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Effect of Chitosan on Hepatic Drug-Metabolizing Enzymes and Oxidative Stress in Rats Fed Low- and High-Fat Diets

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Chitosan is sold worldwide as a lipid-lowering functional food and may be taken with certain medications. To investigate the effect of chitosan on drug-metabolizing enzymes and oxidative stress in the liver, male Wistar rats were fed a low- or high-fat diet with cellulose or chitosan for 4 weeks. A significant decrease in cytochrome P450 (CYP) 3A-catalyzed testosterone 6β-hydroxylation in liver microsomes was observed in rats fed the chitosan with low- and high-fat diets. The expression of CYP 3A1 and 3A2, however, was suppressed by chitosan in rats fed the low-fat diet only. Furthermore, rats fed the low-fat diet with chitosan had lower hepatic glutathione *S*-transferase (GST) activity and superoxide dismutase activity and higher total tissue and microsomal lipid hydroperoxides. Hepatic α-tocopherol was lower in rats fed the chitosan-containing diet. The results suggest that chitosan is likely to modulate CYP 3A activity and protein expression and GST activity partially in a dietary fat-dependent manner. This change may cause a decrease in the metabolism of drugs catalyzed by these enzymes in liver tissues. Moreover, decrease of α-tocopherol level and SOD activity by chitosan partly accounts for the increase of hepatic lipid peroxidation.

KEYWORDS: Chitosan; drug-metabolizing enzymes; CYP; oxidative stress; rats

INTRODUCTION

Oxidation and conjugation are two major reactions involved in the biotransformation of drugs and chemicals in the mammalian system. Mammalian cytochrome P450 (CYP) enzymes are monooxygenases that catalyze the phase I oxidative metabolism of small hydrophobic compounds including steroids, fatty acids, fat-soluble vitamins, drugs, and toxins (*1*). In general, drugs and chemicals undergo phase I oxidative metabolic reactions resulting in the formation of more water-soluble and less toxic metabolites. However, some CYP enzymes such as CYP 1A1, 3A, and 2E1 are involved in the metabolic activations of carcinogens such as benzo(*a*)pyrene, *N*-nitrosodimethylamine, and aflatoxin B_1 (*2*). Furthermore, CYP-mediated oxidative metabolism of the substrates may generate toxic electrophiles and reactive oxygen species (ROS) (*3*). Phase II conjugation enzymes function to eliminate electrophiles and ROS generated by phase I reactions, thereby preventing the increase of oxidative stress (*4*). Microsomal UDP-glucuronosyltransferase (UGT) and cytosolic glutathione *S*-transferase (GST) are two important phase II enzymes that catalyze the conjugation reactions resulting in the formation of water-soluble glucuronate and glutathione conjugates to facilitate the excretion of xenobiotics. Several CYP isoforms and phase II enzymes can be induced or suppressed by micro- and macronutrients. Dietary fats, for example, with different amounts of and/or different fatty acid compositions, can change the activities of certain CYP enzymes, UGT, and GST (*5*, *6*). In addition, vitamin E and retinoids are shown to activate the nuclear receptors and induce CYP3A expression at mRNA as well as enzyme activity (*7*, *8*). Induction and inhibition of these enzymes can change the pharmacological activities and toxicities of drugs and cause drug interactions (*9*). Increasing demands for functional foods and herbal medicines with trends for self-medication are prevalent throughout the world. There are great concerns about drug interactions involving modulation of drug-metabolizing enzymes by functional foods because these natural products may frequently be taken with various drugs prescribed for the treatment of many chronic diseases (*10*, *11*).

Chitosan, a biopolymer of glucosamine derived from chitin, is chemically similar to cellulose. It is not digestible by mammalian digestive enzymes and acts as dietary fiber in the gastrointestinal tract. Chitosan was shown to reduce dietary fat absorption and decrease plasma cholesterol concentrations (*12*). It is sold worldwide as a lipid-lowering functional food. Animal studies showed that chitosan ingestion reduced fat accumulation as well as fat-soluble vitamin levels, especially vitamin E, in the liver due to the lowering of fats absorption (*13*). Co-administration of chitosan with dietary fats had been reported to increase lipid peroxidation in rat liver (*14*, *15*). However, other studies indicated that chitosan reduced lipid peroxidation and protected

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Table 1. Composition (Percent, w/w) of the Experimental Diets[a]

ingredient	LF	LF-C	HF	HF-C
casein	20	20	20	20
soybean oil[b]	5	5	20	20
vitamin mixture[c]	1	1	1	1
salt mixture[c]	5	5	5	5
choline chloride	0.2	0.2	0.2	0.2
cellulose	5		5	
chitosan[d]		5		5
corn starch	63.8	63.8	48.8	48.8

[a] LF, cellulose group fed a low-fat diet; LF-C, chitosan group fed a low-fat diet; HF, cellulose group fed a high-fat diet; HF-C, chitosan group fed a high-fat diet. [b] The composition of the fatty acid in soybean oil was 0.2% myristic acid ($C_{14:0}$), 12.5% palmitic acid ($C_{16:0}$), 0.1% palmitoleic acid ($C_{16:1}$), 3.3% stearic acid ($C_{18:0}$), 22.6% oleic acid ($C_{18:1}$), 56.1% linoleic acid ($C_{18:2}$), and 5.2% linolenic acid ($C_{18:3}$). [c] The AIN 76 vitamin and mineral mixture were procured from ICN Biochemicals (Costa Mesa, CA). [d] The degree of deacetylation and average molecular weight of chitosan were 83% and 6.25×10^5 Da, respectively.

the liver from chemical-induced oxidative damage (*16*, *17*). One possible explanation for this discrepancy may have been due to the differences in the amount and physiochemical properties of the chitosan used in these studies as well as the differences in the amount of dietary fats provided by the experimental diets. Because dietary fats and vitamin E are important regulatory factors in xenobiotic metabolism and lipid peroxidation (*6*, *18*) and their gastrointestinal absorption could be changed by chitosan, it is suggested that hepatic drug-metabolizing enzymes and oxidative stress may be modulated by chitosan when fed together with dietary fats.

The present study was designed to investigate the effects of chitosan co-administered with low or high dietary fat on hepatic drug-metabolizing enzymes including CYP, GST, and UGT and on oxidative stress in rats. Rats fed a 5% cellulose diet were treated as the controls.

MATERIALS AND METHODS

Materials. Testosterone, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, resorufin, *p*-nitrophenol, 4-nitrocatechol, NADPH, glutathione, pyrogallol, glutathione reductase, 1-chloro-2,4-dinitrobenzene, sodium dodecyl sulfate (SDS), cytochrome *c*, heparin, Ponceau S, and cellulose were obtained from Sigma (St. Louis, MO). 6-β-Hydroxytestosterone was purchased from Ultrafine Chemicals (Manchester, U.K.). All other chemicals and reagents were of analytical grade and were obtained commercially. Chitosan, prepared from shrimp shell chitin, was generously supplied by the Taiwan Tanabe Seiyaku Co. (Taipei, Taiwan). The degree of deacetylation and average molecular weight of the chitosan were determined as reported previously (*12*) and were 83% and 6.25×10^5 Da, respectively.

