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炸油攝食對缺鐵大鼠肝微粒體細胞色素 P450 誘發之影響

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Induction of Hepatic Cytochrome P450 Using Dietary Oxidized Oil in Iron Deficient Rats

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ABSTRACT This study was conducted to investigate the effects of mild and severe iron deficiency on the induction of hepatic cytochrome P450 (CYP) using oxidized oil. Oxidized oil was prepared from soybean oil subjected to deep-frying, heated at 205 ± 5℃ for 24 hours. All experimental diets were based on the AIN-76 formula but contained 15% fresh or oxidized soybean oil. Weanling, male Wistar rats were randomly assigned to three iron diets containing fresh oil: control (45 ppm Fe), low Fe (20 ppm) and Fe deficient (6 ppm) groups. After 10 days on the diet, each iron group was further divided into fresh oil and oxidized oil groups, and feeding continued for 32 days. Microsomal CYP concentration was significantly higher in the oxidized oil groups. Among the oxidized oil groups, total hepatic CYP content was not significantly different when normalized on a body weight basis. For all iron groups, consumption of oxidized oil suppressed growth, body weight gain and feed efficiency. This suppression was most severe in the iron deficient group. Hemoglobin concentration in rats fed a low iron diet was also suppressed by feeding oxidized oil. In the control and low Fe group, oxidized oil led to a significant reduction in total amount of hepatic ferritin, but total hepatic iron remained unchanged. Hepatic TBARS value was elevated significantly in the oxidized oil groups, among which the control group had significantly higher TBARS values than the other two groups. These results indicated that CYP induction was not compromised by severe iron deficiency, but was maintained at the expense of other heme proteins and body growth. Consumption of oxidized oil appears to alter iron metabolism and reduce the storage form of iron in the liver.

Keywords: *hepatic cytochrome P450, oxidized oil, iron deficiency, hepatic ferritin, rats*

INTRODUCTION

The hepatic mixed-function oxidase (MFO) system is responsible for the metabolism of xenobiotics to accelerate the elimination of toxic chemicals[1]. Xenobiotic metabolism occurs in two phases[2]. Phase I metabolism involves biotransformation of lipophilic

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xenobiotics by reduction, oxidation and hydrolysis into more hydrophilic metabolites. Phase II metabolism involves conjugation between parent xenobiotics or phase I metabolites and endogenous small molecules to render them further more polar and readily excretable. These enzyme activities are located primarily in the intracellular endoplasmic reticulum (microsomal fraction). The cytochrome P450 (CYP) system is the most important enzyme system catalyzing phase I metabolic reactions and exists as a superfamily of heme-protein forms and displays a broad range of substrate specificity. Both CYP and conjugating enzymes are inducible by many chemicals and drugs, some of which are also substrates for these enzymes[3]. In addition to xenobiotic metabolism, CYP are also exploited by many organisms in the biosynthesis of endogenous compounds, such as steroid hormones, fatty acids, prostaglandins, leukotrienes, vitamins and fatty acids[4].

Iron is essential for all living organisms and is involved in cell proliferation, respiration, oxygen and electron transport, and the regulation of gene expression. Iron deficiency could affect the CYP concentration either directly or indirectly, because iron is a substrate in heme formation and controls the reactions using two key heme-synthesis enzymes, delta-aminolevulinic acid synthetase[5] and ferrochelatase[6]. It has been demonstrated in adult mice[7] and rats[8-12] that chronic iron deficiency leads to stimulation of some MFO enzymes whereas others are unaffected, but that the CYP concentration remains unchanged even though the hemoglobin level may be only 35% normal. However, only the constitutive CYP levels were measured in these studies since the animals did not receive any inducing agents, and no studies dealt with the effect of inferior iron status on the induction of CYP, when the demand for heme increased. Iron is also potentially deleterious, and the reactive oxygen species generated by the iron-mediated Fenton reaction may contribute to major pathological processes such as cancer, atherosclerosis, and neurodegenerative diseases[13,14].

In contrast to the acute effects from drugs frequently reported in literatures, diet is probably the most substantial and chronic exposure route to xenobiotics, including those naturally synthesized by plants or formed during food storage and preparation. Chronic consumption of deteriorated frying oil leads to the induction of hepatic CYP as well as enhanced oxidative stress in various tissues in rats[15,16]. This study was designed to explore the effects of different degrees of iron deficiency on the induction of hepatic CYP and oxidative stress by oxidized oil. Among the CYP isoforms, the CYP4 family plays important roles in the metabolism of fatty acids[1]. A commercially available antibody for CYP4A makes it possible to identify its induction by oxidized oil.

