British Journal of Nutrition (2003), **89**, 189–200
DOI: 10.1079/BJN2002766
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https://doi.org/10.1079/BJN2002766 Published online by Cambridge University Press

The combined effects of garlic oil and fish oil on the hepatic antioxidant and drug-metabolizing enzymes of rats

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(Received 8 November 2001 – Revised 30 August 2002 – Accepted 22 September 2002)

This present study was designed to investigate the combined modulatory effect of garlic oil (GO) and fish oil (FO) on the antioxidant and drug metabolism systems. Rats were fed either a low-maize oil (MO) diet (50 g MO/kg), high-MO diet (235 g MO/kg) or high-FO diet (205 g FO + 30 g MO/kg) and received different doses of GO (0–200 mg/kg body weight) three times per week for 6 weeks. Fatty acid analysis showed that 20 : 5*n*-3 and 22 : 6*n*-3 were incorporated into serum lipid at the expense of 18 : 2*n*-6 and 20 : 4*n*-6 in rats fed the high-FO diet. GO dose-dependently increased hepatic glutathione *S*-transferase (GST), glutathione reductase, superoxide dismutase (SOD) and ethoxyresorufin *O*-deethylase (EROD) activities, but decreased glutathione peroxidase and *N*-nitrosodimethylamine demethylase (NDMAD) activities ($P<0{\cdot}05$). With the exception of glutathione peroxidase, the activities of glutathione reductase, SOD, GST, EROD and NDMAD were modulated by the dietary fat. The high-FO group had greater SOD and EROD activity than either MO-fed group; it also had greater NDMAD activity than the low-MO group ($P<0{\cdot}05$). GST activity was higher in rats fed high-FO or high-MO diets than rats fed the low-MO diet. Change in erythromycin demethylase activity, however, was not caused by either dietary fat or GO. Immunoblot assay showed that GO dose-dependently enhanced the protein level of the Ya, Yb1, Yc isoenzymes of GST and cytochrome P450 (CYP) 1A1 and 3A1, but GO suppressed CYP2E1 expression. Regardless of the dosage of GO, the high-FO diet increased CYP1A1, CYP3A1 and CYP2E1 levels compared with the high- and low-MO diets. Accompanying the changes observed in immunoblots, CYP1A1 and CYP3A1 mRNA levels were increased by GO in a dose-dependent manner and also increased additively in combination with FO feeding. These present results indicate that co-administration of GO and FO modulates the antioxidant and drug-metabolizing capacity of animals and that the effect of GO and FO on drug-metabolizing enzymes is additive.

Fish oil: Garlic oil: Antioxidant enzymes: Cytochrome P450

Drug metabolism systems are composed of phase I and II enzymes. Phase I enzymes, mainly cytochrome P450 (CYP), detoxify a variety of endogenous and exogenous chemicals and activate many carcinogens (Guengerich, 1991). Phase II enzyme systems catalyse the conjugation of phase I metabolites to various water-soluble molecules, such as glutathione (GSH) or glucuronic acid, which accelerates metabolite excretion rate. Glutathione *S*-transferase (GST) is one of the most important phase II enzymes. GST not only conjugates GSH to various electrophilic compounds, but also acts as a Se-independent GSH peroxidase. Oxidative stress of endogenous and exogenous origins is known to be involved in cancer formation and in many of the diseases associated with ageing, such as cardiovascular disease, stroke, arthritis and cataract (Cross *et al.* 1987). An effective defence mechanism is, thus, important to the health of animals and man (Machlin & Bendich, 1987). The inherent antioxidant defence system is composed of antioxidants, such as vitamin E and vitamin C, and antioxidant enzymes, such as GSH peroxidase, catalase and superoxide dismutase (SOD). SOD is involved in quenching the superoxide anion and in the formation of H_2O_2, and H_2O_2 is then decomposed to H_2O by catalase and GSH peroxidase. Because of the

Abbreviations: CYP, cytochrome P450; EROD, ethoxyresorufin *O*-deethylase; GO, garlic oil; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; MO, maize oil; FO, fish oil; NDMAD, *N*-nitrosodimethylamine demethylase; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

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https://doi.org/10.1079/BJN2002766 Published online by Cambridge University Press

important role of antioxidant and drug-metabolizing enzymes against oxidative stress and chemical-induced toxicity, any factors modulating the activity or expression of these enzymes may lead to altered susceptibility of animals to environmental injury.

In recent years, there has been an increasing emphasis on foods and food components in disease prevention. Garlic and its related compounds have attracted a great deal of attention. The significant effects of garlic on disease prevention are in the fields of cardiovascular disease and cancer (Agarwal, 1996; Rahman, 2001). Recently, Fleischhauer & Arab (2001) have summarized the epidemiological evidence and indicated that the consumption of garlic was inversely correlated with the incidence of stomach and colorectal cancer; however, the effect of garlic on lung, breast and prostate cancer is inconsistent and needs further studies. Among various garlic components, the organo-S compounds are considered the most potent in chemoprevention of cancer, and their preventive capabilities are related to their modulation of drug-metabolizing enzymes involved in the activation or detoxification of carcinogens (Yang *et al.* 1994). In an animal study, four garlic-derived organo-S compunds, i.e. allyl methyl trisulfide, allyl methyl disulfide, diallyl trisulfide and diallyl sulfide, were found to inhibit benzo(a)pyrene-induced forestomach neoplasia and were accompanied by an increase in GST activity in the forestomach (Sparnins *et al.* 1988). Garlic components are also effective in modulating GSH peroxidase, GSH reductase and SOD activities (Iqbal & Athar, 1998; Sheen *et al.* 1999) and in inhibiting lipoprotein oxidation (Phelps & Harris, 1993).

Interest in *n*-3 fatty acids arose 30 years ago when studies showed that intake of *n*-3 polyunsaturated fatty acids in marine fish oils (FO) was associated with a low incidence of cardiovascular disease in Greenland Eskimos (Dyerberg *et al.* 1975; Bang *et al.* 1980). In addition to their well-known cholesterol- and triacylglycerol-lowering effects, the following effects of *n*-3 polyunsaturated fatty acids are recognized to be associated with their antiatherosclerotic actions: modulation of thromboxane A_2 and prostacyclin production (Goodnight *et al.* 1982); inhibition of monocyte migration into the plaque, reduction in cytokine and interleukin 1α production; stimulation of endothelial NO production (Shimokawa & Vanhoutte, 1989); reduction in platelet-derived growth factor (Fox & DiCorleto, 1988). However, there is growing concern that high content of polyunsaturated fatty acids increases lipid peroxidation and free-radical production and thus increases the risk of carcinogenesis (Fang *et al.* 1996). To compensate for the damage of oxidative stress, *n*-3 polyunsaturated fatty acids have also been shown to enhance GSH peroxidase, GSH reductase and SOD activities in rat livers (Ruiz-Gutiérrez *et al.* 1999).