Animals and Treatments. Male Wistar rats weighing 250 g (6 weeks old) were obtained from BioLASCO, Taiwan (Ilan, Taiwan). During the adaptation period, rats were fed a chow diet for 1 week. Then the animals were randomly divided into four groups with seven rats in each group: LF group, 5% cellulose with 5% soybean oil diet; LF-C group, 5% chitosan with 5% soybean oil diet; HF group, 5% cellulose with 20% soybean oil diet; and HF-C, 5% chitosan with 20% soybean oil diet. The compositions of the experimental diets given to the test animals are shown in **Table 1**. Rats were housed in individual plastic cages in a room kept at 23 ± 1 °C and 60 ± 5% relative humidity with a 12 h light/dark cycle. Food and drinking water were available ad libitum for 4 weeks. This study was approved by the Animal Center Management Committee of China Medical University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals as issued by the Animal Center of the National Science Council, Taiwan.

Collection of Blood and Tissue Samples. At the end of the experimental period, animals were fasted for 12 h prior to being sacrificed. Animals were killed by exsanguination via the abdominal aorta while under carbon dioxide (70%/30%, CO_2/O_2) anesthesia. Heparin was used as the anticoagulant. Plasma was separated from the blood by centrifugation (1750*g*) at 4 °C for 20 min. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured immediately by commercial kits (Randox Laboratories, Antrum, U.K.). The liver from each animal was immediately removed, weighed, and stored at −80 °C. Microsomal preparation and enzyme assays were performed within 2 weeks after liver collection.

Preparation of Liver Microsomes. The frozen liver samples were homogenized (1:4, w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA. The homogenate was first centrifuged at 10000*g* for 15 min at 4 °C. The supernatant was then centrifuged at 105000*g* for 60 min. The resulting microsomal pellets were suspended in a 0.25 M sucrose solution containing 1 mM EDTA and stored at −80 °C until use.

Drug-Metabolizing Enzyme Assays. The contents of total CYP and cytochrome *b*5 were quantified according to the method of Omura and Sato (*19*). The NADPH-CYP reductase activity was measured according to the procedure described by Phillips and Langdon (*20*) using cytochrome *c* as the substrate. A number of substrates were used to determine each specific CYP enzyme activity as reported previously (*21*). Ethoxyresorufin (2 μM), methoxyresorufin (5 μM), and pentoxyresorufin (5 μM) were respectively used as the probe substrates for ethoxyresorufin *O*-deethylation (CYP 1A1), methoxyresorufin *O*-demethylation (CYP 1A2), and pentoxyresorufin *O*-dependentylation (CYP 2B), and *p*-nitrophenol (50 μM) and testosterone (60 μM) were respectively used as the probe substrates for *p*-nitrophenol 6-hydroxylation (CYP 2E1) and testosterone 6β-hydroxylation (CYP 3A). Microsomal protein concentrations of all metabolic reactions were 0.2 mg/mL. The incubation time for CYP 1A1, 1A2, and 2B was 10 min and that for CYP 2E1 and 3A was 15 min. The metabolites of various CYP enzyme reactions were determined by high-performance liquid chromatography (HPLC)−mass spectrometric (MS) methods as reported previously (*21*). Enzyme activities were expressed as picomoles of metabolite formation per minute per milligram of protein.

The microsomal UGT activity was determined using *p*-nitrophenol as the substrate, where the rate of formation of *p*-nitrophenol glucuronic acid was measured spectrophotometrically (*22*). Cytosolic GST activity was determined spectrophotometrically according to the method of Habig and Jakoby (*23*). Enzyme activity was expressed as nanomoles of CDNB-GSH formation per minute per milligram of protein. The protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL).

Determination of Lipid Peroxide, Glutathione, and Antioxidative Enzyme Activities. Liver homogenates were prepared by homogenizing tissue in ice-cold 1.15% KCl to obtain a 10% solution and used for the determination of lipid hydroperoxide (LPO) and reduced glutathione (GSH). LPO levels in the liver homogenate and liver microsomes were determined by a commercial kit (Cayman Chemicals, Ann Arbor, MI). The GSH concentration in the liver homogenate was assayed as reported previously (*24*). To determine the antioxidative enzyme activities, liver homogenates was obtained as described previously and centrifuged (10000*g* for 15 min) at 4 °C, and the resulting supernatant was used for the assays. The protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL). Glutathione peroxidase (GSH-Px) activity was determined spectrophotometrically according to the method of Mohandas et al. (*25*). GSH-Px activity was expressed as nanomoles of NADPH decrease per minute per milligram of protein. Superoxide dismutase (SOD) activity was determined as described by Marklund and Marklund (*26*). One unit of SOD activity was defined as the amount of protein that inhibits the rate of pyrogallol reduction by 50%.

Western Blot Analysis. Liver microsomes were analyzed for the expression of CYP 3A1 and 3A2, and liver homogenates as described above were analyzed for the expression of heme oxygenase-1 (HO-1) by immunoblotting analysis. Protein from the microsomes (CYP 3A1 and 3A2, 4 μg) and liver homogenates (HO-1, 10 μg) were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. Subsequently, the membranes were blocked with 5% skim milk in PBST buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, and 0.1% Tween-20) for 1 h at room temperature and then hybridized with primary antibody against CYP 3A1 and 3A2 from Chemicon International, Inc. (Temecula, CA), or HO-1 from Calbiochem (San Diego, CA) with gentle agitation overnight at 4 °C . After washing with PBST, the membranes were incubated with HRP-conjugated secondary antibody from Chemicon for 1 h at room temperature. The immunoreactive bands were visualized

Table 2. Body Weight, Liver Weight, and Food Intake in Rats Fed the Low- and High-Fat Diets with Chitosan or Cellulose for 4 Weeks[a]

	diet				*p* value (two-way ANOVA)		
	LF	LF-C	HF	HF-C	chitosan	fat	interaction
initial body wt (g)	296.1 ± 18.1	295.9 ± 18.4	296.7 ± 16.8	297.6 ± 17.5	ns	ns	ns
final body wt (g)	418.7 ± 23.4	395.6 ± 25.3	455.9 ± 31.7 a	373.1 ± 12.9 b	<0.001	ns	0.01
food intake (g/day)	30.2 ± 2.3	30.0 ± 2.1	26.2 ± 1.1	27.1 ± 2.1	ns	0.005	ns
liver wt (g)	12.6 ± 1.3 a	10.7 ± 0.9 b	13.2 ± 0.9 a	9.8 ± 0.3 b	<0.001	ns	ns
liver wt (g/100 g of body wt)	3.0 ± 0.2 a	2.7 ± 0.1 b	2.9 ± 0.1 a	2.6 ± 0.1 b	<0.001	ns	ns

[a] Values are mean ± SD, $n = 7$. Rats were fed one of following diets for 4 weeks: low-fat diet (LF), high-fat diet (HF), low-fat diet plus chitosan (LF-C), high-fat diet plus chitosan (HF-C). Groups receiving the same level of dietary fat diet not sharing the same letter are significantly different, $p < 0.05$. ns, not significantly different at $p > 0.05$.