MATERIALS AND METHODS

Preparation of oxidized oil

Oxidized oil was prepared according to the method of Huang et al.[15]. Soybean oil was purchased from a local supermarket and heated in a cast iron wok (41 cm i.d., 7 cm central depth) on a gas stove. The temperature of the oil was maintained at 205 ± 5℃, with wheat flour dough sheets (10×4.5 ×0.15 cm) fried one at a time. The frying proceeded for 6 hr per day and repeated successively for 4 days. The oil thus prepared was stored at −20℃ for the preparation of the experimental diets.

Experimental diets

Diets were formulated according to AIN-76 with some modifications in the iron and oil content[17]. There were three dietary iron levels: iron salt was omitted in the iron deficient diet, while ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was added to the low iron diet and the control diet at levels of 20 mg and 45 mg of Fe per kg of diet, respectively. Each iron group was further divided into a fresh oil group and oxidized oil group, which contained soybean oil and fried soy-

bean oil at 15% (w/w), respectively. Ingredients used in the diets were purchased from Sigma, except corn starch was from Roquatte (France), and cellulose from J. Betten-Maier & Sokhe (Germany).

Animals

Forty weanling, male Wistar rats (Laboratory Animal Center, College of Medicine, National Taiwan University), weighing 63 ± 7 g, were housed individually in stainless steel cages with wire mesh floors in a temperature-, humidity- and light-controlled room. Animal care and handling conformed to the *NSC Guide for the Care and Use of Laboratory Animals*[17].

Treatments

In the beginning of the experiment, the rats were randomly assigned into three groups of 13 or 14 rats each and allocated to one of the three iron diets, all containing fresh oil, for the monitoring of iron status. Food and de-ionized water were freely available. Body weight and feed intake corrected for spillage were recorded regularly. Blood was collected by amputating the tip of the tail for monitoring hemoglobin concentration. By the 10th day, rats fed the iron deficient diet exhibited iron deficiency anemia with hemoglobin concentrations ranging between 1.24 and 1.55 mmol/L. The average body weight of rats at this time was 131 g and not different among the three iron levels. Each iron group was further divided into fresh oil and oxidized oil groups, both having similar average body weight. After 32 days of dietary treatment, rats were killed by carbon dioxide asphyxiation, and blood and liver were collected for analysis.

Preparation of microsomes

Liver homogenate (25% w/v) was prepared by homogenizing a fraction of liver in 0.01 M potassium-phosphate buffer using a Potter-Elvehjem homogenizer. Hepatic microsomes were prepared according to the method of Huang et al.[15]. Briefly, the liver homogenate was centrifuged at 12,000 × g for 20 min to obtain the post-mitochondrial supernatant, which was further centrifuged at 105,000 × g for 1 hr (Ultra-centrifuge, 50.4 Ti Rotor, Beckman) to obtain the microsomal pellets. Pellets were then suspended in 0.05 M phosphate buffer containing 1 mM EDTA at pH 7.7, stored at −70℃, and used for CYP analysis in 1 month.

Analysis

Hemoglobin was determined by the cyanomethemoglobin method using Drabkin's reagent[19]. Liver iron concentration was measured with an atomic absorption spectrophotometer (Model 3100, Perkin-Elmer Co., Norwalk, CT) with an air-acetylene flame at 248.3 nm. Measurement of TBARS (thiobarbituric acid reactive substances) was carried out according to the procedures described in detail by Liu and Huang[16]. Microsomal CYP was determined using the dithionite CO binding difference spectrum described by Omura and Sato[20]. To correct for liver enlargement and growth suppression observed in rats fed oxidized oil, CYP was expressed in three types of units: concentration based on microsomal protein, total liver content, and total liver content normalized on body weight[16].

Western blot analysis

Microsomal fraction containing 5 μg of protein was resolved using 12% SDS-polyacrylamide gel electrophoresis. Protein was transferred to PVDF membranes (Millipore), which were subsequently incubated with sheep anti-rat CYP4A antibodies. After incubation, the membranes were washed and incubated with anti-sheep IgG-biotinylated species-specific antibodies (Amersham, UK). The immunoreactive protein was then visulalized with an ECL Western blotting detection system (Amersham Pharmacia Biotech) according to the manufacturer's description.

