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Original Article

Effects of dietary fish oil on cytochrome P450 3A expression in the liver of SHR/NDmcr-cp (*cp*/*cp*) rats, an animal model for metabolic syndrome

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ABSTRACT — Pathophysiological and nutritional conditions often affect the expression of drug-metabolizing enzymes. SHR/NDmcr-cp (*cp*/*cp*) rats (SHR/NDcp) are highly suitable as a metabolic syndrome (MS) model. Nevertheless, little is known about the expression profile of cytochrome P450 (CYP) in the liver of SHR/NDcp. We thus attempted to clarify the expression profile of CYP genes and the effect of fish oil (FO) on this profile in the liver of SHR/NDcp. Lower levels of CYP3A2 mRNA and CYP3A activity (testosterone 6β-hydroxylation) were distinctive features in SHR/NDcp compared with their controls (Wistar Kyoto rats (WKY), spontaneously hypertensive rats (SHR), stroke-prone SHR and lean littermates of SHR/NDcp). Differently from CYP3A2, the expression of other CYP isoforms was largely unchanged in SHR/NDcp. The changes in CYP profile observed in SHR/NDcp are similar to those of patients with diabetes and simple hepatic steatosis. Feeding on FO at a high dose (18.8% in the diet) upregulated CYP3A2 gene expression and CYP3A activity in the liver; the extent of these increases was greater in SHR/NDcp than in WKY and lean littermates of SHR/NDcp. This effect was not observed with FO at a normal dose (5% in the diet). These results indicate that, in the context of the CYP profile, SHR/NDcp is an animal model that is suitable for studying MS and imply that FO intake is critical in determining the efficacy or adverse effects of drugs in patients with MS.

Key words: Fish oil, CYP3A, Liver, SHR/NDmcr-cp (*cp*/*cp*) rats, Metabolic syndrome

INTRODUCTION

Metabolic syndrome (MS) is a cluster of metabolic abnormalities: visceral obesity, hypertension, dyslipidemia and insulin resistance, and patients with MS are susceptible to cardiovascular diseases (Alberti *et al*., 2005; Eckel *et al*., 2005). Owing to the worldwide surge in obesity, diabetes and cardiovascular disease, the increased incidence of MS is a major concern for public health. Since there are some difficulties in exploring the mechanisms underlying MS in humans because of the heterogeneous genetic background and/or lifestyle, well-characterized animal models for MS are required. SHR/NDmcr-cp (*cp*/*cp*) rats (SHR/NDcp) have a genetic background from spontaneously hypertensive rats (SHR) and carry a nonsense mutation of the leptin receptor derived from obese Koletsky rats (Koletsky, 1975). Therefore, SHR/NDcp develop severe hypertension compared with their normotensive controls, Wistar Kyoto rats (WKY), and display severe obesity compared with their lean littermates (SHR/ND+). In addition to obesity and hypertension, SHR/NDcp also exhibit several metabolic disorders, such as hyperglycemia, hyperinsulinemia, hyperlipidemia and hepatic steatosis. Since the phenotype of SHR/NDcp is very similar to that seen in patients with MS, SHR/NDcp are thought to be one of the animal models that are highly suitable for studying MS (Miyata *et al*., 2005; Tanaka *et al*., 2012, 2013).

Drug-metabolizing enzymes play a central role in the metabolism and disposition of drugs, and liver is the major organ for drug biotransformation. In particular, hepatic cytochrome P450 (CYP) plays a major role in the metabolism of many drugs. Pathophysiological conditions are considered to affect the expression and activ-

