, 2.5 mM UDP-glucuronic acid, and 1 mg microsomal protein in a final volume of 0.4 ml. UDP-glucuronic acid was omitted from all blanks. The reaction was arrested by the addition of an equal volume of 0.2 M trichloroacetic acid (TCA). The tubes were centrifuged, and a 0.5-ml aliquot of supernatant was added to 1.5 ml of 0.5 M NaOH. The *p*-nitrophenol concentration was monitored at 405 nm in a Gilford spectrophotometer (Gilford Instrument Laboratories Inc., OH). The reaction was found to be linear up to 20 min and 2 mg enzyme protein under these conditions. Na^+ , K^+ -ATPase and Mg^{2+} -ATPase activities of liver microsomal membranes were

assayed according to the method of Post and Sen [24] with a slight modification. Ouabain when required was added at a 1 mM concentration. The reaction mixture contained 140 mM NaCl, 14 mM KCl, 1 mM EGTA, 3 mM MgCl₂, 3 mM Tris-ATP, and 50 μ g enzyme protein in a final volume of 0.5 ml. The reaction was started by the addition of Tris-ATP and arrested by that of 0.5 ml of ice-cold 10% TCA. Na⁺, K⁺-ATPase activity was taken as the difference between the activities obtained in the presence and absence of ouabain in the reaction medium. The activity obtained in the presence of 1 mM ouabain was taken as the Mg²⁺-ATPase activity.

Lipids were extracted from liver microsomes according to the method of Folch *et al.* [25]. Total cholesterol and phospholipid contents were determined as described by Zlatkis *et al.* [26] and Bartlett [27], respectively.

Statistical evaluation was done by analysis of variance.

RESULTS

Effects of dietary groundnut, coconut, safflower or mustard oil on the body and liver weights of rats and on liver microsomal cytochrome P-450 content are shown in Table 3. The body weight of the mustard oil-fed group was significantly lower than that of the other groups. However, the liver weight as well as the

Table 3. Body weight, liver weight, and liver cytochrome P-450 content of rats fed various oil diets.

	Coconut oil	Mustard oil	Groundnut oil	Safflower oil
Body weight(g)	484.9 $\pm$ 8.86 ^{a,c}	335.5 $\pm$ 10.85 ^b	496.3 $\pm$ 1.24 ^a	462.9 $\pm$ 12.33 ^b
Liver weight(g)	13.6 $\pm$ 0.50 ^a	15.3 $\pm$ 0.36 ^b	13.8 $\pm$ 0.31 ^a	13.0 $\pm$ 0.42 ^a
Liver weight (as % of body weight)	2.82 $\pm$ 0.11 ^a	4.59 $\pm$ 0.17 ^b	2.79 $\pm$ 0.09 ^a	2.83 $\pm$ 0.11 ^a
Cytochrome P-450 content (nmol/mg protein)	0.63 $\pm$ 0.06 ^a	0.61 $\pm$ 0.03 ^a	0.79 $\pm$ 0.05 ^b	0.97 $\pm$ 0.06 ^c

Values are Mean $\pm$ SE, $n=8$ observations in each group. Values in the horizontal rows not showing a common superscript are significantly different from each other at the 5% level ($p<0.05$) by analysis of variance.

Table 4. Enzyme activities in liver microsomal membranes from rats fed different oil diets.

	Coconut oil	Mustard oil	Groundnut oil	Safflower oil
Na ⁺ ,K ⁺ -ATPase (μ mol Pi/mg protein/h)	0.38 $\pm$ 0.05 ^a (6)	0.81 $\pm$ 0.13 ^b (7)	0.36 $\pm$ 0.08 ^a (7)	0.21 $\pm$ 0.06 ^a (5)
Mg ²⁺ -ATPase (μ mol Pi/mg protein/h)	3.27 $\pm$ 0.18 ^a (6)	3.23 $\pm$ 0.16 ^a (7)	4.28 $\pm$ 0.17 ^b (7)	2.55 $\pm$ 0.13 ^c (5)
UDP-glucuronyltransferase (nmol conjugated/min/mg protein)	0.49 $\pm$ 0.05 ^a (4)	1.01 $\pm$ 0.16 ^b (8)	0.65 $\pm$ 0.13 ^a (7)	1.00 $\pm$ 0.13 ^b (8)

Values are Mean $\pm$ SE. Numbers in parentheses indicate the number of observations. Values in horizontal rows not sharing the common superscript are significantly different from each other at the 5% level ($p<0.05$) by analysis of variance.