For the analysis of ferritin, liver homogenate was heated at 75℃ for 10 min, then centrifuged at 10,000 × g for 20 min, and protein in the supernatant was resolved using 15% SDS-polyacrylamide

gel electrophoresis. Protein was transferred to PVDF membranes, subsequently incubated with rabbit anti-rat ferritin polyclonal antibodies (produced in our laboratory). After 1-h incubation, the membranes were washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Vectastain) for 1 h. The immunoreactive protein (MW 19～21 KDa) was visualized with an enzymatic color reaction using diaminobenzidine as a substrate (Sigma).

Sandwiched ELISA analysis

The ELISA plate was first coated with chicken anti-rat-ferritin polyclonal antibodies prepared in our laboratory, washed properly with PBST (10 mM K_3PO_4, 0.85% NaCl, 0.5% Tween 20), and blocked with NET (50 mM Tris, pH 8.0, 0.25% gelatin, 0.15 M NaCl, 5 mM EDTA, 0.05% Tween 20). Supernatant from heat-treated liver homogenate was added to react overnight with the antibodies. Finally, immobilized ferritin was detected by ELISA procedures with rabbit anti-rat ferritin antibodies (prepared in our laboratory) as the primary antibody, and VECTASTAIN ABC kit (VECTOR Laboratories, USA) containing marker enzyme horseradish peroxidase and substrate o-phenylenediamine dihydrochloride. Quantitative measurements were made at 490 nm in a Microplate autoreader (EL311, Bio-Tek Instrument) using purified rat liver ferritin (Sigma) as the standard.

Statistical analysis

The effects of dietary iron and oil type were tested by two-way ANOVA. The differences among the six groups were tested by Duncan's multiple range test. Statistical significance level was set at $p < 0.05$. All statistics were carried out on the SAS System (Version 6, SAS Institute, Cary, NC).

RESULTS

Growth and hemoglobin

The growth performances of the rats are listed in Table 1. At each dietary Fe level, consumption of oxidized oil significantly suppressed body weight, weight gain, and feed intake in rats when compared with the corresponding fresh oil group. Among the fresh oil groups or oxidized oil groups, rats fed Fe deficient diets had significantly suppressed body weight, weight gain, and feed efficiency than those fed the other two iron diets. Rats fed the control and the low Fe diets had similar growth performance and feed efficiency. A dose-response effect of dietary iron on feed intake occurred in the fresh oil groups but not in the oxidized oil groups. Hemoglobin was affected mainly by dietary iron levels. Rats fed the Fe deficient diets had significantly reduced hemoglobin concentration compared with rats fed the other two iron diets (Table 1). Consumption of oxidized oil resulted in depressed hemoglobin concentration only in the low iron groups, but not in the control and the iron deficient groups.

CYP and TBARS

As listed in Table 2, at each iron level, rats fed oxidized oil had liver weight 30% to 40% higher than those fed fresh oil. The main effects of iron intake and oil type on microsomal CYP concentration were significant. Rats fed the Fe deficient diet had the lowest microsomal CYP concentrations among the fresh oil groups. At each iron level, the microsomal CYP concentrations of the oxidized oil groups were 30 to 50% higher than that of the corresponding fresh oil groups. For the control and the low Fe rats, the oxidized oil groups had significantly higher total hepatic CYP contents than the fresh oil groups, but there was no such significant change for the Fe deficient groups. When the hepatic CYP contents were normalized on body weight basis, the major effect of oil type was significant ($p = 0.0001$), while that of iron intake was not ($p = 0.38$). The normalized CYP contents were significantly higher in the oxidized oil groups at all three iron levels, and were also comparable among the three iron groups in response to oxidized oil. A major CYP isoform induced by oxidized oil consumption was CYP 4A as detected by Western immunoblotting (Fig. 1).

Table 1. Hemoglobin, body weight and feed efficiency of rats as a function of dietary iron intake and oil type[1]