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ity of CYPs. In fact, it is known that the expression of CYPs is altered in the liver of humans and animal models with diabetes mellitus, obesity and/or hepatic steatosis. With regard to humans, decreased CYP3A activity is associated with hepatic steatosis (Kolwankar *et al*., 2007). Moreover, CYP3A activity and CYP3A4 mRNA level are reduced and CYP2E1 activity is increased in the liver of subjects with diabetes (Dostalek *et al*., 2011). CYP2E1 activity in the liver of patients with obesity and nonalcoholic fatty liver disease is significantly higher than that in controls (Emery *et al*., 2003; Orellana *et al*., 2006). With regard to animal models, CYP2E1 expression is decreased or unchanged in the liver of obese Zucker rats and *ob*/*ob* mice; CYP4A2 expression in obese Zucker rats and the expression of CYP2B10, CYP4A10 and CYP4A14 in *ob*/*ob* mice are up-regulated (Cheng *et al*., 2008; Enriquez *et al*., 1999). The expression of CYP2B10, CYP2C29 and CYP4A10 is increased in the liver of *db*/*db* mice, but no significant changes are found in the expression of either CYP3A11 or CYP2E1(Yoshinari *et al*., 2006). Thus, the findings obtained from studies with experimental animals indicate that the expression or activities of CYPs vary among animal models of MS and that the expression profile of CYPs in these animal models is not necessarily identical to that in patients with MS. With regard to SHR/NDcp, the expression profile of CYPs in the liver has been little studied so far. In addition to pathophysiological states, nutritional conditions may influence the expression of CYPs. In rodents, feeding on fish oil (FO) produces less obesity, suppresses hepatic steatosis and lowers triacylglycerol levels in circulation (Cunnane *et al*., 1986; Saraswathi *et al*., 2009; Sato *et al*., 2010). In humans, increased intake of FO shows protective effects against cardiovascular disease and MS (Belalcazar *et al*., 2010; Carpentier *et al*., 2006). These findings imply that patients with MS should intake FO concurrently with the administration of therapeutic drugs. Nevertheless, to our knowledge, no information is available about the effect of dietary FO on the expression of CYPs in the liver of SHR/NDcp. It is important, therefore, to determine whether increased intake of dietary FO influences the profile of CYPs in the liver of animal models because changes in the activities of CYPs are likely to be critical in determining the efficacy or adverse effects of drugs in these patients. In this context, the current study was designed (1) to characterize the expression profile of CYPs and (2) to explore the effect of FO feeding on the expression of CYPs in the liver of SHR/NDcp.

MATERIALS AND METHODS

Chemicals and reagents

Testosterone and 11α-hydroxyprogesterone were purchased from Tokyo Chemical Industry (Tokyo, Japan). 6β-Hydroxytestosterone was from Sigma-Aldrich (St. Louis, MO, USA). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and $NADP^+$ were from Oriental Yeast (Tokyo, Japan). Safflower oil (SO) was from Summit Oil Mill (Chiba, Japan). FO was from Maruha Nichiro Corporation (Tokyo, Japan). All other chemicals used were of analytical grade.

Animals

All animal procedures were approved by Josai University's Institutional Animal Care Committee in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan). Sixteen-week-old male WKY, SHR, stroke-prone SHR (SHRsp), SHR/ND+ and SHR/NDcp were obtained from SLC (Hamamatsu, Japan). Animals were fed on a standard diet (CE-2; Clea Japan, Tokyo, Japan) *ad libitum* and allowed free access to water. The standard chow contained 4.6% fat. For estimation of the effects of dietary oils (SO and FO) on the expression of CYPs, WKY, SHR/ND+ and SHR/NDcp were fed on a modified AIN-93M diet containing SO or FO at a concentration of 5% (w/w) (normal-fat diet, NFD) or 18.8% (w/w) (high-fat diet, HFD) for 4 weeks. The compositions of these experimental diets and their fatty acid composition are shown in Tables 1 and 2. At the age of 22 weeks, rats were anesthetized with diethyl ether, blood was withdrawn and the liver was immediately excised. One portion of the liver was frozen in liquid nitrogen and stored at -80°C until analysis. The rest (about 10 g) was perfused with ice-cold saline and homogenized with 4 volumes of 0.25 M sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.4) in a Potter-glass homogenizer. The homogenates were subjected to differential centrifugation, as described previously (Karahashi *et al*., 2013). The protein concentrations of microsome preparations were determined by a previously reported method (Lowry *et al*., 1951) with bovine serum albumin (Sigma-Aldrich) as a standard.

Reverse transcription and quantitative real-time polymerase chain reaction

Total RNA was extracted from the liver using QIAzol reagent and RNeasy kit (QIAGEN; Hilden, Germany). Complementary DNA was synthesized from 500 ng of total RNA with avian myeloblastosis virus reverse transcriptase (Takara, Shiga, Japan). Polymerase chain reac-

Table 1. Composition of experimental diets.