FO and garlic-derived products are popular health supplements. However, few studies have investigated how garlic components interact with dietary lipids to modulate the physiological and biological actions. In a recent study, we have reported that co-administration of garlic oil (GO) and FO additively increased both CYP2B1 and the placental form of GST protein and mRNA expression (Chen *et al.* 2001). This finding implies the possible cross-reaction of these two health supplements in their physiological activities. This has also been demonstrated by the anti-hyperlipidaemic and anti-hypercholesterolaemic effect of co-administration of GO and FO (Alder & Holub, 1997; Morcos, 1997). Based on this evidence, it is of interest to examine the effects of FO on the antioxidant and drug-metabolizing system, and whether these effects are modulated by garlic components.

In this present study, male Sprague-Dawley rats were fed three diets varying in the amount and type of fat, and given oral administrations of 0–200 mg GO/kg body weight three times per week, to investigate the individual and combined effects of dietary lipid and GO on antioxidant and drug-metabolizing enzymes.

Materials and methods

Materials

GO was prepared with a steam distillation technique, as previously described (Chen *et al.* 2001). 2,4-Chloro-dinitrobenzene, GSH, oxidized GSH (GSH disulfide, GSSG), ethoxyresorufin, erythromycin, *N*-nitrosodimethylamine and other biochemical reagents were purchased from Sigma Chemical (St Louis, MO, USA). Antibodies against GST Ya, Yb1 and Yc isoenzymes were purchased from Biotrin (Dublin, Republic of Ireland). Anti-CYP1A1, CYP2E1 and CYP3A1 polyclonal antibodies were purchased from Oxford Biomedical Research (Oxford, MI, USA).

Animals and treatments

Male Sprague-Dawley rats (4 weeks old) were purchased from the National Animal Breeding and Research Centre (Taipei, Taiwan). After 1 week of acclimatization, rats were randomly assigned to each experimental group by weight and housed in plastic cages with a 12 h light–dark cycle. Rats were fed a diet containing 50 g maize oil (MO)/kg (low MO), 235 g MO/kg (high MO) or 205 g FO + 30 g MO/kg (high FO) (Table 1) as described by

Table 1. The composition of experimental diets

Ingredients* (g/kg)	Low MO	High MO	High FO
Casein	20·0	23·5	23·5
Sucrose	49·5	25·63	25·63
Maize starch	15·0	15·0	15·0
MO†	5·0	23·5	3·0
FO†‡	—	—	20·5
Cellulose	5·0	5·9	5·9
AIN76 mineral mix	3·5	4·11	4·11
AIN76 vitamin mix	1·0	1·18	1·18
Methionine	0·3	0·35	0·35
Choline bitartrate	0·2	0·24	0·24
Cholesterol	0·5	0·59	0·59

MO, maize oil; FO, fish oil.
* All diet ingredients, except FO, were purchased from Harlan Teklad (Madison, MI, USA).
† Vitamin E content of the FO and MO was 490 and 170 mg α-tocopherol equivalents/kg respectively.
‡ FO was purchased from Tama Biochemical Co. (Tokyo, Japan).

Reddy & Sugie (1988) for 6 weeks. The composition of all experimental diets was adjusted so that rats in all dietary groups would receive the same dosage of vitamins, minerals and fibre (Reddy & Sugie, 1988). During the entire experiment, rats were administered 0, 30, 80 and 200 mg GO/kg body weight (MO as a vehicle) by oral intubation three times per week. Rats were allowed free access to water and diet, and treated in compliance with National Research Council (1985) guidelines. Body weight was measured weekly.

After 6 weeks, rats were fasted overnight and killed with CO_2. The livers, spleens, hearts and jugular lymph nodes were removed and weighed immediately. Fresh livers were used for microsome and cytosol preparations or quickly freeze-clamped in liquid N_2 and stored at −80°C. For cytosol and microsome preparations, livers were homogenized in 4 vol. buffer (pH 7·4, containing 10 mM-potassium phosphate and KCl (11·5 g/l)), and centrifuged at 10 000 ***g*** for 30 min at 4°C. The resultant supernatant fraction was further ultracentrifuged at 105 000 ***g*** for 1 h, and the final cytosolic and microsomal fractions were stored at −80°C until analysis.

Serum fatty acid and liver fat analysis

Serum fatty acid analysis was performed by the method described by Lepage & Roy (1986). Fatty-acid methyl esters were quantified by GC (Hitachi G3000; Hitachi, Tokyo, Japan). A 30 m fused silica column with an internal diameter of 0·25 mm (Supelco, Bellefonte, PA, USA) was used. The column was wall-coated with 0·2 mm SP-2330 (Supelco). Peaks were identified by comparing the retention times with those of authentic fatty-acid methyl ester standards (Sigma). Peak areas of each individual fatty acid were determined and the percentages calculated on the basis of total peak areas of all detectable fatty acids. The total lipid content of rat livers was determined as described by Folch *et al.* (1957).

Enzyme activity assays

Hepatic GST activity was assayed by the method of Habig *et al.* (1974), with 2,4-choloro-dinitrobenzene as the substrate. GSH peroxidase activity was determined spectrophotometrically with a coupled procedure using H_2O_2 as the substrate (Lawrence & Burk, 1976). GSH reductase activity was measured by the method of Bellomo *et al.* (1987). SOD activity was determined with the Randox SOD kit according to the manufacturer's instructions (Randox Laboratories Ltd, Antrim, UK) and was calculated by the degree of inhibition of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride reaction with superoxide anion generated by xanthine–xanthine oxidase. One unit SOD activity is defined as the amount of enzyme required to inhibit the rate of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride oxidation by 50 %. *N*-nitrosodimethylamine demethylase (NDMAD) and erythromycin demethylase activities were determined by the method of Nash (1953). Briefly, 0·35 ml microsomal protein (1 mg/ml) was mixed with 0·05 ml 10 mM-$MgCl_2$ and either 0·05 ml 50 mM-*N*-nitrodimethylamine or 0·05 ml 10 mM-erythromycin, then 0·05 ml 10 mM-NADPH was added to start the reaction. The reaction mixture was incubated at 37°C for 10 min. The reaction was stopped by adding 0·1 ml $ZnSO_4$ (50 g/l) and 0·1 ml saturated $Ba(OH)_2$ solutions. Ethoxyresorufin *O*-deethylase (EROD) activity was measured with a fluorescence spectrophotometer (F4500; Hitachi) as previously described (Poul & Fouts, 1980).

Glutathione S*-transferase, α-tocopherol and lipid peroxidation assays*

Fresh liver tissues were used to measure GSH redox status and α-tocopherol levels. Hepatic GSH and GSSG concentrations were determined by HPLC as described by Reed *et al.* (1980). α-Tocopherol was measured by means of a modification of the procedure previously described (Catignani & Bieri, 1983). Lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances (TBARS) as described by Fraga *et al.* (1988). Fresh livers were homogenized in 14 vol. 10 mM-potassium phosphate buffer, pH 7·4. Cell homogenates were then used for determination of the endogenous TBARS or incubated with an exogenous oxidant, i.e. 50 μM-$FeSO_4$, at 37°C for 30 min to induce lipid peroxidation. Butylated hydroxytoluene was added to terminate lipid peroxidation. The fluorescence of samples was detected at an excitation wavelength of 515 nm and an emission wavelength of 555 nm in a F4500 fluorescence spectrophotometer (Hitachi). 1,1,3,3-Tetramethoxypropane was used as a malondialdehyde precursor and a TBARS standard.