Table 3. Activities of Drug-Metabolizing Enzymes in Rat Liver[a]

	diet				*p* value (two-way ANOVA)		
	LF	LF-C	HF	HF-C	chitosan	fat	interaction
cytochrome P450 (pmol/mg of protein)	762.1 ± 90.5	659.9 ± 144.2	927.1 ± 177.1 a	623.7 ± 173.7 b	<0.001	ns	0.019
cytochrome *b*5 (pmol/mg of protein)	296.7 ± 44.7	302.9 ± 58.1	405.1 ± 63.3 a	256.7 ± 45.8 b	0.002	ns	0.001
NADPH-cytochrome P450 reductase (μmol/min/mg of protein)	87.1 ± 5.0	94.2 ± 10.5	91.6 ± 7.8	94.5 ± 10.5	ns	ns	ns
ethoxyresorufin *O*-deethylase (CYP 1A1) (pmol/min/mg of protein)	107.8 ± 30.7	93.7 ± 16.8	135.5 ± 32.0	104.2 ± 18.8	0.046	ns	ns
methoxyresorufin *O*-demethylase (CYP 1A2) (pmol/min/mg of protein)	81.2 ± 20.2	64.0 ± 10.4	97.8 ± 32.2	91.1 ± 26.2	ns	0.041	ns
pentoxyresorufin *O*-depentylase (CYP 2B) (pmol/min/mg of protein)	26.4 ± 2.8	25.9 ± 3.5	31.8 ± 5.2	28.8 ± 7.7	ns	ns	ns
p-nitrophenol 6-hydroxylase (CYP 2E1) (pmol/min/mg of protein)	568.0 ± 82.8	469.0 ± 75.9	542.4 ± 181.9	458.3 ± 105.3	ns	ns	ns
testosterone 6β-hydroxylase (CYP3A) (pmol/min/mg of protein)	1826.8 ± 283.6 a	1147.2 ± 269.4 b	1898.0 ± 210.2 a	1388.8 ± 410.1 b	<0.001	ns	ns
glutathione *S*-transferase (nmol/min/mg of protein)	710.8 ± 88.3 a	373.9 ± 131.3 b	782.3 ± 225.9	700.2 ± 215.9	0.018	0.023	ns
UDP-glucurosyltransferase (nmol/min/mg of protein)	1.4 ± 0.2	1.3 ± 0.1	1.6 ± 0.3	1.3 ± 0.1	ns	ns	ns

[a] Values are mean ± SD, $n = 7$. Rats were fed one of following diets for 4 weeks: low-fat diet (LF), high-fat diet (HF), low-fat diet plus chitosan (LF-C), high-fat diet plus chitosan (HF-C). Groups receiving the same levels of dietary fat diet not sharing the same letter are significantly different, $p < 0.05$. ns, not significantly different at $p > 0.05$.

using Western LightningR Western blot chemiluminescence reagents (Blossom Biotechnologies, Inc., TX). Densitometric measurement of the bands were made using an image analysis system with Image pro plus 5.1 software (Media Cybernetics, Silver Spring, MD). Equal loading across the lanes was confirmed by staining the blot with Ponceau S solution.

RNA Extraction and Quantitative Polymerase Chain Reaction (q-PCR). The rat liver samples were sectioned and ground by a homogenizer. Total RNA was extracted with TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. Subsequently, cDNA was synthesized from 5 μg of total RNA in a reaction mixture containing 2.5 μM oligo(dT) primer, 0.5 mM dNTP mix, 200 U SuperScript III reverse transcriptase, and 40 U RNaseOUT, an RNase inhibitor (all from Invitrogen). After incubation at 50 °C for 50 min, the reaction mixture was heat inactivated at 85 °C for 5 min and then treated with 2 U RNase H at 37 °C for 20 min.

The q-PCR was performed with Probes Master and the probes of Universal ProbeLibrary (UPL) (Roche, Mannhein, Germany) by the LightCycler 480 apparatus (Roche). Primers were designed using ProbeFinder software (https://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp?id=UP030000). The primer sets and the matched UPL probe numbers are as follows: UPL probe 12, HO-1, 5′-ctaagaccgccttcctgct-3′ (forward), 5′-tgtctgtgagggactctggtc-3′ (reverse); and UPL probe 6, Actb, 5′-ctggctcctagcaccatga-3′ (forward), 5′-tagagccaccaatccacaca-3′ (reverse). The PCR was performed in a reaction mixture containing 0.5 μM of each primer, 0.1 μM UPL probe, and 1-fold concentration of Probes Master reagent. The amplification conditions were initial denaturation at 94 °C for 10 min, followed by 45 cycles of 94 °C for 10 s, 55 °C for 30 s, and 72 °C for 1 s. The fluorescent signal was detected at the 72 °C step of each cycle. The relative quantification of each gene was normalized by β-actin and calculated by the value of cross-point (CP) of each fluorescence curve.

Determinations of Hepatic Lipid and α-Tocopherol Concentrations. Liver total lipids were extracted with a chloroform/methanol (2: 1, v/v) mixture according to the method of Folch et al. (*27*). The hepatic total cholesterol, triglyceride, and phospholipid contents in the liver extract were solubilized in Triton X-100 (*28*) and assayed enzymatically using kits purchased from Audit Diagnostics (Cork, Ireland). To determine the hepatic α-tocopherol level, the extracted lipids in livers were dissolved in methanol containing 0.1% (w/v) BHT and determined by HPLC (*29*). The concentration of α-tocopherol was measured from preparative standard curves detected at 290 nm.

Determinations of the Fatty Acid Composition in Liver Microsomes. Lipids were extracted from the liver microsomes according to the method of Folch et al. (*27*). The fatty acid composition was determined according to the method of Huang et al. (*30*). In brief, extracted lipids were dissolved with 1 mL of 14% boron trifluoride−methanol (BF_3−methanol, Sigma) for the fatty acid methylation reaction. Fatty acid methyl esters were analyzed by gas chromatography (Hitachi GC, G-3000, Osaka, Japan) equipped with a capillary column (Supelco SP2330, 0.32 mm i.d. × 30 m, Bellefonte, PA) and a flame ionization detector. Fatty acid profiles were identified according to the retention times of appropriate standard fatty acid methyl esters.