Component	SO-NFD	SO-HFD	FO-NFD	FO-HFD
		(%)		
Cornstarch	45.57	31.77	45.57	31.77
Casein	14.00	14.00	14.00	14.00
α-Cornstarch	15.50	15.50	15.50	15.50
Sucrose	10.00	10.00	10.00	10.00
Cellulose	5.00	5.00	5.00	5.00
L-Cystine	0.18	0.18	0.18	0.18
SO	5.00	18.80	1.10	1.10
FO	0.00	0.00	3.90	17.70
Mineral mix (AIN 93M)	3.50	3.50	3.50	3.50
Vitamin mix (AIN 93VX)	1.00	1.00	1.00	1.00
Choline bitartrate	0.25	0.25	0.25	0.25

Dietary oils contained 0.0010 g of *tert*-butylhydroquinone per 5.0 g of oil and 0.00376 g of *tert*-butylhydroquinone per 18.8 g of oil.

Table 2. Fatty acid composition of experimental diets.

Fatty acid	CE-2	SO-NFD	SO-HFD	FO-NFD	FO-HFD
			(mol %)		
16:0	23.29	7.09	7.09	11.40	9.28
16:1*n*-7	1.96	0.15	0.15	9.96	10.38
18:0	2.42	2.52	2.52	6.20	5.67
18:1*n*-9	21.93	13.69	13.69	13.69	12.89
18:2*n*-6	42.18	75.78	75.78	24.20	7.96
18:3*n*-3	3.60	0.16	0.16	0.73	0.76
20:4n-6	0.16	0.01	0.01	1.14	1.65
20:5*n*-3	2.87	0.28	0.28	21.31	32.88
22:5*n*-3	0.26	0.15	0.15	1.79	3.42
22:6*n*-3	1.34	0.17	0.17	6.98	12.98

The fatty acids are designated by the numbers of carbon atoms and double bonds; palmitic acid, 16:0; palmitoleic acid, 16:1*n*-7; stearic acid, 18:0; oleic acid, 18:1*n*-9; linoleic acid, 18:2*n*-6; α-linolenic acid, 18:3*n*-3; arachidonic acid, 20:4*n*-6; 5,8,11,14,17-eicosapentaenoic acid, 20:5*n*-3; 7,10,13,16,19-docosapentaenoic acid, 22:5*n*-3; 4,7,10,13,16,19-docosahexaenoic acid, 22:6*n*-3.

tion amplification was carried out using SYBR Premix ExTaq (Takara). The amplification and detection were performed with a Step One Plus real-time PCR system (Life Technologies, Carlsbad, CA, USA). The pair of forward and reverse primers used in this study was designed using Primer Express 2.0 software (Life Technologies). Each primer was searched for using an NCBI BLAST homology search to ensure specificity to the target mRNA transcript. The primers were synthesized by Sigma Genosys (Ishikari, Hokkaido, Japan). The sequences of the primers used in this study are listed in Table 3. The thermal cycling program was as follows: a 15 sec denaturation step at 95°C, followed by 50 cycles of 5 sec denaturation at 95°C and 34 sec annealing and extension at 60°C. Changes in gene expression were calculated by using the comparative threshold cycle (Ct) method. Ct values were first normalized by subtracting the Ct value obtained from β-actin (control).

Assay for testosterone 6β-hydroxylation activity

Testosterone 6β-hydroxylation activity was determined by the quantification of 6β-hydroxytestosterone forma-

Table 3. Sequences of primers used for real-time PCR.

Gene	Accession No.	Sequence	Product size
CYP1A2	NM_012541	F : CCATGATGAGAAGCAGTGGA	113
		R : CCGAAGAGCATCACCTTCTC	
CYP2C11	NM_019184	F : TGGGATGCAATGGAAGGAGA	70
		R : TCTTGCCCATCCCAAAAGCT	
CYP2D1	NM_153313	F : AGAGTTTGACAGAAGTCTCTG	139
		R : AGCCAGCAGGTTATCCAGTAA	
CYP2D2	NM_012730	F : GGCAAAGTCTTCCCCAAGCTCA	117
		R : GGAAGGCATCAGTCATGTCTCG	
CYP2D4	NM_138515	F : CAGTGCCGAGTACAACAGGA	230
		R : CAGCACTGAGGACAGGTTGA	
CYP2E1	NM_031543	F : CGTGTGTGTGTTGGAGAAGG	139
		R : TACTGCCAAAGCCAACTGTG	
CYP3A1	NM_013105	F : TACTGCCAAAGCCAACTGTG	168
		R : CATAGGTGGGAGGTGCCTTA	
CYP3A2	NM_153312	F : GCTCATAATAATTCCAAAGACGAAGTG	147
		R : GGTGAGTGGCCAGGAAATACAA	
CYP4A10	NM_153307	F : TCAGGTGGATGGAAATTACA	108
		R : ATAGATGGTATCATTCTGAT	
CYP4A14	BC098705	F : ATTCCAGGTCCTACACCAAG	112
		R : GCCATCAGAGGACATATTGT	
β-actin	V01217	F : TGCAGAAGGAGATTACTGCC	220
		R : CGCAGCTCAGTAACAGTCC	