Immunoblot assay

Protein levels in liver cytosols and microsomes were determined by the method of Lowry *et al.* (1951). Equal amounts of liver cytosolic and microsomal protein of each animal in each group were pooled and then were subjected to SDS–polyacrylamide gel electrophoresis (Laemmli, 1970). Following electrophoresis, proteins separated on gels were transferred to polyvinylidiene difluoride membranes. The membranes were incubated with non-fat dried milk (50 g/l in 15 mM-Tris–150 mM-NaCl buffer, pH 7·4) at 4°C overnight, to block non-specific binding. Then, membranes were incubated with CYP1A1 or CYP2E1 or CYP3A1 antibody at 37°C for 1 h followed by peroxidase-labelled goat anti-rabbit immunoglobulin G. For GST isoenzyme detection, membranes were co-incubated with GST Ya, Yb1 and Yc antibodies. H_2O_2 and tetrahydrochloride diaminbenzidine were used for colour development.

Northern blot analysis

Total RNA was extracted by homogenizing 50 mg fresh liver tissues in 1 ml Trizol reagent (Life Technologies Inc., Grand Island, NY, USA) using a Potter-Elvehjem homogenizer (Glas-Col Co., Texxe Haute, IN, USA). For Northern blot analysis, two pairs of oligonucleotide primers were designed based on the published sequences of

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CYP1A1 (forward 5′-CTGCCTTGGATTCTGGGTGGTT-3′, backward 5′-CAAAGGATGAATGTCGGA-AGGT-3′) and CYP3A1 (forward 5′-TTGCCATCACGGACACAG-AAAT-3′, backward 5′-ATGCTGC-CCTTGTTCTCC-TTGC-3′). Reverse transcription–polymerase chain reaction was performed as previously described (Chen *et al.* 2001). The DNA fragment of CYP1A1 and CYP3A1 were labelled with α-deoxy-cytidine 5′-[^{32}P]triphosphate and used as probes for Northern blot analysis. From each sample, 20 μg RNA was electrophoretically separated by agarose (10 g/l) gel containing formaldehyde (60 ml/l) and transferred to HyBond membrane (Amersham Pharmacia Biotech, Arlington Heights, IL, USA). For hybridization with cDNA, the membrane was prehybridized for 2 h at 42°C in a solution containing Denhardt's reagent (g/l: Ficoll 20, polyvinylpyrolidone 20, bovine serum albumin 20), sodium chloride-sodium phosphate-EDTA buffer (750 mM-NaCl, 50 mM-NaH_2PO_4, 5 mM-EDTA), SDS (20 g/l), formamide (500 g/l), and 100 μg single-strand sheared salmon sperm DNA/ml. The membrane was then hybridized in the same solution with ^{32}P-labelled CYP1A1 cDNA probe at 42°C overnight. After washing, autoradiography was performed by exposing the membrane to Kodak SuperRx x-ray film (Rochester, NY, USA) at −80°C with an intensifying screen. For rehybridization with CYP3A1 cDNA probe, the membrane was deprobed by washing twice with boiling SDS (1 g/l). The bands on the x-ray film were measured with an AlphaImager™ 2000 (Alpha Innotech Co., San Leandro, CA, USA).

Statistical analysis

Data were analysed by means of ANOVA and Tukey's test was used to test the significance of GO treatments in each dietary-fat-fed group. Two-way ANOVA was used to test the effects of both dietary fat and GO and their interaction. All statistical analyses were performed with commercially available software (SAS Institute Inc., Cary, NC, USA).

Results

Growth characteristics

There was no significant difference in the body-weight gain of rats fed different dietary lipid amount and source. No effect of GO administration on the growth of rats was found either. The average food intakes in rats fed the low-MO, high-MO or high-FO diets were 20·8 (SD 3·2), 18·1 (SD 2·7) and 16·7 (SD 2·9) g/d respectively, and there was no difference among GO treatments in each dietary group (results not shown). Different dietary lipid amount significantly affected the relative liver weight of rats ($P<0·05$) (Table 2). Rats fed the high-fat diet, either high-MO or high-FO, had significantly greater relative liver weight than rats fed a low-MO diet ($P<0·05$); however, there was no significant difference between rats fed either high-fat diet. Different doses of GO had no effect on the relative liver weight of rats. Relative heart and kidney weights were not changed by dietary lipid and GO. Relative spleen weight of rats was not affected by dietary lipid amount and source; however, it was significantly changed by GO. In each dietary lipid group, GO dose-dependently increased the relative spleen weight (Table 2). Rats that received 200 mg GO/kg body weight doubled the relative spleen weight as compared with rats receiving no GO. The relative weight of the jugular lymph nodes was related to the amount but not the

Table 2. Effect of garlic oil (GO) on the growth characteristics of rats receiving different types and amounts of dietary fat for 6 weeks†
(Mean values and standard deviations for five rats per group)

		Body-wt gain (g)		Liver wt/body wt (%)		Heart wt/body wt (%)		Kidney wt/body wt (%)		Spleen wt/body wt (%)		Jugular lymph-node wt/body wt (%)	
Diet	GO (mg/kg)‡	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low MO	0	265	22	4·00	0·27	0·25	0·03	0·79	0·04	0·20[c]	0·03	0·037	0·005
	30	248	30	4·01	0·34	0·27	0·01	0·79	0·06	0·21[c]	0·01	0·035	0·009
	80	235	30	4·13	0·39	0·28	0·03	0·87	0·09	0·31[c]	0·07	0·038	0·005
	200	259	21	3·98	0·31	0·28	0·02	0·87	0·11	0·42[a]	0·04	0·043	0·010
High MO	0	283	36	4·29	0·23	0·26	0·02	0·75	0·09	0·19[c]	0·01	0·029	0·003
	30	285	12	4·45	0·36	0·26	0·01	0·77	0·09	0·21[c]	0·01	0·030	0·004
	80	271	18	4·40	0·26	0·26	0·03	0·75	0·14	0·28[b]	0·03	0·027	0·003
	200	246	21	4·48	0·30	0·30	0·03	0·82	0·02	0·42[a]	0·08	0·033	0·003
High FO	0	286	42	4·36	0·36	0·28	0·01	0·83	0·06	0·23[c]	0·03	0·026	0·004
	30	269	32	4·47	0·27	0·27	0·01	0·81	0·05	0·23[c]	0·02	0·030	0·006
	80	252	39	4·45	0·40	0·28	0·04	0·82	0·06	0·33[b]	0·04	0·034	0·007
	200	254	57	4·40	0·55	0·26	0·03	0·83	0·13	0·43[a]	0·07	0·034	0·009
Statistical significance of effect (two-way ANOVA): *P*													
Dietary fat		NS		0·004*		NS		NS		NS		0·0002**	
GO		NS		NS		NS		NS		0·0001		NS	
Dietary fat × GO		NS		NS		NS		NS		NS		NS	

wt, weight; MO, maize oil; FO fish oil.
[a,b,c]Mean values within a column and within a dietary group with unlike superscript letters were significantly different: $P<0·05$.
Values for high-MO and high-FO diets were significantly greater than for the low-MO diet (Tukey's test): *$P<0·05$.
Values for high-MO and high-FO diets were significantly less than for low-MO diet: **$P<0·01$.
† For details of diets and procedures, see Table 1 and p. 190.
‡ GO was given by oral intubation three times per week.