Statistical Analysis. Two-way ANOVA was used to test the effects of both chitosan and dietary fat and their interaction (SPSS 10.0.7; SPSS Inc., Chicago, IL). Student's *t* test was used to test the significance of the effect of chitosan treatments in each dietary fat-fed group. A value of $p < 0.05$ was considered to be significant.

RESULTS

Growth Characteristics. As shown in **Table 2**, after 4 weeks of treatment, the final body weight was reduced by chitosan ($p < 0.001$). Moreover, chitosan significantly decreased the liver weight and ratio of liver weight to body weight ($p < 0.001$). Although the amount of dietary fat did not affect the final body weight, it enhanced the decrease of body weight by chitosan ($p = 0.01$). No significant changes on plasma aminotransaminases (AST and ALT) among the rats of the four groups were detected (data not shown), indicating that chitosan caused no hepatotoxicity.

Drug-metabolizing Enzyme Activities. Table 3 shows the drug-metabolizing enzyme activities in rat liver. As indicated, total CYP ($p < 0.001$) and cytochrome *b*5 ($p = 0.002$) contents were decreased by chitosan. Dietary fat alone did not change and the contents of total CYP and cytochrome *b*5, but it interacted with chitosan ($p = 0.019$ and $p = 0.001$, respectively). Decreases of total CYP and cytochrome *b*5 by chitosan were pronounced in rats fed the high-fat diet ($p < 0.05$, HF vs

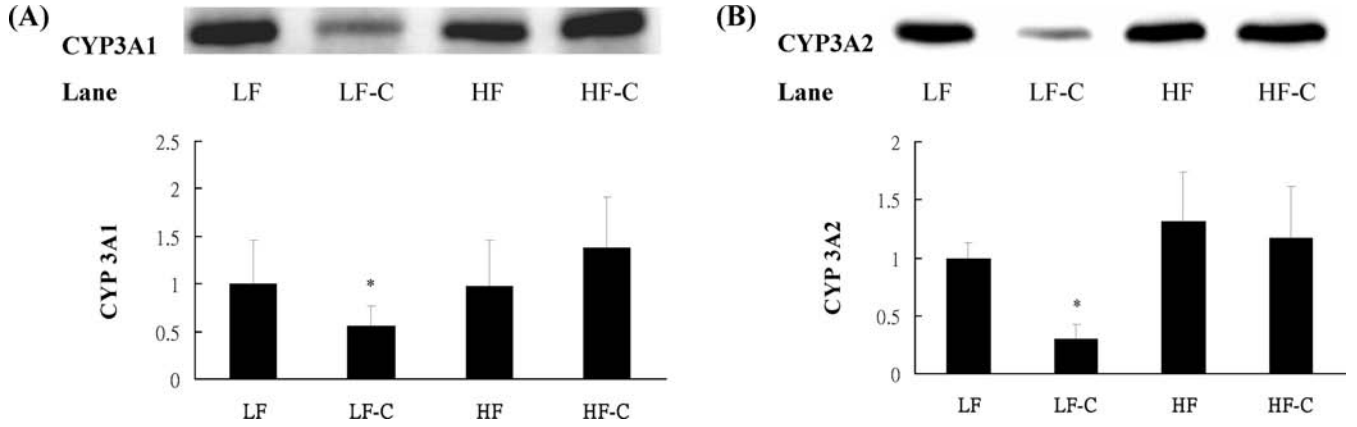

Figure 1. Expression of CYP 3A1 and 3A2 proteins in the liver of rats fed the low- and high-fat diets with chitosan or cellulose. The bolt was representative of the pooled average of individual samples in each group. The detailed experimental conditions are described under Materials and Methods. Values are means ± SD from six or seven animals. ∗, significantly different from the LF group at $p < 0.05$.

Table 4. Concentrations of Lipid Hydroperoxides, Glutathione, α-Tocopherol, and Antioxidative Enzyme Activities in Rat Liver[a]

	diet				*p* value (two-way ANOVA)		
	LF	LF-C	HF	HF-C	chitosan	fat	interaction
total LPO (nmol/g)	22.6 ± 3.0 a	26.1 ± 2.9 b	28.0 ± 3.6	30.1 ± 3.1	0.012	<0.001	ns
microsomal LPO (nmol/mg of protein)	6.3 ± 2.2 a	11.0 ± 3.8 b	13.0 ± 4.5	14.7 ± 3.7	0.042	0.002	ns
α-tocopherol (μg/g)	68.7 ± 13.5 a	29.9 ± 5.9 b	58.0 ± 10.3 a	22.7 ± 8.6 b	<0.001	ns	ns
GSH (μmol/g)	1.7 ± 0.5	1.5 ± 0.5	2.2 ± 0.6 a	1.5 ± 0.6 b	0.043	ns	ns
GSH-Px (NADPH nmol/min/mg)	46.4 ± 13.3	43.4 ± 7.9	42.2 ± 6.0	39.6 ± 10.2	ns	ns	ns
SOD (IU/mg)	2.9 ± 0.6 a	1.5 ± 0.6 b	2.1 ± 0.9	2.1 ± 0.9	0.046	ns	0.025

[a] Values are mean ± SD, $n = 7$. Rats were fed one of following diets for 4 weeks: low-fat diet (LF), high-fat diet (HF), low-fat diet plus chitosan (LF-C), high-fat diet plus chitosan (HF-C). Groups receiving the same levels of dietary fat diet not sharing the same letter are significantly different, $p < 0.05$. ns, not significantly different at $p > 0.05$. LPO, lipid hydroperoxides; GSH, glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase.

HF-C). With regard to the CYP activity, hepatic testosterone 6β-hydroxylase ($p < 0.001$) and ethoxyresorufin *O*-deethylase ($p = 0.046$) activities were significantly decreased by chitosan. The amounts of dietary fat had no effect on the activity of both testosterone 6β-hydroxylase and ethoxyresorufin *O*-deethylase. Higher methoxyresorufin *O*-demethylase activity was noted in rats fed the high-fat diet ($p = 0.041$). No differences in the activities of pentoxyresorufin *O*-dependentylase (CYP 2B), *p*-nitrophenol 6-hydroxylase (CYP2E1), and NADPH-CYP reductase were observed by dietary fat and chitosan. These results indicate that chitosan treatment with either the low- or the high-fat diet may reduce the metabolism of drugs catalyzed by CYP 3A and 1A1.

Change in glutathione *S*-transferase activity resulted from both chitosan ($p = 0.018$) and the levels of dietary fat ($p = 0.023$). Rats fed the low-fat or chitosan-based diet had lower activity of this phase II conjugation enzyme than those fed the high-fat or cellulose-based diet. UDP-glucurosyltransferase activity was not affected by chitosan and dietary fat.

Figure 1 shows the immunoblots of liver microsomal proteins of CYP 3A1 (**A**) and 3A2 (**B**). Rats in the LF-C group had lower CYP 3A1 (−44%) and CYP 3A2 (−70.2%) proteins than those in the LF group. This phenomenon was not observed between rats in the HF-C and HF groups.