Oil group	Fe group	Fe (ppm)	n	Hemoglobin (mmol/L)	Final body weight (g/rat)	Weight gain (g/day/rat)	Food intake (g/day/rat)	Feed efficiency[2] (g /g feed)
Fresh soybean oil	Control	45	7	2.6 ± 0.1^{a}	381 ± 23^{a}	5.8 ± 0.5^{a}	16.6 ± 0.8^{a}	0.35 ± 0.03^{a}
	Low Fe	20	6	2.4 ± 0.2^{a}	376 ± 20^{a}	5.8 ± 0.6^{a}	15.6 ± 0.6^{b}	0.37 ± 0.03^{a}
	Fe deficient	6	6	0.6 ± 0.1^{c}	247 ± 17^{c}	3.4 ± 0.5^{c}	11.0 ± 0.9^{d}	0.30 ± 0.02^{b}
Oxidized soybean oil	Control	45	7	2.5 ± 0.1^{a}	333 ± 16^{b}	4.9 ± 0.2^{b}	13.9 ± 0.6^{c}	0.35 ± 0.03^{a}
	Low Fe	20	7	2.3 ± 0.2^{b}	329 ± 14^{b}	4.8 ± 0.5^{b}	13.9 ± 0.7^{c}	0.34 ± 0.02^{a}
	Fe deficient	6	7	0.7 ± 0.1^{c}	162 ± 16^{d}	1.8 ± 0.4^{d}	7.5 ± 0.7^{e}	0.24 ± 0.04^{c}
				p values from Two-way ANOVA				
	Fe effect			0.0001	0.0001	0.0001	0.0001	0.0001
	Oil effect			0.17	0.0001	0.0001	0.0001	0.008
	Fe × oil			0.07	0.01	0.15	0.009	0.02

[1] Each value represents mean ± SD. In each column, values sharing different superscript letters are significantly different from one another by Duncan's multiple range test at $p < 0.05$.

[2] Feed efficiency = g body weight gain/g food intake.

Table 2. Rat hepatic contents of microsomal cytochrome P450 as a function of dietary iron intake and oil type[1]

Oil group	Fe group	Fe (ppm)	n	Liver weight (g/rat)	Hepatic cytochrome P450		
					Concentration (nmol/mg microsomal protein)	Total (nmol/liver)	Relative to body weight (nmol/100 g)
Fresh soybean oil	Control	45	7	16.4 ± 2.0^{b}	0.53 ± 0.07^{c}	215 ± 56^{b}	56 ± 14^{b}
	Low Fe	20	6	15.2 ± 1.8^{b}	0.51 ± 0.70^{c}	212 ± 45^{b}	57 ± 12^{b}
	Fe deficient	6	6	7.8 ± 0.7^{d}	0.41 ± 0.05^{d}	191 ± 41^{b}	77 ± 15^{b}
Oxidized soybean oil	Control	45	7	22.7 ± 2.0^{a}	0.69 ± 0.06^{ab}	533 ± 69^{a}	161 ± 23^{a}
	Low Fe	20	7	21.3 ± 2.8^{a}	0.70 ± 0.08^{a}	521 ± 125^{a}	158 ± 34^{a}
	Fe deficient	6	7	10.0 ± 0.9^{c}	0.60 ± 0.15^{bc}	255 ± 54^{b}	156 ± 22^{a}
				p values from Two-way ANOVA			
	Fe effect			0.0001	0.0055	0.0001	0.38
	Oil effect			0.0001	0.0001	0.0001	0.0001
	Fe × oil			0.01	0.9	0.0001	0.28

[1] Each value represents mean ± SD. In each column, values sharing different superscript letters are significantly different from one another by Duncan's multiple range test at $p < 0.05$.