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source of dietary lipid. Rats fed the high-MO or high-FO diet had significantly lower relative lymph-node weight than rats fed a low-MO diet ($P<0{\cdot}01$); however, there was no significant difference between rats fed the high-fat diets. In each diet group, different doses of GO had no effect on the relative jugular lymph node weight of rats.

Serum fatty acids and hepatic lipid content

Dietary lipid amount and source had no significant effect on serum 14 : 0 and 18 : 0 (Table 3). Rats fed a low-MO diet had greater serum 16 : 0 than rats fed a high-MO diet ($P<0{\cdot}01$). Rats fed a low-MO diet had greater serum 18 : 1*n*-9 than rats fed the high-MO or high-FO diet ($P<0{\cdot}01$). Serum 18 : 2*n*-6 and 20 : 4*n*-6 were significantly greater in rats fed the MO diets than rats fed FO ($P<0{\cdot}001$), and they were significantly greater in rats fed a high-MO diet than rats fed a low-MO diet ($P<0{\cdot}05$). Serum 20 : 5*n*-3 and 22 : 6*n*-3 were significantly higher in rats fed the high-FO diet than rats fed the low-MO or high-MO diet ($P<0{\cdot}001$).

Total lipid content in rat livers was affected by the amount and source of dietary fat (Fig. 1). Rats fed the high-MO diet had a significantly greater lipid level than those fed the low-MO diet ($P<0{\cdot}001$). Rats fed the high-FO diet, which had the same amount of dietary fat as high-MO diet, had a lipid level similar to those fed the low-MO diet and was significantly lower than for those fed the high-MO diet ($P<0{\cdot}001$). Different dosage of GO had no effect on the lipid content in rat liver tissues.

Antioxidant status

Dietary lipid source and amount had no effect on hepatic GSH peroxidase activity. Instead, GO significantly decreased GSH peroxidase activity (Table 4). In each dietary group, rats administered 200 mg GO/kg body weight had the lowest enzyme activity, and rats that received 0 mg GO/kg body weight had the greatest. Regardless of dietary lipid, GO caused a dose-dependent increase in hepatic GSH reductase activity. Moreover, the activity of GSH reductase was greater in rats fed a high-FO or low-MO diet than rats fed a high-MO diet ($P<0{\cdot}05$) (Table 4). Higher hepatic SOD activity was also noted in rats fed FO than those fed the MO diets ($P<0{\cdot}001$). In the high-FO group, rats administered 200 mg GO/kg body weight had greater SOD activity than rats receiving the other doses of GO ($P<0{\cdot}05$). In addition, there was an interaction between dietary fat and GO on the SOD activity in rat livers.

Dietary fat, but not GO, affected the α-tocopherol content of rat liver. Rats fed the high-FO diet had a greater α-tocopherol level than rats fed the high-MO diet ($P<0{\cdot}05$). Hepatic GSH level was also noted to be affected by dietary fat but not by GO (Table 4). Rats fed the low-MO or high-FO diet had significantly greater hepatic GSH level than rats fed a high-MO diet ($P<0{\cdot}01$). There was no effect of dietary fat or GO on GSH:GSSG ratio. The TBARS levels in the liver tissues, however, were significantly higher in rats fed FO than rats fed the MO diets ($P<0{\cdot}001$) (Table 4). In the low-MO group, rats administered 200 mg GO/kg had lower TBARS production than rats receiving 0 mg GO/kg ($P<0{\cdot}05$).

In addition to the endogenous TBARS, the protection of GO on lipid peroxidation in rats fed the low-MO diet was also examined in the presence of exogenous oxidant (Fig. 2). With 50 μM-$FeSO_4$ incubation, 3-fold higher TBARS levels were produced compared with conditions in which the oxidant was absent (0·77 *v.* 0·20 nmol/mg protein). The increase of lipid peroxidation by $FeSO_4$, however, was significantly suppressed in those prepared from rats given 80 and 200 mg GO/kg body weight ($P<0{\cdot}05$).

Drug-metabolizing enzyme activities

The results indicate that both dietary fat and GO significantly modulated EROD and NDMAD activities (Table 5). Rats fed the high-FO diet had significantly higher

Table 3. Serum fatty acid compositions (g/100 g total fatty acids) in rats fed low- or high-maize-oil (MO) diet or high-fish-oil (FO) diet*

(Mean values and standard deviations for five rats per group)

	Low MO		High MO		High FO	
Fatty acids	Mean	SD	Mean	SD	Mean	SD
14 : 0	0·67	0·26	0·35	0·21	0·72	0·34
16 : 0	23·18[a]	2·76	15·73[b]	3·28	18·81[ab]	3·89
18 : 0	13·97	4·29	12·93	2·47	18·91	4·41
18 : 1	19·11[a]	4·07	12·07[b]	1·11	10·16[b]	2·11
18 : 2	19·50[b]	4·20	27·35[a]	2·50	12·50[b]	2·50
20 : 4	22·84[b]	3·62	30·48[a]	6·58	11·44[c]	2·28
20 : 5	0·05[b]	0·12	0·05[b]	0·08	14·31[a]	2·36
22 : 6	0·67[b]	0·31	0·97[b]	0·33	13·15[a]	3·33

[a,b,c]Mean values within a row with unlike superscript letters were significantly different: $P<0{\cdot}05$.

* For details of diets and procedures, see Table 1 and p. 190.

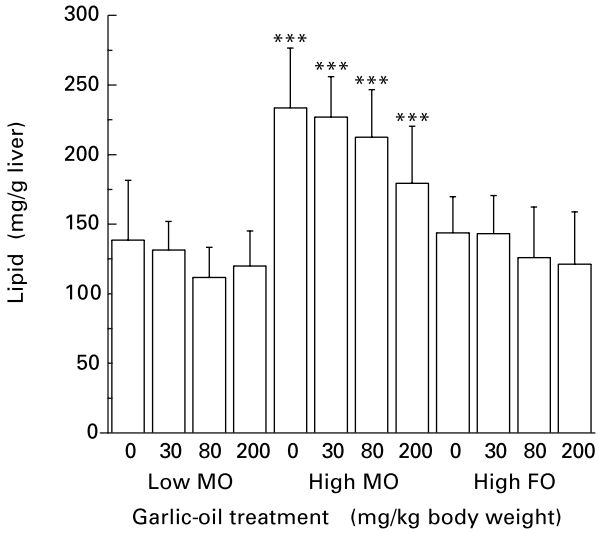

Fig. 1. Effect of dose of garlic oil (0–200 mg/kg body weight three times per week) on the liver lipid concentration in rats fed a high maize-oil (MO) or high-fish oil (FO) diet for 6 weeks. For details of diets and procedures, see Table 1 and p. 192. Values are mean for five rats per group with standard deviations shown by vertical bars. Mean values were significantly greater than those for the low-MO and high-FO diets for each dose of garlic oil: ***$P<0{\cdot}001$.