Oxidative Stress Markers. The total liver and microsomal LPO levels were affected by both chitosan and dietary fat (**Table 4**). Rats fed the high-fat diet showed higher total liver ($p < 0.001$) and microsomal LPO ($p = 0.002$) levels than rats fed the low-fat diet. Increases of total liver ($p = 0.012$) and microsomal LPO ($p = 0.042$) were also noted in rats fed the chitosan. This increase of LPO production by chitosan was accompanied by a lower hepatic α-tocopherol ($p < 0.001$), GSH ($p = 0.043$), and SOD activity ($p = 0.046$). Moreover, decrease of SOD by chitosan was dependent on the amounts of dietary fat ($p = 0.025$), by which a significant decrease of SOD resulted only in rats fed the low-fat diet (LF vs LF-C). No change in GSH-Px activity was found in rats among the four test groups. HO-1, an antioxidative enzyme, is known to be inducible under oxidative stress. However, there was no difference in the expression of HO-1 protein and mRNA among the four groups (**Figure 2**).

Hepatic Lipid Levels. Table 5 shows the contents (mg/g) of lipids in the liver. The concentrations of triglycerides ($p < 0.001$) and cholesterol ($p < 0.001$) in rats fed the high-fat diet were higher than in the rats fed the low-fat diet. Chitosan decreased hepatic triglycerides level ($p = 0.006$), but had no effect on the content of cholesterol. It is interesting to note there is an interaction between chitosan and dietary fat on the triglycerides ($p < 0.001$) and cholesterol ($p < 0.001$) contents. Chitosan-decreased hepatic triglycerides and cholesterol contents were found only in rats fed the high-fat diet (HF-C vs HF, $p < 0.05$). With regard to the membrane phospholipids, chitosan but not dietary fat increased the concentrations of membrane-associated lipids ($p = 0.031$).

Fatty Acid Composition of Microsomal Lipids. To elucidate whether the changes in CYP activity and expression were related to microsomal fatty acid profile, the fatty acid composition of microsomal lipids was analyzed by gas chromatography (**Table 6**). As indicated, linoleic acid was the sole fatty acid in microsomal membranes affected by the amounts of dietary fat ($p = 0.009$). The percentage of linoleic acid was higher in rats fed the 20% soybean oil diet than in those fed the 5% soybean oil diet. Chitosan had no effect on fatty acid profile of microsomal lipids.

DISCUSSION

In this study, we evaluated for the first time the activities of drug-metabolizing enzymes and the oxidative stress in the liver of rats fed chitosan with low-fat (LF-C) and high-fat (HF-C) diets. Our data showed that chitosan reduced CYP 3A-catalyzed testosterone 6β-hydroxylation, CYP 1A1-catalyzed ethoxyresorufin O-deethylation, GST, and SOD activities and had lower α-tocopherol and GSH levels in rat livers, suggesting that chitosan administration may change hepatic drug metabolism and enhance oxidative stress.

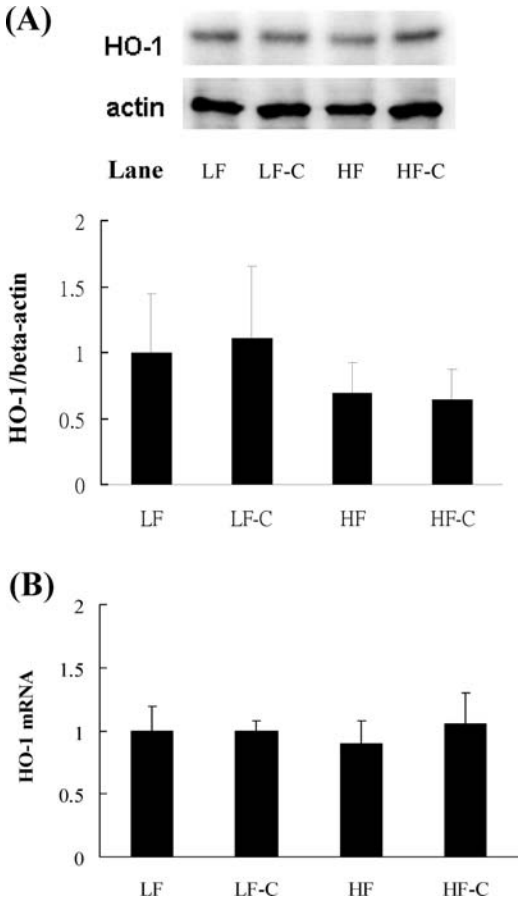

Figure 2. Expressions of HO-1 protein and mRNA in the liver of rats fed the low- and high-fat diets with chitosan or cellulose. The bolt was representative of the pooled average of individual samples in each group. The detailed experimental conditions are described under Materials and Methods. Values are means ± SD from six or seven animals.

Several studies indicated that the amount and kind of fatty acids in dietary fats may modulate certain CYP enzyme activities (*6*). Some CYP enzyme activities are shown to be depressed by diet deficit in linoleic acid (*31*). A diet enriched in polyunsaturated fatty acids (PUFAs) may enhance the drug-metabolizing enzyme system by increased proportion of PUFAs in the microsomal membrane, thus enhancing membrane fluidity and increasing hepatic CYP enzyme activity (*6*, *31*). In the present study, however, total CYP content and CYP-catalyzed enzyme activities, except for CYP 1A2-catalyzed methoxyresorufin O-demethylation ($p = 0.041$), were not changed by the amounts of dietary fat despite a higher linoleic acid proportion in microsomal lipids observed in the HF group than in the LF group. This finding was similar to the result of Norred and Wade (*32*), showing no difference in the liver microsomal CYP content and activities between rats fed the 3 and 10% corn oil diets. Our results suggested that the 5% soybean oil diet might provide sufficient linoleic acid to maintain CYP contents and activities in rat liver regardless of whether the diet was fed with cellulose or chitosan. Therefore, in this study, reduced dietary fat absorption by chitosan appeared not to be the major reason for the change of CYP enzyme activity.