All sequences are shown in the 5' to 3' direction.

tion from testosterone, as described previously (Guo *et al*., 2000), with some modifications. Briefly, the reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), 250 μM testosterone, an NADPH-generating system (1.5 mM $NADP^+$, 15 mM glucose-6-phosphate, 15 mM $MgCl_2$, 0.1 units/mL glucose-6-phosphate dehydrogenase) and 0.1 mg of microsomal protein in a final volume of 0.5 mL. Incubation was carried out at 37°C for 10 min. The reaction was terminated by the addition of 2 mL of ice-cold ethyl acetate. To the mixture, an internal standard (11α-hydroxyprogesterone, 1.5 nmol) was also added at the end of the incubation, and then the samples were immediately vigorously vortex-mixed. After centrifugation at 1200 g for 5 min, the organic layer was transferred and evaporated to dryness under a stream of nitrogen. 6β-Hydroxytestosterone was quantified by high-performance liquid chromatography, as described previously (Brunner *et al*., 1996), with some modifications. In brief, a Shimadzu high-performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) was employed. This system consisted of an LC-10AT pump, a system controller (SCL 10Avp) and a UV detector (SPD-10A). The column, an Inertsil-ODS2 column (4.6 mm × 150 mm I.D.) (GL Sciences, Tokyo, Japan), was kept at a constant temperature of 40°C. The separation of testosterone and 6β-hydroxytestosterone was achieved at a flow rate of 1.5 mL/min by gradients of mobile phase A consisting of methanol-water-acetonitrile (39:60:1, v/v/v) and mobile phase B consisting of methanol-water-acetonitrile (80:18:2, v/v/v). The gradient of mobile phase B was as follows: 0 min, 10%; 0 to 22 min, 10 to 85%; and 22 to 30 min, 85%. The analytes were monitored at a wavelength of 238 nm. 6β-Hydroxytestosterone was quantified using a calibration curve plotted as the peak area ratios (6β-hydroxytestosterone: internal standard) *vs*. the amount of 6β-hydroxytestosterone with those obtained using authentic standards.

Statistical analysis

Data are reported as mean ± S.D. One-way ANOVA was used to test for significant differences between groups. Where differences were significant, the statistical significance ($p < 0.05$) between any two means was determined using Scheffé's multiple range test as a *post-hoc* analysis (Stat View; SAS Institute, Cary, NC, USA).

RESULTS

Profiling of CYP gene expression in the liver of SHR/NDcp

The differences in the expression levels of mRNA encoding CYP in the liver among WKY, SHR, SHRsp, SHR/ND+ and SHR/NDcp were estimated (Table 4). The levels of CYP1A2 mRNA in SHR, SHR/ND+ and SHR/NDcp were significantly lower than that in WKY. The expression level of the CYP3A2 gene in SHR/NDcp was markedly low (35% of that in WKY); in addition, the mRNA level of CYP3A1 tended to be slightly higher than those in other four groups. There were no significant differences in the levels of CYP3A1 and CYP3A2 mRNA among the other four groups of rats. The expression of the CYP4A10 gene in SHR/NDcp was 2.8-fold higher than that in WKY, but there was no significant difference in CYP4A10 expression between SHR/NDcp and SHR/ND+. In contrast to CYP4A10, the mRNA level of CYP4A14 in SHR/NDcp was 28% of that in WKY, but there were no significant differences in the levels among SHR, SHRsp, SHR/ND+ and SHR/NDcp. No significant differences were found in the mRNA levels of CYP2C11, CYP2D1, CYP2D2, CYP2D4 and CYP2E1 among the five groups. The activities for testosterone 6β-hydroxylation in the hepatic microsomes of WKY, SHR, SHRsp, SHR/ND+ and SHR/NDcp were measured (Fig. 1). The testosterone 6β-hydroxylation activity in hepatic microsomes of SHR/NDcp was significantly lower than those in the other four groups (75% of that in WKY).