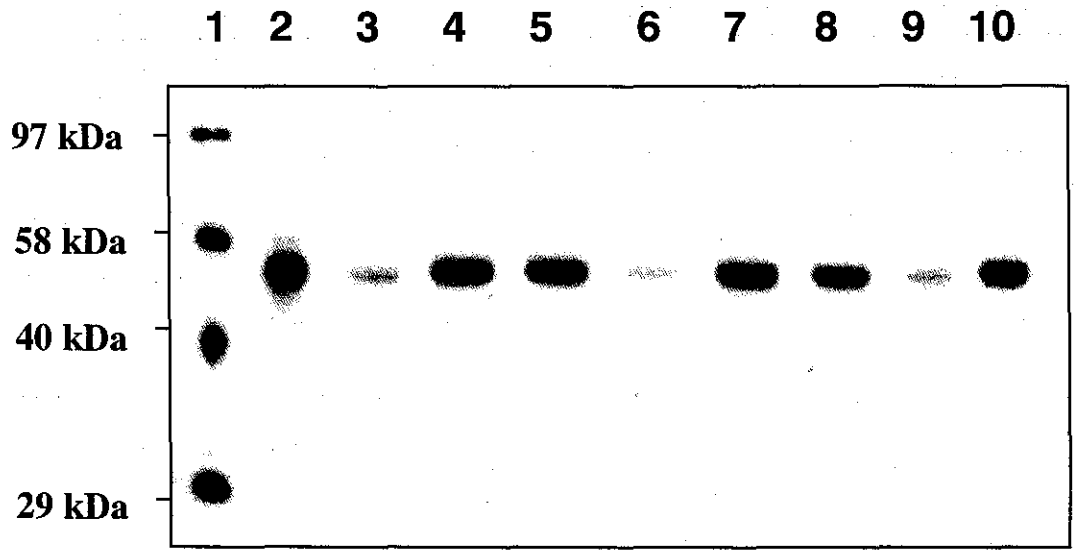

Fig. 1 Induction of hepatic expression of microsomal CYP4A in rats by dietary oxidized oil. Hepatic microsome was prepared from rats fed on either fresh or oxidized soybean oil, and subjected to 12% SDS-PAGE gel electrophoresis and immunoblotting using commercial western blotting kit of rat CYP4A. Lane 1, molecular mass standards; lane 2, positive control using purified CYP4A; lane 3, 6 and 9, microsomes from rats fed fresh soybean oil; lane 4, 5, 7, 8 and 10, microsome from rats fed oxidized soybean oil; each lane represents one rat.

Table 3. Effect of dietary iron intake and oxidized oil on hepatic contents of iron, ferritin and thiobarbituric acid reactive substances (TBARS) in rats[1]

Oil group	Fe group	Fe (ppm)	n	Hepatic Fe		Hepatic ferritin		TBARS (nmol/g liver)
				Concentration (μg/g tissue)	Total (μg/liver)	Concentration (μg/g protein)	Total (μg/liver)	
Fresh soybean oil	Control	45	7	59.9 ± 7.6^{a}	287 ± 89^{a}	2.42 ± 0.65^{a}	4389 ± 1580^{a}	1.61 ± 0.39^{c}
	Low Fe	20	6	31.9 ± 8.2^{c}	128 ± 29^{b}	1.06 ± 0.37^{b}	1826 ± 639^{c}	1.63 ± 0.37^{c}
	Fe deficient	6	6	20.3 ± 8.1^{d}	64 ± 26^{c}	0.08 ± 0.03^{c}	69 ± 42^{d}	2.93 ± 0.61^{bc}
Oxidized soybean oil	Control	45	7	40.4 ± 5.0^{b}	271 ± 31^{a}	1.09 ± 0.23^{b}	3139 ± 582^{b}	6.51 ± 2.40^{a}
	Low Fe	20	7	21.1 ± 3.8^{d}	135 ± 17^{b}	0.26 ± 0.17^{c}	738 ± 511^{d}	4.27 ± 0.59^{b}
	Fe deficient	6	7	17.5 ± 4.6^{d}	107 ± 27^{bc}	0.06 ± 0.03^{c}	78 ± 25^{d}	4.36 ± 1.39^{b}
				p values from Two-way ANOVA				
	Fe effect			0.0001	0.0001	0.0001	0.0001	0.1142
	Oil effect			0.0001	0.4	0.0001	0.003	0.0001
	Fe × oil			0.006	0.23	0.0002	0.14	0.0062

[1] Each value represents mean ± SD. In each column, values sharing different superscript letters are significantly different from one another by Duncan's multiple range test at $p < 0.05$.