It was noteworthy that, in this study, rats fed the chitosan-containing diet had significantly lower CYP 3A and 1A1 activities, but there were little or no differences on the activities of CYP 2E1, 1A2, and 2B and UGT enzymes. These results suggested that chitosan might reduce the biotransformation of xenobiotics that were catalyzed by CYP 3A and 1A1. However, the reduction of the activity of CYP 3A by chitosan (−32%, $p < 0.001$) is more significant than that of the CYP 1A1 (−19%, $p = 0.046$). These results indicated that CYP 3A, a major CYP enzyme in human liver (*10*), was likely to be the target enzyme modulated by chitosan. To investigate whether chitosan reduced CYP 3A through the reduction of PUFA proportion in microsomal membrane, the fatty acid composition in microsomal lipids was determined. Our results showed that the proportions of linoleic acid ($C_{18:2}$), arachidonic acid ($C_{20:4}$), and docosahexaenoic acid

Table 5. Hepatic Lipid Concentrations in Rats[a]

	diet				*p* value (two-way ANOVA)		
	LF	LF-C	HF	HF-C	chitosan	fat	interaction
triglycerides (mg/g)	26.6 ± 5.0	29.7 ± 5.8	50.0 ± 11.0 a	29.7 ± 5.6 b	0.006	<0.001	<0.001
cholesterol (mg/g)	5.7 ± 0.9 a	6.9 ± 1.1 b	8.7 ± 0.7 a	7.0 ± 0.8 b	ns	<0.001	<0.001
phospholipids (mg/g)	21.0 ± 1.8 a	24.2 ± 2.5 b	23.3 ± 2.4	24.5 ± 3.0	0.031	ns	ns

[a] Values are mean ± SD, $n = 7$. Rats were fed one of following diets for 4 weeks: low-fat diet (LF), high-fat diet (HF), low-fat diet plus chitosan (LF-C), high-fat diet plus chitosan (HF-C). Groups receiving the same levels of dietary fat diet not sharing the same letter are significantly different, $p < 0.05$. ns, not significantly different at $p > 0.05$.

Table 6. Fatty Acid Compositions of Liver Microsomal Lipids of Rats Fed the Low- and High-Fat Diets with Chitosan or Cellulose for 4 Weeks[a]

	diet				*p* value (two-way ANOVA)		
	LF	LF-C	HF	HF-C	chitosan	fat	interaction
16:0 (palmitic acid) (%)	23.9 ± 6.2	24.5 ± 3.2	20.8 ± 1.1 a	17.7 ± 1.2 b	ns	ns	ns
18:0 (stearic acid) (%)	21.8 ± 1.8	21.5 ± 2.9	22.1 ± 3.3	25.7 ± 4.3	ns	ns	ns
18:1 (n-9) (oleic acid) (%)	8.7 ± 3.2	11.4 ± 3.8	9.7 ± 3.0	8.8 ± 2.4	ns	ns	ns
18:2 (n-6) (linoleic acid) (%)	13.0 ± 2.0	15.0 ± 1.4	18.8 ± 3.2	18.9 ± 0.5	ns	0.009	ns
20:4 (n-6) (arachidonic acid) (%)	27.0 ± 3.9	23.0 ± 4.0	23.9 ± 5.7	24.9 ± 1.8	ns	ns	ns
22:6 (n-3) (docosahexaenoic acid) (%)	5.6 ± 1.9	4.5 ± 2.0	4.7 ± 0.6	4.0 ± 0.7	ns	ns	ns

[a] Values are mean ± SD, $n = 7$. Rats were fed one of following diets for 4 weeks: low-fat diet (LF), high-fat diet (HF), low-fat diet plus chitosan (LF-C), high-fat diet plus chitosan (HF-C). Groups receiving the same levels of dietary fat diet not sharing the same letter are significantly different, $p < 0.05$. ns, not significantly different at $p > 0.05$.

($C_{22:6}$) in the liver microsomal lipids of rats were not changed by chitosan treatment. These results suggest that the decrease of CYP 3A activity in rats fed the diet with chitosan might not be result from changing microsomal fatty acid composition, which, in turn, changed the membrane fluidity. Fat-soluble vitamins including vitamins A, D, and E were shown to play an important role in modulating CYP 3A expression (*7*, *8*). In the case of vitamin E, it was demonstrated that a vitamin E-deficient diet reduced hepatic drug metabolism (*33*) and that α-tocopherol concentration in the liver is positively correlated to CYP 3A level (*34*). Chitosan was shown to reduce hepatic fat-soluble vitamins, especially α-tocopherol, presumably by reducing fat absorption (*13*). In the present study, a lower hepatic α-tocopherol level was noted in rats fed the chitosan-containing diet. This observation is consistent with the observation that rats fed the chitosan-containing diet had lower activity of testosterone 6*β*-hydroxylase, a marker of the CYP 3A enzyme (**Table 3**). Immunoblots revealed that rats fed the LF-C diet had reduced expressions of CYP 3A1 and 3A2 proteins compared with rats fed the LF diet. However, immunoblots did not reveal any difference in CYP 3A1 and 3A2 protein expressions between rats fed the HF-C and HF diets. The reason for this discrepancy is unknown. Rowe and Wills (*35*) indicated that linoleic acid and vitamin E are the essential components in endoplasmic reticulum for the oxidative metabolism of aminopyridine in rat liver. Our results show that rats fed the diet with chitosan decreased hepatic α-tocopherol which may, in turn, reduce the α-tocopherol in microsomal membranes and, thus, result in a lower metabolism of drugs catalyzed by CYP 3A.

To investigate the role of chitosan on the oxidative stress in liver, in addition to measuring the α-tocopherol level, other parameters including antioxidative enzyme activities and GSH and LPO levels in the liver were also determined. In this study, the amounts of dietary fat did not affect enzymatic and nonenzymatic antioxidants such as α-tocopherol, GSH, GSH-Px, SOD, and GST. However, chitosan treatment significantly decreased α-tocopherol and GSH levels and SOD activity. Accompanied by the decrease of antioxidants, liver total LPO and microsomal LPO levels were increased by chitosan. Moreover, this negative association between antioxidants and LPO production was more apparent in rats fed the low-fat diet. Our results show that rats fed the LF-C diet had significantly higher total and microsomal LPO and lower hepatic α-tocopherol level and lower activities of SOD (**Table 4**) and GST (**Table 3**) than rats fed the LF diet. SOD is an important defense enzyme that catalyzes the dismutation of superoxide radicals (*36*). GST not only acts as a phase II detoxifying enzyme but also catalyzes the reduction of organic hydroperoxides to nontoxic products (*37*). It is known that lipid peroxidation increases when free radical production overwhelms the total antioxidant defense. In this study, chitosan, however, increased LPO production to a lesser extent in rats fed the high-fat diet than in those animals fed the low-fat diet. This is consistent with the findings that rats fed the chitosan with the high-fat diet had significantly decreased α-tocopherol and GSH levels only, but minor effects on SOD and GST activities. Taken together, it is reasonable to speculate that the higher total and microsomal LPO levels observed in the liver of rats fed the chitosan can be partly attributed to the lower antioxidant defense capacity.