Effect of FO feeding on mRNA expression of CYPs in the liver of SHR/NDcp

The effects of dietary FO on the mRNA levels of CYP3A1 and CYP3A2 in the liver were compared among WKY, SHR/ND+ and SHR/NDcp in comparison with SO feeding (Fig. 2). In the present experiment, to prevent essential fatty acid deficiency in the animals, NFD and HFD were supplemented with 1.1 g of SO per 100 g diet. There was no significant difference in the mRNA levels of CYP3A2 between SO-NFD-fed and SO-HFD-fed groups (Fig. 2B). The mRNA level of CYP3A2 in WKY fed on FO-NFD was 2.3-fold greater than that in WKY fed on SO-NFD. However, in SHR/ND+ and SHR/NDcp, no significant differences were observed in the expression of the CYP3A2 gene between the SO-NFD-fed and FO-NFD-fed groups. Feeding on FO-HFD significantly up-regulated the gene expression of CYP3A2 in WKY (1.7-fold) and SHR/ND+ (2.1-fold) compared with that in the corresponding SO-HFD group. The mRNA level of CYP3A2 in SHR/NDcp fed on FO-HFD was 5.8-fold greater than that in SHR/NDcp fed on SO-HFD. On the other hand, the expression of CYP3A1 in the liver of SHR/NDcp was not considerably changed by feeding on FO-HFD (Fig. 2A). Moreover, feeding on FO-HFD left the gene

Table 4. Gene expression of CYPs in the liver of WKY, SHR, SHRsp, SHR/ND+ and SHR/NDcp.

Genes	WKY	SHR	SHRsp	SHR/ND+	SHR/NDcp
CYP1A2	1.00 ± 0.20^{a}	0.47 ± 0.12^{b}	0.84 ± 0.02^{ab}	0.48 ± 0.02^{b}	0.48 ± 0.17^{b}
CYP2C11	1.00 ± 0.46	1.28 ± 0.13	0.93 ± 0.22	1.17 ± 0.14	1.04 ± 0.16
CYP2D1	1.00 ± 0.40	1.44 ± 0.09	1.08 ± 0.17	1.50 ± 0.30	1.35 ± 0.11
CYP2D2	1.00 ± 0.44	1.62 ± 0.07	1.37 ± 0.13	1.24 ± 0.02	1.41 ± 0.09
CYP2D4	1.00 ± 0.57	1.33 ± 0.25	1.14 ± 0.26	1.39 ± 0.05	1.12 ± 0.24
CYP2E1	1.00 ± 0.28	1.18 ± 0.14	1.20 ± 0.29	1.23 ± 0.17	0.82 ± 0.19
CYP3A1	1.00 ± 0.11^{ab}	0.96 ± 0.13^{ab}	0.91 ± 0.49^{a}	0.89 ± 0.10^{a}	1.66 ± 0.31^{b}
CYP3A2	1.00 ± 0.36^{a}	1.33 ± 0.09^{a}	0.96 ± 0.13^{ab}	1.25 ± 0.42^{a}	0.35 ± 0.03^{b}
CYP4A10	1.00 ± 0.34^{a}	0.75 ± 0.45^{a}	1.53 ± 0.26^{ab}	2.21 ± 0.44^{bc}	2.83 ± 0.70^{c}
CYP4A14	1.00 ± 0.26^{a}	0.21 ± 0.10^{b}	0.42 ± 0.07^{b}	0.31 ± 0.10^{b}	0.28 ± 0.05^{b}

Rats were fed on a standard diet. Values represent mean ± S.D. of four rats. Differences in means in the same row without a common superscript (a, b, c) are statistically significant ($p < 0.05$). In the absence of a superscript, the differences in the means are not significant ($p > 0.05$).