Table 5. Lipid composition of liver microsomal preparations.

	Coconut oil	Mustard oil	Groundnut oil	Safflower oil
Cholesterol ($\mu\text{mol}/\text{mg}$ protein)	0.14 ± 0.01^a	0.29 ± 0.01^b	0.20 ± 0.01^c	0.26 ± 0.03^c
Phospholipid ($\mu\text{mol}/\text{mg}$ protein)	0.19 ± 0.01^a	0.38 ± 0.01^b	0.23 ± 0.01^c	0.30 ± 0.05^c
Cholesterol/phospholipid molar ratio	0.74 ± 0.04^a	0.76 ± 0.02^a	$0.86 \pm 0.04^{a,b}$	0.89 ± 0.06^b

Values are Mean $\pm$ SE. $n=6$ observations in each group. Values in the horizontal rows not sharing a common superscript are significantly different from each other at the 5% level ($p < 0.05$) by analysis of variance.

proportion of liver weight to body weight were markedly higher in this group. The results on the activities of Na^+, K^+ -ATPase, Mg^{2+} -ATPase, and UDP-glucuronyl-transferase are presented in Table 4. Mustard and safflower oil feeding resulted in enhanced UDP-glucuronyltransferase activity as compared with feeding with groundnut and coconut oils. The activity of Mg^{2+} -ATPase was markedly lower in the safflower oil-fed group than in the rest of the groups. Na^+, K^+ -ATPase activity was distinctly higher in the mustard oil-fed group. The results on the lipid composition of liver microsomes from the various groups are presented in Table 5. Total cholesterol and phospholipid contents were significantly higher in safflower oil- and mustard oil-fed groups. However, safflower oil feeding alone resulted in an enhanced molar ratio of cholesterol to phospholipid.

DISCUSSION

The results of the present study indicate that feeding of mustard oil caused a significant growth depression compared with groundnut, coconut or safflower oil feeding, which can be attributed to the presence of erucic (22:1) acid in the mustard oil [28]. Further, the liver weight and liver weight as a percentage of body weight were significantly higher in this group, probably due to lipid infiltration.

Changes in the lipid composition of microsomal membrane are known to affect the structure and dynamics of the bilayer and to produce diverse effects on the kinetic behavior of intrinsic proteins of the membrane [29-34]. The influence of quality and quantity of dietary fat on mixed-function oxidases has been studied extensively [35-40]. Further, the direct relationship between the dietary content of linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids and cytochrome P-450 content of liver microsomal membranes has been well documented [17, 38]. The present study also confirms some of these earlier observations. The diet containing safflower oil (rich in linoleic (18:2) acid) showed the highest level of cytochrome P-450 followed by groundnut oil having the next highest level of linoleic (18:2) acid. Mustard and coconut oil diets had the lowest level of cytochrome P-450 content. A previous study on a high erucic acid-containing rapeseed oil diet indicated no effect on liver microsomal cytochrome P-450 content

[41], and the present study corroborates this earlier observation.

Although in the present study the liver microsomes were not analyzed for fatty acid composition, our own studies on fatty acid profiles of membrane preparations from other sources such as erythrocytes and cardiac tissue [42, 43] and those of others [5-7] suggest that the fatty acid composition of membranes does reflect that of the dietary fat. Based on such observations changes in the fatty acid composition could be envisaged even with respect to liver microsomal membranes. In fact, Caster *et al.* [44] demonstrated a relationship between the fatty acid composition of the diet and that of hepatic tissue.

The data on lipid composition of the liver microsomal membranes indicate that feeding of highly unsaturated fats such as safflower and mustard oils results in high absolute amounts of cholesterol and phospholipids. Similar observations were made by us with respect to the erythrocyte membrane preparation of these dietary groups [42]. A similar increase in cholesterol content of liver microsomes of safflower oil-fed rats was reported by Lee *et al.* [45]. This observed increase in cholesterol content could be a compensatory mechanism to maintain optimal fluidity of liver microsomal membranes in the event of increased levels of unsaturated fatty acid-containing phospholipid.