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Table 4. Effect of dietary fat and garlic oil (GO) fed for 6 weeks on hepatic antioxidant states in rat livers§
(Mean values and standard deviations for four or five rats per group)

Diet	GO (mg/kg)‖	GSH peroxidase (μmol/min per g liver)		GSH reductase (μmol/min per g liver)		SOD (U/min per g liver)		α-Tocopherol (μg/g liver)		GSH (μmol/g liver)		GSH:GSSG ratio		TBARS (nmol/g liver)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low MO	0	$13{\cdot}0^{a}$	1·7	$2{\cdot}53^{b}$	0·37	3·44	0·40	0·60	0·37	4·81	1·25	16·0	5·7	37^{a}	13
	30	$11{\cdot}1^{ab}$	1·7	$2{\cdot}70^{ab}$	0·36	3·40	0·47	0·68	0·27	5·16	0·88	18·0	6·5	30^{ab}	15
	80	$9{\cdot}7^{bc}$	0·9	$2{\cdot}84^{ab}$	0·22	3·64	0·48	0·54	0·13	5·76	1·43	16·5	1·8	32^{ab}	8
	200	$8{\cdot}2^{c}$	1·1	$3{\cdot}20^{a}$	0·34	3·84	0·62	0·57	0·45	5·22	1·01	15·7	6·4	19^{b}	6
High MO	0	$11{\cdot}5^{a}$	2·1	$2{\cdot}18^{b}$	0·26	3·87	0·50	0·45	0·24	4·06	0·91	14·7	4·7	60	8
	30	$10{\cdot}1^{ab}$	1·8	$2{\cdot}35^{ab}$	0·22	4·16	0·48	0·40	0·18	3·94	0·56	14·2	4·0	55	9
	80	$8{\cdot}9^{ab}$	2·0	$2{\cdot}49^{ab}$	0·10	4·47	0·72	0·45	0·32	4·32	0·83	14·2	4·1	53	10
	200	$7{\cdot}3^{b}$	1·5	$2{\cdot}57^{a}$	0·18	3·81	0·51	0·46	0·23	4·15	0·84	12·2	4·1	65	13
High FO	0	$10{\cdot}8^{a}$	1·5	$2{\cdot}37^{b}$	0·45	$4{\cdot}11^{b}$	0·41	0·84	0·47	5·33	1·22	17·8	4·3	1344	159
	30	$10{\cdot}1^{a}$	1·9	$2{\cdot}67^{b}$	0·37	$5{\cdot}12^{b}$	0·66	0·77	0·15	5·57	1·26	18·9	5·9	1123	422
	80	$9{\cdot}0^{ab}$	1·9	$2{\cdot}94^{ab}$	0·37	$5{\cdot}43^{b}$	0·65	0·65	0·35	4·90	0·54	15·1	1·8	1037	560
	200	$6{\cdot}9^{b}$	1·9	$3{\cdot}16^{a}$	0·42	$8{\cdot}12^{a}$	1·46	0·91	0·29	5·44	0·63	14·9	5·8	909	388
Statistical significance of effect (two-way ANOVA): *P*															
Dietary fat		NS		0·0171*		0·0001†††		0·0192‡		0·0005‡‡		NS		0·0001†††	
GO		0·0001		0·0213		0·0001		NS		NS		NS		NS	
Dietary fat × GO		NS		NS		NS		NS		NS		NS		NS	

GSH, glutathione; SOD, superoxide dismutase; GSSG, glutathione disulfide; TBARS, thiobarbituric acid-reactive substances; MO, maize oil; FO, fish oil.
[a,b,c]Mean values within a column and within a dietary group with unlike superscript letters were significantly different (Tukey's test): $P<0{\cdot}05$.
Values for high-FO and low-MO diets were significantly greater than for high-MO diet: *$P<0{\cdot}05$.
Value for high-FO diet was significantly greater than for high-MO and low-MO diets: †††$P<0{\cdot}001$.
Value for high-FO diet was significantly greater than for high-MO diet: ‡$P<0{\cdot}05$; ‡‡$P<0{\cdot}01$.
§ For details of diets and procedures, see Table 1 and p. 190.
‖ GO was given by oral intubation three times per week.

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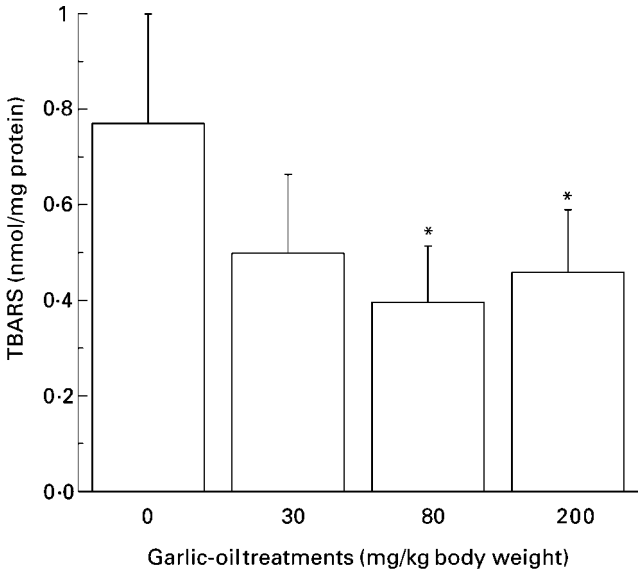

Fig. 2. Effect of dose of garlic oil (0–200 mg/kg body weight three times per week) on the iron sulfate-induced thiobarbituric acid-reactive substances (TBARS) in rats fed a low maize-oil diet for 6 weeks. Livers were homogenized with 14 vol. potassium phosphate buffer, pH 7·4. Cell homogenates were then incubated with 50 μM-$FeSO_4$ at 37°C. After 30 min, butylated hydroxytoluene was added to terminate lipid peroxidation. For details of diets and procedures, see Table 1 and p. 190. Values are mean for five rats per group with standard deviations shown by vertical bars. Mean values were significantly different from those of the control rats (Tukey's test): *$P<0·05$.

EROD activity than rats fed the high-MO or low-MO diet ($P<0·05$), and also had higher NDMAD activity than rats fed a low-MO diet ($P<0·05$). GO caused a dose-dependent increase of EROD activity, but a decrease of NDMAD activity. The activity of erythromycin demethylase, however, was not affected by either dietary fat or GO. Hepatic GST activity was also significantly affected by both dietary lipid and GO. Rats fed the high-MO or high-FO diet had greater enzyme activity than rats fed a low-MO diet ($P<0·001$). In each dietary group, a dose-dependent increase of GST activity by GO was noted. Rats receiving 80 or 200 mg GO/kg had higher GST activities than rats receiving 0 mg GO/kg ($P<0·05$). No interaction of dietary fat and GO on drug-metabolizing enzyme activities was found.