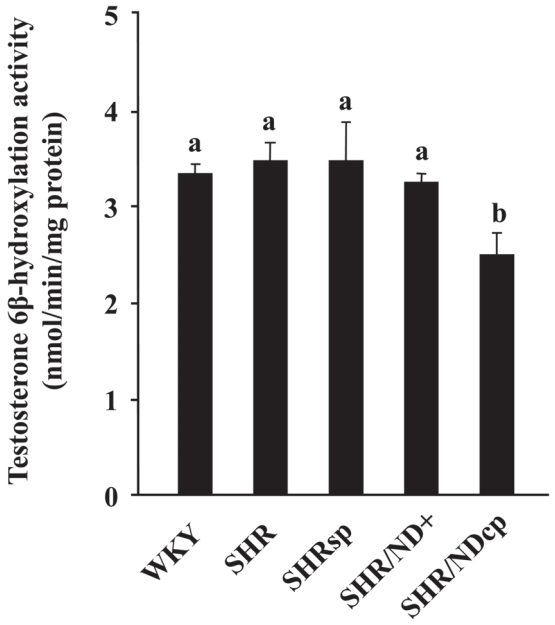

Fig. 1. Testosterone 6β-hydroxylation activities in hepatic microsomes of WKY, SHR, SHRsp, SHR/ND+ and SHR/NDcp. Rats were fed on a standard diet. Values represent mean ± S.D. of four rats. [a,b]Differences in the means without a common superscript are statistically significant ($p < 0.05$).

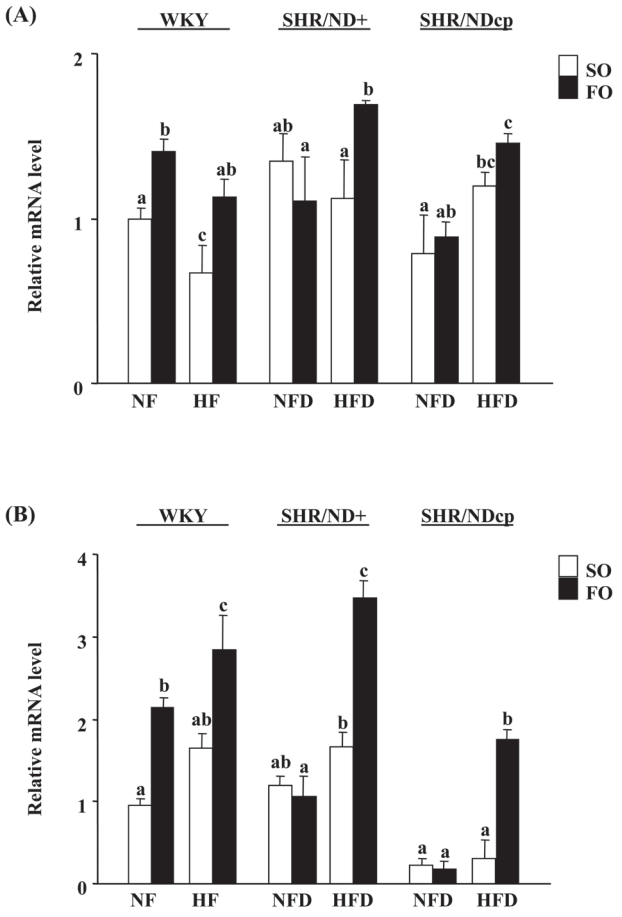

Fig. 2. Effects of dietary FO on the levels of mRNA encoding CYP3A1 and CYP3A2 in the liver of WKY, SHR/ND+ and SHR/NDcp. Rats were fed on SO-NFD, SO-HFD, FO-NFD or FO-HFD for 4 weeks. (A) CYP3A1; (B) CYP3A2. Values represent mean ± S.D. of four rats. [a,b,c]Differences in the means without a common superscript are statistically significant ($p < 0.05$). Open column, SO; closed column, FO; SO, safflower oil; FO, fish oil; NFD, normal-fat diet (diet containing 5% dietary oil); HFD, high-fat diet (diet containing 18.8% dietary oil).

expression of other CYP isoforms such as CYP1A2, CYP2E1, CYP4A10 and CYP4A14 largely unchanged in the liver of SHR/NDcp (unpublished data).

Effect of FO feeding on CYP3A-mediated testosterone 6β-hydroxylation in the liver of SHR/NDcp

The effect of FO-HFD on testosterone 6β-hydroxylation activity in microsomes of the liver was estimated in comparison with SO feeding (Fig. 3). In WKY, SHR/ND+ and SHR/NDcp, the testosterone 6β-hydroxylation activity was not increased by SO-HFD feeding; the activities rather tended to be decreased in WKY, SHR/ND+ and SHR/NDcp. The activity in SHR/NDcp fed on FO-NFD was 1.5-fold higher than that fed on SO-NFD, whereas no significant changes were observed in WKY and SHR/ND+ in response to FO-NFD feeding. Feeding on FO-HFD elevated the activity in WKY (2.0-fold), SHR/ND+ (1.7-fold) and SHR/NDcp (3.8-fold) compared with that in the corresponding rats fed on SO-HFD. Figure 4 shows the relationship between the mRNA level of CYP3A2 in the liver and the testosterone 6β-hydroxylation activity in hepatic microsomes. There was a strong correlation between these two variables ($r^2 = 0.6313$). On the other hand, no significant correlation was observed between the mRNA level of CYP3A1 in the liver and the testosterone 6β-hydroxylation activity ($r^2 = 0.2333$). These results strongly indicated that the testosterone 6β-hydroxylation activity in the liver reflects the mRNA level of CYP3A2, but not CYP3A1.