It was noteworthy that the contents of microsomal CYP and cytochrome *b*5 were decreased by chitosan in rats fed the high-fat diet (HF-C vs HF) (**Table 3**). CYP enzymes, especially CYP 3A, 2B, and 2E1, are known to utilize molecular oxygen to oxidize their substrates and comprise an important source of ROS formation in the liver cells, which may increase lipid peroxidation (*3*). Therefore, despite lower hepatic α-tocopherol level, the increment of CYP enzyme-mediated ROS production might be reduced by chitosan, as evidenced by no significant change in LPO level (HF-C vs HF) (**Table 4**). Certain CYP enzymes are known to be susceptible to damage by microsomal lipid peroxidation. For instance, loss of CYP 3A function is more significant than that of other CYP enzymes (*38*). In this study, although rats fed the high-fat diet had increased microsomal LPO levels, it did not affect CYP 3A activity (HF vs LF). A similar extent of increase in microsomal LPO level was noted between rats fed the low-fat diet with cellulose and chitosan (LF vs LF-C). Therefore, our results suggest that the extent of increase in microsomal LPO level in rats fed the chitosan-containing diet might not reach a level high enough to damage the CYP 3A protein. It is thus suggested that reduced CYP 3A activity by chitosan might result from a lower hepatic α-tocopherol level but not an increase in lipid peroxidation. Because chitosan acts as a dietary fiber in the gastrointestinal tract, the modulations on drug-metabolizing enzymes and oxidative stress by chitosan may possibly have originated from the reduction of dietary fat and fat-soluble vitamins absorption in the small intestine.

HO-1 is an inducible isoform in response to oxidative damage. It can be induced by products of lipid peroxide, and the induction of HO-1 is an adaptive response against oxidative damage (*39*). To evaluate whether the extent of increased lipid peroxidation by chitosan in rats fed the low-fat diet can induce HO-1 expressions, HO-1 protein and mRNA expressions in liver were determined. In the present study, no significant difference in HO-1 protein and mRNA expression in liver was observed among the four test groups. Malaguarnera et al. (*39*) indicated that the elevation of lipid peroxidation correlated with HO-1 induction in the liver is observed only in patients with steatosis but not in normal subjects. Thus, it appears that the extent of increased hepatic lipid peroxidation by chitosan is mild and seems unlikely to produce a significant degree of oxidative damage in liver.

Chitosan feeding has been shown to lower hepatic lipids in animals (*12*, *14*). In the present study, lower hepatic triglyceride and cholesterol levels were observed only in the liver of rats fed the chitosan with high-fat diets (HF-C vs HF). In contrast, in rats fed the low-fat diet, the cholesterol level in rat liver was increased by chitosan. There are two possible explanations for this observation: (1) Reduced fat digestibility by chitosan is dependent on the dietary fat content. In the high-fat diet, chitosan decrease of fat absorption is more significant than that in the low-fat diet. (2) The decrease of fat absorption caused by chitosan may trigger hepatic lipogenesis by increasing the activities of fatty acid synthetase and glucose-6-phosphate dehydrogenase (*14*, *40*), and this compensation reaction is more prominent in animals fed the low-fat diet than in those fed the high-fat diet. Therefore, the reduction in fat absorption by chitosan may be compensated by the increase of fatty acid synthesis in the liver of rats given the low-fat diet, resulting in no change of hepatic triglyceride level.

In summary, the present study demonstrates that chitosan is likely to modulate CYP 3A activity and protein expression and GST activity partially in a dietary fat-dependent manner. This change may cause a decrease in the metabolism of drugs catalyzed by these enzymes in liver tissues. Moreover, decreases of α-tocopherol level and SOD activity by chitosan partly account for the increase of hepatic lipid peroxidation. Because CY3A is the most important isoform related to the oxidative biotransformation of numerous medicines in humans, further studies will be needed to investigate their possible interactions.

ABBREVIATIONS USED

CYP, cytochrome P450; UGT, UDP-glucuronosyl transferase; GST, glutathione-*S*-transferase; LPO, lipid hydroperoxides; GSH, reduced glutathione; GSH-Px, glutathione peroxidase;

SOD, superoxide dismutase; SDS, sodium dodecyl sulfate; HO-1, heme oxygenase-1; ROS, reactive oxygen species.

LITERATURE CITED

(1) Nebert, D. W. Proposed role of drug-metabolizing enzymes: regulation of steady state levels of the ligands that effect growth, homeostasis, differentiation, and neuroendocrine functions. *Mol. Endocrinol.* **1991**, *5*, 1203–1214.

(2) Gonzalez, F. J.; Gelboin, H. V. Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metab. Rev.* **1994**, *26*, 165–183.

(3) Serron, S. C.; Dwivedi, N.; Backes, W. L. Ethylbenzene induces microsomal oxygen free radical generation: antibody-directed characterization of the responsible cytochrome P450 enzymes. *Toxicol. Appl. Pharmacol.* **2000**, *164*, 305–311.

(4) Krajka-Kuźniak, V. Induction of phase II enzymes as a strategy in the chemoprevention of cancer and other degenerative diseases. *Postepy Hig. Med. Dosw.* **2007**, *61*, 627–638.

(5) Dannenberg, A. J.; Yang, E. K.; Aharon, D. Dietary lipids induce phase 2 enzymes in rat small intestine. *Biochim. Biophys. Acta* **1993**, *1210*, 8–12.

(6) Chen, H. W.; Tsai, C. W.; Yang, J. J.; Liu, C. T.; Kuo, W. W.; Lii, C. K. The combined effects of garlic oil and fish oil on the hepatic antioxidant and drug-metabolizing enzymes of rats. *Br. J. Nutr.* **2003**, *89*, 189–200.

(7) Traber, M. G. Vitamin E, nuclear receptors and xenobiotic metabolism. *Arch. Biochem. Biophys.* **2004**, *423*, 6–11.

(8) Wang, K.; Chen, S.; Xie, W.; Wan, Y. J. Retinoids induce cytochrome P450 3A4 through RXR/VDR-mediated pathway. *Biochem. Pharmacol.* **2008**, *75*, 2204–2213.

(9) Guengerich, F. P. Role of cytochrome P450 enzymes in drug-drug interactions. *Adv. Pharmacol.* **1997**, *43*, 7–35.

(10) Fujita, K. Food-drug interactions via human cytochrome P450 3A (CYP3A). *Drug Metab. Drug Interact.* **2004**, *20*, 195–217.

(11) Gurley, B. J.; Gardner, S. F.; Hubbard, M. A.; Williams, D. K.; Gentry, W. B.; Cui, Y.; Ang, C. Y. Clinical assessment of effects of botanical supplementation on cytochrome P450 phenotypes in the elderly: St John's wort, garlic oil, *Panax ginseng* and *Ginkgo biloba*. *Drugs Aging* **2005**, *22*, 525–539.

(12) Yao, H. T.; Huang, S. Y.; Chiang, M. T. A comparative study on hypoglycemic and hypocholesterolemic effects of high and low molecular weight chitosan in streptozotocin-induced diabetic rats. *Food Chem. Toxicol.* **2008**, *46*, 1525–1534.

(13) Deuchi, K.; Kanauchi, O.; Shizukuishi, M.; Kobayashi, E. Continuous and massive intake of chitosan affects mineral and fat-soluble vitamin status in rats fed on a high-fat diet. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1211–1216.