UDP-glucuronyltransferases are a group of enzymes of the endoplasmic reticulum responsible for the phase II step of bio-transformation of drugs, xenobiotics, and endogenous substrates [46]. These are phospholipid-dependent integral proteins of the membrane, with their activity depending on the interaction with lipids containing phosphoryl groups [47, 48]. The kinetic properties of UDP-glucuronyltransferase in liver microsomes of the guinea pig have been studied extensively [20, 32, 33]. Essential fatty acid deficiency induced a reduction in the double-bond index of liver microsomal lipids of the guinea pig and resulted in depressed UDP-glucuronyltransferase activity without altering the cholesterol to phospholipid molar ratio [20]. On the other hand, the altered UDP-glucuronyltransferase activity due to cholesterol supplementation has been attributed to a higher membrane ordering effect produced by cholesterol that could not be offset by a decreased phosphatidylethanolamine to phosphatidylcholine ratio [32].

In the present study feeding of highly unsaturated fats such as safflower and mustard oil resulted in higher UDP-glucuronyltransferase activity, thereby suggesting that it is the total unsaturated fatty acid content of the oil rather than the polyunsaturated fatty acid fraction that is responsible for the stimulation of this drug-metabolizing enzyme that occurs by alteration of the lipid milieu of the membrane. The early work of Mounie *et al.* [49] suggested that the vegetable fats, viz., coconut oil-, groundnut oil-, and corn oil-containing diets, had no effect on liver microsomal cytochrome P-450 content. However, corn oil and fish oil diets have been shown to stimulate UDP-glucuronyltransferase activity with substrates such as 4-nitrophenol and 2-naphthol 4 methyl umbelliferone (group I substrates), which activity is considered to be an indicator of hepatotoxicity [50]. A similar increase in the activity of UDP-glucuronyltransferase with 4-nitrophenol was

observed in safflower oil- and mustard oil-fed rat liver microsomes in the present study, which is suggestive of the potential hepatotoxic nature of these oils. Enhanced glucose-6-phosphatase activity due to feeding with high erucic acid-containing rapeseed oil, which is yet another indicator of hepatotoxicity, was also reported earlier [41]. Thus the results of the present study along with the earlier observations [41] substantiate the potential hepatotoxic nature of high erucic acid-containing oils.

The effect of dietary fat on other membrane-bound enzymes such as adenylate cyclase and 5'-nucleotidase of the plasma membrane of various tissues such as cardiac tissue has been reported [1-3]. There are also reports on the influence of dietary fat on liver microsomal fatty acid synthetase, desaturase, and Ca^{2+} -ATPase [51-53]. Nutritional manipulations that induce higher saturation of membrane fatty acyl chains result in higher desaturase activity [54]. However, Ca^{2+} -transport ATPase and Ca^{2+} -uptake in liver microsomes were significantly depressed in essential fatty acid-deficient animals [53]. These effects have been attributed mainly to changes in fatty acid composition rather than to altered contents of membrane cholesterol and phospholipids [53]. However, to our knowledge there are no reports describing the possible effects of dietary fat on other ion-transporting enzymes of liver microsomes.

Our results on microsomal membrane ATPase suggest that feeding with safflower oil, which is rich in polyunsaturated fatty acids, has a depressing effect on both Na^+, K^+ -ATPase and Mg^{2+} -ATPase, whereas mustard oil (rich in erucic acid) feeding enhanced the activity of these two enzymes. Similar observation was made by us on the activities of these enzymes in the erythrocyte membrane of rats in response to feeding with safflower and mustard oil diets [42]. The depressed Na^+, K^+ -ATPase and Mg^{2+} -ATPase activities of safflower oil-fed animals is due to higher polyunsaturated fatty acid content of this oil, since higher levels of PUFA are known to inhibit Na^+, K^+ -ATPase activity [1]. This contention was further substantiated by the observations that essential fatty acid-deficient diet enhanced the Na^+, K^+ -ATPase activity of kidney, submaxillary gland, heart and liver plasma membranes [1, 55, 56]. The present study, in addition to establishing the inhibitory effect of increased polyunsaturated fatty acid content on some membrane-bound enzymes such as Na^+, K^+ -ATPase and Mg^{2+} -ATPase [1], also suggests a stimulatory effect of highly unsaturated fats on some of the drug-metabolizing enzymes.

We can conclude from the present study that the activities of various membrane-bound enzymes of liver microsomes are influenced by the quality of the dietary fat, which in turn alters the lipid microenvironment of these enzymes.

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