Immunoblot assay

Accompanying the changes of EROD and GST activities, protein levels of CYP1A1 and three GST isoenzymes, Ya, Yb1 and Yc, were elevated by increasing the dosage of GO in each dietary group (Fig. 3). The expression of CYP2E1, however, was dose-dependently decreased in the presence of GO. Meanwhile, the protein level of CYP1A1 and CYP2E1 was increased in rats fed the high-FO diet compared with those fed the high-MO or low-MO diet. Such an increase by high-FO, however, was not noted for GST Ya, Yb1 and Yc. Although changes of erythromycin demethylase activity was not noted, CYP3A1 level was dose-dependently increased in the presence of GO and was increased in the high-FO group. It

Table 5. Effect of garlic oil (GO) and dietary fat fed for six weeks on the hepatic ethoxyresorufin *O*-deethylase (EROD), *N*-nitrosodimethylamine demethylase (NDMAD), erythromycin demethylase (EMD), and glutathione *S*-transferase (GST) activities§

(Mean values and standard deviations for four to five rats per group)

		EROD (pmol/min per mg protein)		NDMAD (nmol/min per mg protein)		EMD (nmol/min per mg protein)		GST (nmol/min per mg protein)	
Diet	GO (mg/kg)‖	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low MO	0	$7·8^{a}$	3·0	2·5	1·0	0·37	0·54	1959^{c}	173
	30	$12·2^{ab}$	3·9	2·5	1·0	0·53	0·20	2251^{bc}	258
	80	$12·6^{ab}$	6·3	1·9	0·4	0·62	0·93	2655^{ab}	358
	200	$21·0^{b}$	8·0	1·7	0·6	0·54	0·40	2906^{a}	472
High MO	0	11·7	3·1	3·1	1·8	0·94	0·58	2761^{c}	370
	30	13·5	6·7	2·5	1·5	0·58	0·23	3164^{bc}	265
	80	13·1	1·8	2·0	0·8	1·03	0·92	3254^{b}	244
	200	15·6	7·1	2·2	0·7	1·78	2·32	3993^{a}	305
High FO	0	17·4	10·2	4·3	1·2	1·23	0·44	2686^{c}	219
	30	20·7	10·5	2·3	0·7	1·32	0·90	3100^{b}	279
	80	15·4	6·4	3·2	1·9	1·07	0·91	3112^{b}	116
	200	23·9	9·2	2·6	1·3	0·78	0·35	3620^{a}	123
Statistical significance of effect (two-way ANOVA): *P*									
Dietary fat		0·0169*		0·0392†		NS		0·0001‡‡‡	
GO		0·0353		0·0498		NS		0·0001	
Dietary fat × GO		NS		NS		NS		NS	

MO, maize oil; FO, fish oil.
[a,b,c]Mean values within a column and within a dietary group with unlike superscript letters were significantly different (Tukey's test): $P<0·05$.
Value for high-FO diet was significantly greater than for high-MO and low-MO diets: *$P<0·05$.
Value for high-FO diet was significantly greater than for low-MO diet: †$P<0·05$.
Values for high-FO and high-MO diets were significantly greater than for low-MO diet: ‡‡‡$P<0·001$.
§ For details of diets and procedures, see Table 1 and p. 190.
‖ GO was given by oral intubation three times per week.

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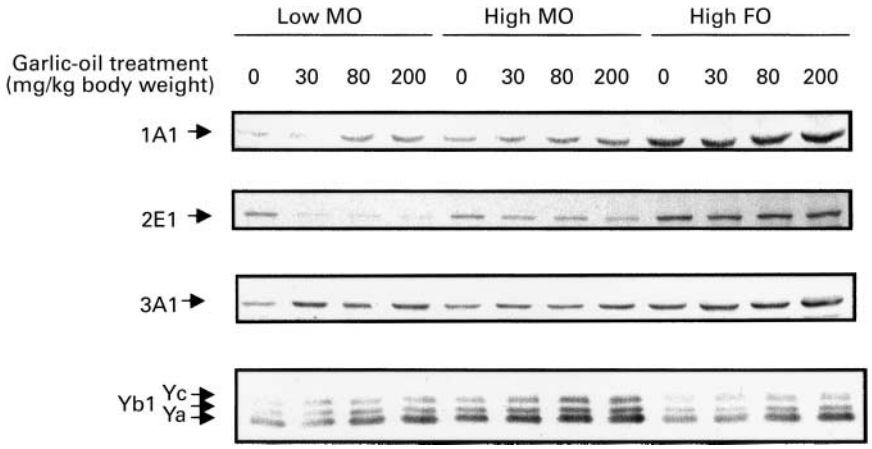

Fig. 3. Immunoblot analysis of hepatic cytochrome P450 (CYP) 1A1, CYP2E1, CYP3A1 and glutathione *S*-transferase isoenzymes Ya, Yb1 and Yc. Microsomal and cytosolic proteins were pooled from five rats' livers per group. Each group of rats was fed a low-maize oil (MO), high-MO diet or a high-fish oil (FO) diet for 6 weeks. Proteins were electrophoretically separated on SDS–polyacrylamide gels and were transferred to polyvinylidiene difluoride membranes. Immunoreactive bands were stained with primary antibodies and the peroxidase-conjugated secondary antibody. Microsomal and cytosolic proteins of individual rats were tested in triplicate in each group and similar results were obtained. For details of diets and procedures, see Table 1 and p. 190.

was also noted that the combined changes of CYP1A1, CYP2E1 and CYP3A1 protein level by GO and FO are additive.

mRNA expression

Northern blots revealed that the changes of CYP1A1 and CYP3A1 mRNA levels (Fig. 4) were consistent with the protein levels stated previously. The transcripts of hepatic CYP1A1 and CYP3A1 were greater in rats fed the high-FO diet than in those fed the low-MO or high-MO diet (Fig. 4(A)). The dose-dependent increase by GO treatment was found in all dietary groups. In addition, CYP1A1 and CYP3A1 mRNA levels were increased additively in rats fed the high-FO diet and receiving GO. The CYP1A1 transcripts of rats fed the low-MO, high-MO or high-FO diet and receiving 200 mg GO/kg body weight were 5·7 (SD 1·4), 5·4 (SD 2·9) and 22·4 (SD 7·3)-fold (*n* 3) that of rats fed the low-MO diet without GO respectively; the CYP3A1 transcripts were 5·2 (SD 2·5), 4·9 (SD 1·4) and 14·6 (SD 2·7)-fold (*n* 3) respectively (Fig. 4(B)).