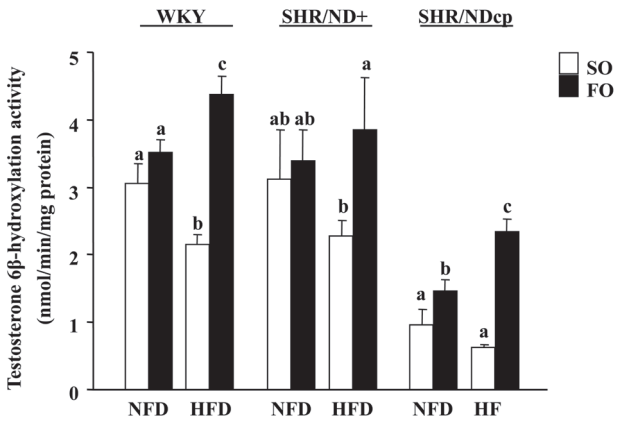

Fig. 3. Effects of FO on the testosterone 6β-hydroxylation activities in hepatic microsomes of WKY, SHR/ND+ and SHR/NDcp. Rats were fed on SO-NFD, SO-HFD, FO-NFD or FO-HFD for 4 weeks. Values represent mean ± S.D. of four rats. [a,b,c]Differences in the means without a common superscript are statistically significant ($p < 0.05$). Open column, SO; closed column, FO; SO, safflower oil; FO, fish oil; NFD, normal-fat diet (diet containing 5% dietary oil); HFD, high-fat diet (diet containing 18.8% dietary oil).

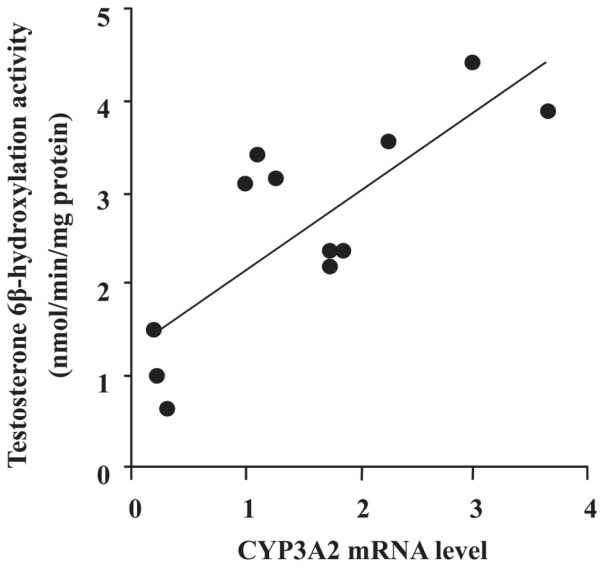

Fig. 4. Relationship between CYP3A2 mRNA level and testosterone 6β-hydroxylase activity in hepatic microsomes. The relationship between CYP3A2 mRNA level (data from Fig. 2B) and testosterone 6β-hydroxylation activity (data from Fig. 3) was determined as $y = 0.8557x + 1.2847$ ($r^2 = 0.6313$).