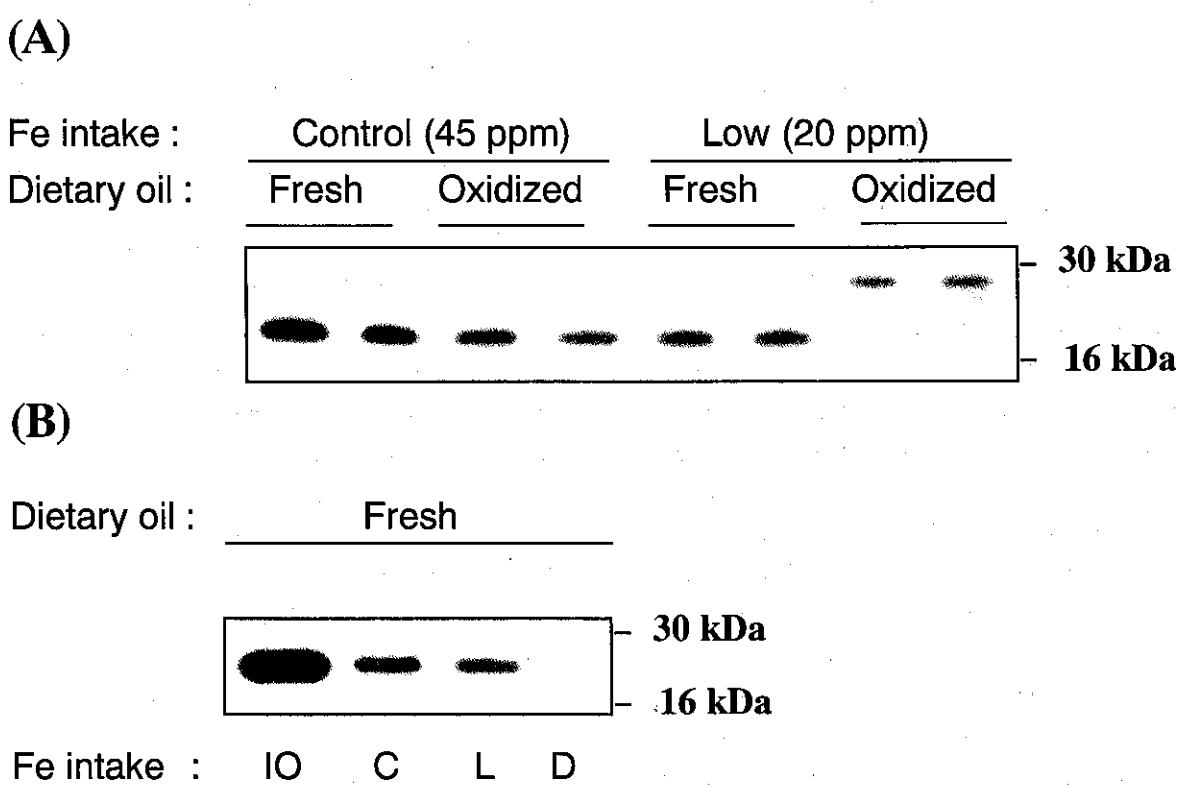

Fig. 2 (A) Effects of dietary iron intake and oxidized soybean oil on hepatic ferritin protein (MW 19～21 kDa) in rats. (B) Expression of hepatic ferritin protein in rats fed conrtrol (C, 45 ppm Fe), low Fe (L, 20 ppm Fe) and Fe deficient (D, 6 ppm Fe) diets; hepatic homogenate from an iron-loaded rat (IO, fed on a diet containing 5000 ppm Fe) served as a positive control. Supernatant from heated liver homogenate was prepared from rats fed on fresh as well as oxidized soybean oil, and subjected to 15% SDS-PAGE gel electrophoresis and immunoblotting using rabbit anti-rat ferritin antibodies and horseradish peroxidase-conjugated goat anti-rabbit IgG. The immunoreactive protein was visualized with an enzymatic color reaction using diaminobenzidine as the substrate. Each lane represents one rat. The near 30 kDa bands of the low Fe, oxidized group were due to nonspecific activity of the polyclonal antibodies used in the assay.

Hepatic iron and ferritin

Decreased hepatic ferritin levels by consumption of oxidized oil were observed only in the control and the low iron groups (panel A, Fig. 2), while hepatic ferritin in the deficient groups was below the detection limit of the Western blot analysis (panel B, Fig. 2). As listed in Table 3, both iron intake and oil type exerted a significant main effect on total hepatic ferrtin, but only iron intake exerted a significant main effect on total hepatic iron. Among the fresh oil groups, both concentrations and total amounts of hepatic iron as well as those of ferritin increased according to dietary iron levels. Among the oxidized oil groups, total amounts and concentrations of hepatic iron as well as those of ferritin were significantly higher in the control rats than in the other two iron groups. It is noted that oxidized oil reduced the total amount of hepatic ferritin, but maintained a constant total amount of hepatic iron in the control and the low Fe groups. On the contrary, oil type did not led to differences in total hepatic iron and total ferritin between the two Fe deficient groups.

At all three iron levels, oxidative stress was ele-

vated by oxidized oil as indicated by TBARS concentration (Table 3). Among the oxidized oil groups, the control group had significantly higher TBARS values than the other two iron groups.

DISCUSSION

Nutrients and diet play a key role in the metabolism of foreign compounds. Alternation of macro-components and many micronutrients have been shown to alter the rate of drug metabolism and influence the toxicological outcome of the ingested foreign compound[21-23]. However, severe iron depletion, either short term (6 wk) or long term (17 wk), does not affect hepatic CYP content[10-12]. These earlier studies did not apply any inducing agents and measured only the constitutive level of CYP. In contrast, a more stringent dietary treatment including a combination of chronic consumption CYP-inducing agents and severe iron restriction was employed in this study. The major finding in this study was that CYP induction is not compromised by mild or severe iron deficiency and the isoform CYP 4A is inducible by oxidized oil.