Discussion

In this 6-week experiment, with the exception of the relative spleen and jugular lymph-node weight, the body-weight gain, relative heart and kidney weights were not affected by the dietary lipid and GO manipulation. Among three GSH-related enzymes studied, the activity of GSH peroxidase was affected only by GO, whereas the activity of GSH reductase (Table 4) and GST (Table 5) were significantly modulated by both GO and dietary fat. As shown, GO dose-dependently decreased GSH peroxidase activity, but the activities of GSH reductase and GST were dose-dependently increased. The decrease of GSH peroxidase activity was consistent with our previous report (Sheen *et al.* 1999). Iqbal & Athar (1998), however, reported that renal GSH peroxidase activity in rats pre-treated with GO (50 and 100 mg/kg body weight) and then injected intraperitoneally with iron-nitrilotriacetate (9 mg/kg body weight) was significantly increased compared with rats without GO. It seemed that GO pre-treatment offers protection to rats against iron-nitrilotriacetate-induced nephrotoxicity. The discrepancy between these studies may be due to the target organ studied and the exposure of animals to toxin. Although GSH peroxidase activity was decreased by GO, the increase of GST activity, which acts as a Se-independent GSH peroxidase that reduces a variety of organic hydroperoxides to alcohols, may partly compensate for the loss of Se-dependent GSH peroxidase. In the study of Iqbal & Athar (1998), they also found that GO pre-treatment significantly increased renal GSH reductase activity of rats exposed to iron-nitrilotriacetate compared with rats receiving no GO pre-treatment. Taken together, GO-suppressed $FeSO_4$-induced lipid peroxidation can be partly attributed to its modulation of GSH-related antioxidant enzyme activities (Fig. 2).

In addition to GO, FO increased the hepatic GSH reductase and GST activities. GSH reductase is responsible for the regeneration of GSH from oxidized GSH. A lower GSH reductase activity may partly explain the slightly lower GSH level and GSH:GSSG ratio noted in the high-MO group (Table 4). In contrast, FO had no effect on GSH peroxidase in rat livers. FO enhanced intrinsic GSH peroxidase activity in human vascular endothelial cells and such an induction may be an important mechanism protecting cells against oxidative damage (Crosby *et al.* 1996). Although no change in GSH peroxidase activity was noted, FO-induced an increase in the activity of GST that can take over the role of GSH peroxidase, at least in

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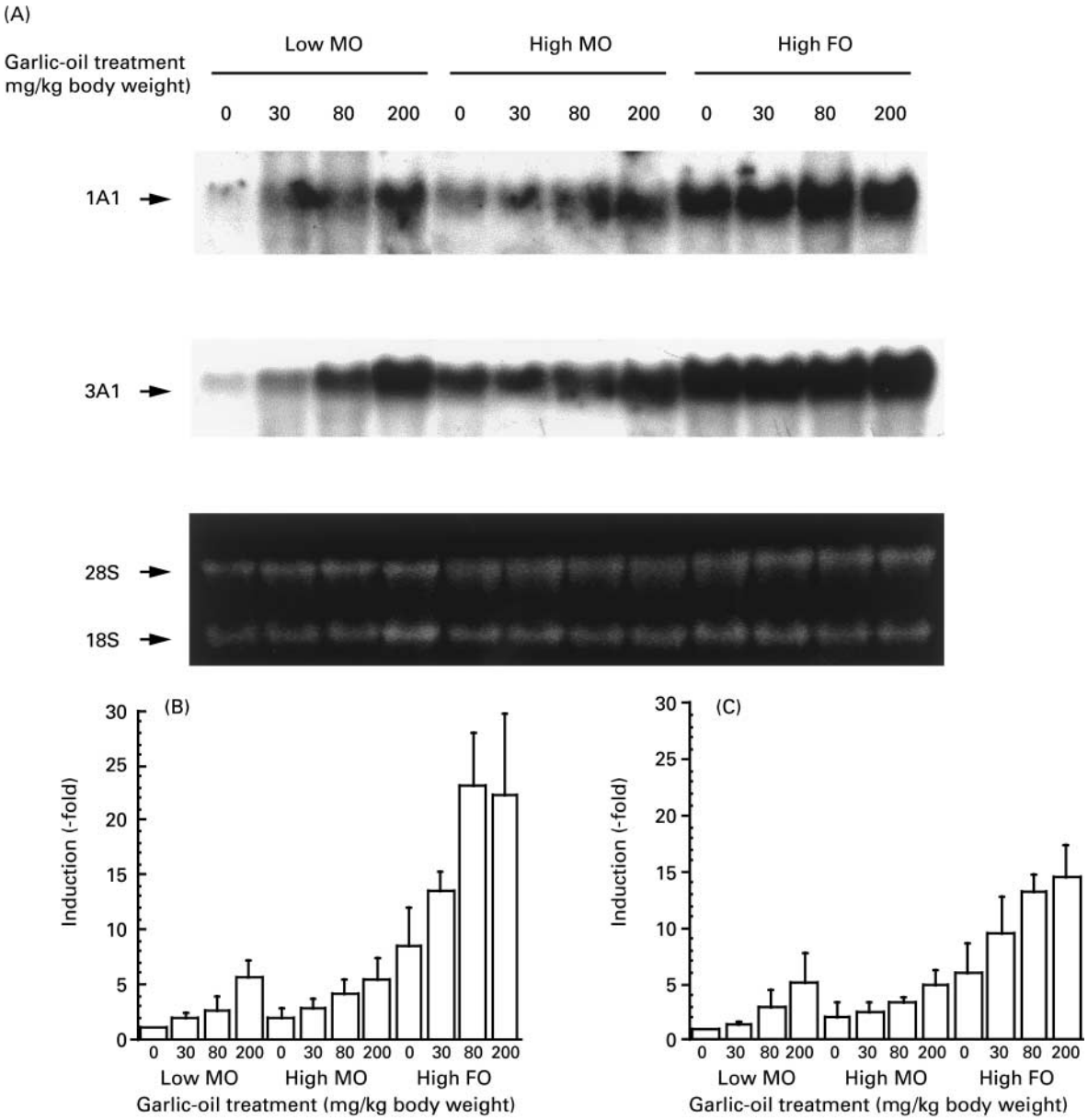

Fig. 4. Northern blot analysis of hepatic cytochrome P450 (CYP) 1A1 and CYP3A1 levels in rats dosed with garlic oil (0–200 mg/kg body weight three times per week) and fed a low-maize oil (MO), high MO or a high-fish oil (FO) diet for 6 weeks. Total RNA was extracted by Trizol. For details of diets and procedures, see Table 1 and p. 190. An equal amount of RNA was subjected to Northern blot analysis (20 ug per lane; (A)). The filter was first hybridized with a CYP3A1 cDNA and then rehybridized with a CYP1A1 cDNA after deprobing. The induction (-fold) of CYP1A1 (B) and CYP3A1 (C) mRNA was quantitated by densitometry based on the relative amount of 18S RNA.

part. The high content of polyunsaturated fatty acids in the FO increases lipid peroxidation and production of free radicals (Crosby *et al.* 1996; Ruiz-Gutiérrez *et al.* 1999). Induction of the activity of NDMAD and expression of CYP2E1 (Table 5), which is known to be involved in fatty acid oxidation and stimulate lipid peroxidation (Leclercq *et al.* 2000), also accelerated the endogenous oxidative stress in the high-FO-fed rats. As a consequence, there is growing concern that habitual intake of high quantities of FO may induce oxidative damage. However, the effectiveness of GO on the modulation of antioxidant enzyme activity and on the suppression of CYP2E1 expression suggests that the co-administration of GO may compensate for the risk of oxidative-related diseases.