DISCUSSION

Previous studies have shown the altered gene expression of CYPs in the liver of genetically obese and/or diabetic animals (Cheng *et al*., 2008; Enriquez *et al*., 1999; Yoshinari *et al*., 2006). However, little information is available about the expression profile of CYPs in the liver of rodents of an MS model because of the lack of model animals that are adequate for displaying combined metabolic disorders, a symptom that is characteristic of MS. Recently, SHR/NDcp have been shown to be highly suitable as an MS model (Kawai *et al*., 2012; Tanaka *et al*., 2013). It is therefore of interest to understand the alteration occurring in the CYP profile in the liver of SHR/NDcp. The present study showed that there were considerable differences in the profile of hepatic expression of CYP genes between SHR/NDcp and the corresponding controls (WKY, SHR, SHRsp and SHR/ND+) when the rats fed on standard chow. From the comparison, the most notable observation was that the expression of the CYP3A2 gene and CYP3A activity, estimated as testosterone 6β-hydroxylation, in the liver of SHR/NDcp were significantly lower than those of the other four groups of rats. SHR/NDcp is known to exhibit insulin resistance and hyperinsulinemia (Kawai *et al*., 2012); moreover, our previous study revealed that SHR/NDcp is associated with simple hepatic steatosis but not steatohepatitis (Tanaka *et al*., 2013). Therefore, these results are consistent with the findings of previous studies with human subjects, results that show lower CYP3A activity in the liver of diabetes (Dostalek *et al*., 2011) and a significant association between CYP3A activity and the severity of hepatic steatosis (Kolwankar *et al*., 2007). In contrast to SHR/NDcp, decreased expression of CYP3A is not evident in other animal models such as obese Zucker rats, *ob/ob* mice and *db/db* mice (Cheng *et al*., 2008; Enriquez *et al*., 1999; Yoshinari *et al*., 2006). Since it is considered that CYP3A4 is responsible for the metabolism of about half of all drugs in use (Evans and Relling, 1999; Guengerich, 1999), it should be taken into consideration that the reduced activity of CYP3A may play a role in the altered efficacy and adverse effects of drugs that are substrates for CYP3A. In this context, SHR/NDcp is an adequate model for MS in basic research on drug metabolism under MS.

In the present study, we also investigated the effect of FO intake on the expression of the CYP3A2 gene in the liver employing SHR/NDcp as an MS model because *n*-3 polyunsaturated fatty acids, which are enriched in FO, have beneficial effects on the systemic homeostasis of lip-

id and glucose in MS (Carpentier *et al*., 2006). Although docosahexaenoic acid has been demonstrated to inhibit CYP3A activity *in vitro* (Hirunpanich *et al*., 2006, 2007), little is known about the *in vivo* effects of the chronic intake of FO on drug-metabolizing enzymes in SHR/NDcp. The present study clearly showed that FO-HFD (diet containing 18.8% oil) feeding resulted in up-regulation of the expression of the CYP3A2 gene and the activity of CYP3A-mediated testosterone 6β-hydroxylation in the liver of WKY, SHR/ND+ and, to the most marked extent, SHR/NDcp. It is noteworthy that this recovery effect was not observed with FO-NFD (diet containing 5% oil). With regard to regulation of the expression of CYP genes, it is widely considered that the nuclear receptors participate in the transcription of their genes. A previous study demonstrated that linoleic acid or SO up-regulates CYP3A expression through the activation of constitutive androstane receptor (Finn *et al*., 2009); however, FO rather contains less linoleic acid (Table 2). Pregnane X receptor is also known to be involved in the induction of CYP3A (Aleksunes and Klaassen, 2012; Sueyoshi and Negishi, 2001; Waxman, 1999). To our knowledge, however, evidence that fatty acids are capable of binding to pregnane X receptor has not been reported. At present, therefore, the precise mechanism by which the expression of the CYP3A2 gene is down-regulated in the liver of SHR/NDcp and dietary FO recovers the suppressed CYP3A2 expression remains to be elucidated.

In conclusion, the present study has produced two notable findings. Firstly, in the context of the expression profile of CYPs in the liver, SHR/NDcp is likely to be an adequate model for MS. Importantly, CYP3A2 gene expression and CYP3A-mediated testosterone 6β-hydroxylation activity were significantly down-regulated in the liver of SHR/NDcp. Secondly, the present study clearly demonstrated that FO intake at a large dosage recovered the suppressed CYP3A2 gene expression and CYP3A-mediated testosterone 6β-hydroxylation activity in the liver of SHR/NDcp. The inducing effects of FO on the expression of the CYP3A 2 gene in SHR/NDcp were much more prominent than those in WKY and SHR/ND+. Taking these findings together, the suppressed activity of CYP3A in patients with MS is likely to be causally linked to the adverse effects due to overdoses of many drugs that are substrates of CYP3A, because CYP3A is the major drug-metabolizing enzyme in human liver. Moreover, it is conceivable that the dietary intake of FO can recover the suppressed CYP3A activity in the liver; however, this is not necessarily beneficial because this recovery may enhance the metabolism of drugs in use, so FO intake concurrently with drug treatment seems to be likely to cause a decrease in therapeutic efficiency of such drugs.

Conflict of interest---- The authors declare that there is no conflict of interest.

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