(14) Chiang, M. T.; Yao, H. T.; Chen, H. C. Effect of dietary chitosans with different viscosity on plasma lipids and lipid peroxidation in rats fed on a diet enriched with cholesterol. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 965–971.

(15) Hossain, S.; Rahman, A.; Kabir, Y.; Shams, A. A.; Afros, F.; Hashimoto, M. Effects of shrimp (*Macrobracium rosenbergii*)-derived chitosan on plasma lipid profile and liver lipid peroxide levels in normo- and hypercholesterolaemic rats. *Clin. Exp. Pharmacol. Physiol.* **2007**, *34*, 170–176.

(16) Jeon, T. I.; Hwang, S. G.; Park, N. G.; Jung, Y. R.; Shin, S. I.; Choi, S. D.; Park, D. K. Antioxidative effect of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. *Toxicology* **2003**, *187*, 67–73.

(17) Santhosh, S.; Sini, T. K.; Anandan, R.; Mathew, P. T. Effect of chitosan supplementation on antitubercular drugs-induced hepatotoxicity in rats. *Toxicology* **2006**, *219*, 53–59.

(18) Chen, H. W.; Lii, C. K.; Sung, W. C.; Ko, Y. J. Effect of vitamin E on rat hepatic cytochrome P-450 activity. *Nutr Cancer*. **1998**, *31*, 178–183.

(19) Omura, T.; Sato, R. The carbon monoxide-binding pigment of liver microsomes. l. Evidence for its hemeprotein nature. *J. Biol. Chem.* **1964**, *239*, 2370–2379.

(20) Phillips, A. H.; Langdon, R. G. Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: isolation, characterization, and kinetic studies. *J. Biol. Chem.* **1962**, *237*, 2652–2660.

(21) Yao, H. T.; Chang, Y. W.; Lan, S. J.; Yeh, T. K. The inhibitory effect of tannic acid on cytochrome P450 enzymes and NADPH-CYP reductase in rat and human liver microsomes. *Food Chem. Toxicol.* **2008**, *46*, 645–653.

(22) D'Andrea, V.; Pérez, L. M.; Sánchez Pozzi, E. J. Inhibition of rat liver UDP-glucuronosyltransferase by silymarin and the metabolite silibinin-glucuronide. *Life Sci.* **2005**, *77*, 683–692.

(23) Habig, W. H.; Jakoby, W. B. Assays for differentiation of glutathione S-transferases. *Methods Enzymol.* **1981**, *77*, 398–405.

(24) Yao, H. T.; Lin, P.; Chang, Y. W.; Chen, C. T.; Chiang, M. T.; Chang, L.; Kuo, Y. C.; Tsai, H. T.; Yeh, T. K. Effect of taurine supplementation on cytochrome P450 2E1 and oxidative stress in the liver and kidneys of rats with streptozotocin-induced diabetes. *Food Chem. Toxicol.* **2009**, *47*, 1703–1709.

(25) Mohandas, J.; Marshall, J. J.; Duggin, G. G.; Horvath, J. S.; Tiller, D. J. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. *Cancer Res.* **1984**, *44*, 5086–5091.

(26) Marklund, S.; Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **1974**, *47*, 469–474.

(27) Folch, J.; Lees, M.; Sloane-Stanley, G. M. A purification of total lipid from animal tissue. *J. Biol. Chem.* **1957**, *226*, 497–509.

(28) Carlson, E.; Goldford, S. A sensitive enzymatic method for determination of free and esterified tissue cholesterol. *Clin. Chim. Acta* **1979**, *79*, 575–582.

(29) Shih, C. K.; Chang, J. H.; Yang, S. H.; Chou, T. W.; Cheng, H. H. β-Carotene and canthaxanthin alter the pro-oxidation and antioxidation balance in rats fed a high-cholesterol and high-fat diet. *Br. J. Nutr.* **2008**, *99*, 59–66.

(30) Huang, S. Y.; Yang, H. T.; Chiu, C. C.; Pariante, C. M.; Su, K. P. Omega-3 fatty acids on the forced-swimming test. *J. Psychiatr. Res.* **2008**, *42*, 58–63.

(31) Hietanen, E.; Laitinen, M.; Vainio, H.; Hanninen, O. Dietary fats and properties of endoplasmic reticulum. II. Dietary lipid induced changes in activities of drug metabolizing enzymes in liver and duodenum of rat. *Lipids* **1975**, *10*, 467–472.

(32) Norred, W. P.; Wade, A. E. Dietary fatty acid-induced alterations of hepatic microsomal drug metabolism. *Biochem. Pharmacol.* **1972**, *21*, 2887–2897.

(33) Zannoni, V. G.; Sato, P. H. The effect of certain vitamin deficiencies on hepatic drug metabolism. *Fed. Proc.* **1976**, *35*, 2464–2469.

(34) Traber, M. G.; Siddens, L. K.; Leonard, S. W.; Schock, B.; Gohil, K.; Krueger, S. K.; Cross, C. E.; Williams, D. E. α-Tocopherol modulates Cyp3a expression, increases γ-CEHC production, and limits tissue γ-tocopherol accumulation in mice fed high γ-tocopherol diets. *Free Radical Biol. Med.* **2005**, *38*, 773–785.

(35) Rowe, L.; Wills, E. D. The effect of dietary lipids and vitamin E on lipid peroxide formation, cytochrome P-450 and oxidative demethylation in the endoplasmic reticulum. *Biochem. Pharmacol.* **1976**, *25*, 175–179.

(36) Afonso, V.; Champy, R.; Mitrovic, D.; Collin, P.; Lomri, A. Reactive oxygen species and superoxide dismutases: role in joint diseases. *Jt. Bone Spine* **2007**, *74*, 324–329.

(37) Hurst, R.; Bao, Y.; Jemth, P.; Mannervik, B.; Williamson, G. Phospholipid hydroperoxide glutathione peroxidase activity of human glutathione transferases. *Biochem. J.* **1998**, *332*, 97–100.

(38) Kitada, M.; Komori, M.; Ohi, H.; Imaoka, S.; Funae, Y.; Kamataki, T. Form-specific degradation of cytochrome P-450 by lipid peroxidation in rat liver microsomes. *Res. Commun. Chem. Pathol. Pharmacol.* **1989**, *63*, 175–188.

(39) Malaguarnera, L.; Madeddu, R.; Palio, E.; Arena, N.; Malaguarnera, M. Heme oxygenase-1 levels and oxidative stress-related parameters in non-alcoholic fatty liver disease patients. *J. Hepatol.* **2005**, *42*, 585–591.

(40) Yao, H. T.; Chiang, M. T. Plasma lipoprotein cholesterol in rats fed a diet enriched in chitosan and cholesterol. *J. Nutr. Sci. Vitaminol.* **2002**, *48*, 379–383.

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