Although both GO and dietary fat modulated GSH-related enzyme activity, lack of interaction revealed that GO and dietary fat act independently. In contrast, GO interacted with dietary fat on the hepatic SOD activity. Rats fed FO not only had significantly greater SOD activity than rats fed the MO diets, but GO also synergistically enhanced the induction of FO on this enzyme activity. Venkatraman *et al.* (1994) and Fernandes *et al.* (1996) reported that the autoimmune-prone NZB × NZW F_1 mice fed FO had significantly greater hepatic Cu,Zn-SOD activity than rats fed MO; they suggested that *n*-3 lipids

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delay the onset of autoimmune disease in B/W mice, perhaps via the enhancement of hepatic antioxidant enzyme activities and expression and increase of programmed cell death to prevent accumulation of self-reactive immune cells in lymphoid organs.

The present study demonstrated that both GO and FO are two food components that modulate drug-metabolizing systems. The results showed that FO increased EROD and NDMAD activities compared with MO, and high-fat diets increased GST activity (Table 5) compared with 50 g MO/kg diet. Meanwhile, regardless of dietary fat, GO dose-dependently increased GST and EROD and decreased NDMAD activities. Northern and Western blot results further demonstrated that the modulation by GO and dietary fat of CYP1A1, CYP2E1 and CYP3A1 and GST isoenzymes occur at the transcriptional and/or post-transcriptional stages. Moreover, the combined effects of GO and FO on CYP1A1, CYP2E1 and CYP3A1 protein and/or mRNA expression appeared to be additive. The combined effect of GO and FO on drug metabolism, obtained in the present study and in our previous study (Chen *et al.* 2001), demonstrated that the usefulness of co-administration of GO and FO is not only due to the decrease in blood cholesterol (Alder & Holub, 1997; Morcos, 1997), but also to a modulation of drug metabolism. The modulation of drug-metabolizing enzymes by dietary components may also have a major impact on drug pharmacokinetics, drug–drug interactions, toxicity and carcinogenicity of foreign chemicals, and on the activity and disposition of endogenous compounds (Conney, 1982). In the present study, changes in drug-metabolizing enzyme activities were noted to be inconsistent with the CYP protein and mRNA levels, especially for erythromycin demethylase activity and CYP3A1 expression. This discrepancy is attributed, at least in part, to the lack of enzyme substrate specificity.

Steatosis of the liver is an important factor influencing hepatic drug-metabolizing enzyme activity and expression. In two rat models of non-alcoholic steatohepatitis and microvesicular steatohepatitis that were induced by refeeding a high-glucose, fat-free diet following 48 h fasting and orotic acid respectively, CYP2E1, CYP3A and CYP2A protein level and activity were shown to be inversely correlated with the degree of hepatic fat content (Leclercq *et al.* 1998; Su *et al.* 1999). This suggests that lipid deposition in the liver can change the drug metabolism. However, in a mouse non-alcoholic steatohepatitis model produced by the intake of methionine- and choline-deficient diet, CYP2E1 induction accompanied steatosis (Leclercq *et al.* 2000). This controversy indicates that the relationship between liver fat deposition and drug-metabolizing enzyme expression is more complex and may not be simply attributed to the fat content. In the present study, rats fed the high-MO diet had 70 % higher liver lipid content than those fed the low-MO diet, but no changes in CYP1A1, CYP2E1 and CYP3A1 proteins and mRNA levels were noted. Further study is required to clarify the effect of diet-induced hepatosteatosis on the drug metabolism.

Although dietary lipids were once thought to modulate microsomal drug-metabolizing enzymes by changing the lipid environment of the microsomal membrane matrix, it is now known that the mechanisms of this modulation are more complex. Numerous studies have suggested that dietary fatty acids can directly and indirectly modulate receptor-mediated signalling pathways at multiple levels and therefore the gene expression (Hwang & Rhee, 1999). One well-known example is that the gene products involved in lipid metabolism, such as acyl-CoA oxidase, fatty acid synthase and CYP4A, are regulated by certain polyunsaturated fatty acids and their metabolites through the peroxisome proliferator-activated receptor (Jump, 2002), a member of nuclear receptor 1 gene family. Pregnane X receptor and constitutive androstane receptor are two members of the nuclear receptor 1 family, and are known to participate in the induction of CYP3A and CYP2B expression respectively, in the presence of a broad range of drugs and steroids (Waxman, 1999). Although there is no evidence that indicates that fatty acids are capable of binding to pregnane X receptor and constitutive androstane receptor, it is clear that CYP3A1 and CYP2B1are up-regulated in rats fed FO (Chen *et al.* 2001). The existence of undiscovered receptor-mediated pathways cannot be excluded. Moreover, α-tocopherol modulation on the drug-metabolizing enzyme activity and expression is an alternative explanation. Recently, we demonstrated that α-tocopherol up-regulates CYP2B1 expression via prostaglandin E_2-mediated pathways (Tsai *et al.* 2001). Such an effect may explain in part the higher CYP protein and mRNA content in the high-FO group, which had a greater α-tocopherol level than the MO-fed groups. GST and many CYP enzymes have been shown to be inducible in the presence of various drugs and food components (Vos & Van Bladeren, 1990; Guengerich, 1995). The molecular mechanisms involved in the regulation of GST isoenzyme genes are mediated by an antioxidant-responsive element and the activator protein-1-responsive element; both are located on GST gene promoter and/or enhancer regions (Rushmore & Pickett, 1990; Bergelson *et al.* 1994; Fotouhi-Ardakani & Batist, 1999). However, the molecular mechanism of garlic's effect is not clear and it is compelling to investigate whether GO mediates GST genes through the antioxidant-responsive element pathway and/or the Fos/Jun binding to AP-1 binding site. Elucidating the molecular and cellular mechanisms of such responses obtained in the present study requires further study. In conclusion, in addition to the well-known functions of GO and FO, the effect of these two popular health supplements on disease prevention may be due to their regulation of antioxidant- and drug-metabolizing enzyme activity and expression. The combined effect of GO and FO on drug-metabolizing enzymes is additive.

Acknowledgements

This research was supported by the Department of Health, Republic of China, Grant DOH 91-TD-1010. Our thanks also go to Dr Suzanne Hendrich, Department of Food Science and Human Nutrition, Iowa State University, for critically reading this manuscript and for stimulating discussions.

https://doi.org/10.1079/BJN2002766 Published online by Cambridge University Press

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https://doi.org/10.1079/BJN2002766 Published online by Cambridge University Press