The microsomal CYP concentration increased at all three iron levels in response to oxidized oil, indicating that the enhanced expression of the CYP proteins was not restricted by iron deficiency. The significantly higher hepatic CYP contents in the control and the low Fe rats reflected the increased metabolic capacity of the liver toward the oxidized oil challenge. Although the total hepatic CYP content did not increase in the iron deficient rats fed oxidized oil, the normalized hepatic CYP content was as high as that of the other two iron groups, implying that the rats would maintain a relative metabolic capacity toward the oxidized oil challenge regardless of the iron status.

This conservation of CYP-inducing ability was at the expense of health. Between the oil groups, slight but further significant hemoglobin reduction occurred only in the low Fe groups when the rats were fed oxidized oil (Table 1), presumably because the hepatic iron store was at the margin of exhaustion (Table 3) and the iron supply to the erythropoietic tissue became limited. Dietary iron deficiency is generally considered to progress in a sequence of three stages, ranging from initial depletion of iron stores, then a decrease in transport iron, and finally diminished production of functional proteins. Dallman et al. demonstrated in a rat study that there is a marked overlap between depletion of storage iron and transport iron, and that hemoglobin depletion could occur before the exhaustion of potentially mobilizable iron stores[24]. Hemoglobin concentration was not affected by oxidized oil in the control rats, because there was ample iron stores as represented by ferritin contents (Table 3) and the iron supply to erythropoiesis was not limited. On the other hand, the hemoglobin concentration in the iron deficient rats was only 25% normal, already too low to be reduced further. Greater growth suppression in the iron deficient rats was demonstrated by a much greater reduction in final body weight, weight gain, and feed efficiency than observed in the control and the low Fe groups. The oxidized oil suppressed the average body weight, weight gain and feed efficiency by 35%, 47% and 20% in the iron deficient rats, while they were suppressed by only 12%, 17% and $<8\%$ in the control and the low Fe groups (Table 1).

CYP conservation in face of a stimulating agent does not necessary imply normal xenobiotic metabolism in iron deficient rats. Diminished aryl hydrocarbon hydroxylase and microsomal epoxide hydrolase activities in phase I reaction and UDP-glucuronyl transferase in phase II reaction occurred in rats subjected to iron deficiency for 17 weeks[11]. Because foreign chemicals may be threatening to life, for the sake of survival, animals may strive to maintain the xenobiotic metabolism capacity in the liver when challenged, but finally, combination of malnutrition and stress will surpass the metabolic adaptation capacity and result in toxicity and tissue damage. These results demonstrated that the conserva-

tion of CYP-inducing ability appeared to occur at the expense of body growth in severe iron restriction and possibly at the expense of other heme proteins in mild iron deficiency, which may exert temporary protection for survival.

CYP isoforms in the liver have been classified into four gene families, designated 1 through 4, based primarily on their amino acid sequences[1]. CYP4 family members have little or no activity towards xenobiotics, but have important roles in the metabolism of endogenous substrates including fatty acids, prostaglandins, and leukotrienes[25]. Hepatic CYP4A in this study was mainly induced by consumption of oxidized oil, because it was extremely low in the fresh oil groups. The inducing agents in the oxidized oil have not been identified. Agents that induce CYP4A members also exhibit peroxisome proliferating activities[25]. It has been suggested that peroxisomes are involved in the gluconeogenetic processes and probably afford the potential of alternative pathways for the conversion of triacylglycerols to glucose, and for the conversion of amino acids and lactate to carbohydrates[26]. In severe iron deficient rats, lactic acidosis occurs as a result of diminished mitochondrial respiratory chains[27]. Therefore, we speculate that peroxisome proliferation may be more or less stimulated in the oxidized oil groups. This process may be transiently beneficial to rats fed on diets containing as high as 15% soybean oil because of the complementary role of peroxisomes and mitochondria in beta-oxidation and gluconeogenesis[26,28].

Similar to the previous report[16], oxidative stress was mainly exerted by oxidized oil, but reduction in hepatic iron and ferritin contents help to slightly reduce the oxidative stress as shown by reduced TBARS values in the low Fe and Fe deficient rats (Table 3). Iron has long been recognized as playing a catalytic role in free radical mediated oxidation of lipids, proteins and DNA[14]. Under normal physiological conditions, the concentration of iron capable of catalyzing these reactions is low, because iron metabolism is tightly controlled and regulated[29]. Ferritin is a protein for iron storage and serves to sequester iron in a relatively inert form. Iron within ferritin exists as a hydrous ferric oxide core that may contain as many as 4500 atoms Fe per molecule, but is normally 20% saturated[30]. Ferritin iron can be mobilized by superoxide-dependent reactions and serve as a source of free iron[13]. When microsomal CYP was induced in rats by treatment with phenobarbital, 3-methylcholanthrene, or 4-methylpyrazole, ferritin increased production of reactive oxygen species with all microsomal preparations, and the increase was completely prevented by superoxide dismutase, indicating that ferritin iron is released by CYP via superoxide production[31]. In the presence of oxidizing agents, ferritin at physiological concentrations may contribute to the risk of oxidative damage, especially in the presence of CYP-inducing agents such as alcohol, polychlorinated biphenyls and oxidized oil as well[32,33].

Consumption of oxidized oil appeared to alter hepatic iron metabolism, since total hepatic ferritin, but not total hepatic iron, was suppressed by oxidized oil in the control and the low Fe rats (Table 3). Homeostasis of intracellular iron in mammalian cells is mostly maintained by a coordinated regulation of the expression of transferrin receptor and ferritin, and iron regulatory proteins (IRPs) play a central role in this regulatory pathway[34]. The activity of IRPs can be mediated by signals other than iron, including cytokines, reactive oxygen species and reactive nitrogen species[34,35]. It has been recently reported that extracellular hydrogen peroxide can activate IRP1 activity and is accompanied by inhibition of ferritin synthesis and by stabilization of the transferrin receptor (TfR) mRNA[36]. In our preliminary studies, we observed that oxidized oil can alter IRP activities and TfR mRNA concentration and contribute to the inhibition of ferritin synthesis (data not shown).

In summary, oxidized oil significantly induces the hepatic expression of CYP. This induction is not compromised by severe iron deficiency, but rather

maintained at the expense of other heme proteins and body growth. Consumption of oxidized oil appears to reduce the hepatic iron stores.

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炸油攝食對缺鐵大鼠肝微粒體細胞色素 P450 誘發之影響

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摘要　本研究探討炸油攝食對輕微與嚴重缺鐵大鼠肝臟微粒體細胞色素 P450 誘發之影響。實驗用炸油爲新鮮黃豆油經 205±5℃油炸麵粉片共 24 小時而得。離乳雄性 Wistar 大鼠隨機分配爲體重相當之三組，分別餵食鐵正常（45 ppm Fe），低鐵（20 ppm Fe）與缺鐵飼料（6 ppm Fe）；飼料依據 AIN-76 配方，其中含黃豆油 15%，並依實驗需要以硫酸亞鐵調整其鐵濃度。經 10 天飼養後缺鐵大鼠血紅素降爲 1.55 mmol/L 而呈貧血症狀，各組再隨機分爲新鮮油與炸油兩組，炸油組飼料以等量炸油取代黃豆油，而鐵量維持不變，繼續飼養 32 天後，犧牲動物取其血液與肝臟進行生化與化學分析。結果可見：炸油組之肝微粒體細胞色素 P450 濃度顯著高於新鮮油組（$p = 0.0001$），炸油各組之肝微粒體細胞色素 P450 總量對體重之比例並不因缺鐵程度而有差異（$p = 0.38$）；鐵營養狀況相同時，炸油明顯抑制體重與飼料效率，尤以對缺鐵組的影響最大；炸油使低鐵組的血紅素濃度明顯降低，也使鐵正常與低鐵組之肝鐵蛋白總量降低，但是不影響其肝鐵總量；炸油各組肝臟之 TBARS 值顯著較新鮮油各組爲高，其中又以鐵正常組明顯高於低鐵與缺鐵組。根據以上結果而知：長期炸油攝食時，肝微粒體細胞色素 P450 之誘發不因輕微或嚴重缺鐵而受限，但是膳食炸油加上缺鐵會嚴重阻礙成長與飼料之利用效率，並且有降低血紅素之虞慮，而且肝鐵的儲存與代謝有因炸油而改變的現象。

關鍵詞：肝臟細胞色素 P450、炸油、缺鐵、肝鐵蛋白、大鼠