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Characterization of Fatty Acid Profile in the Liver of SHR/NDmcr-cp (*cp*/*cp*) Rats, a Model of the Metabolic Syndrome

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The fatty acid profile of hepatic lipid in spontaneously hypertensive rats (SHR)/NDmcr-cp (*cp*/*cp*) rats (SHR/NDcp), which offer an animal model of the metabolic syndrome, was characterized by comparing those in Wistar Kyoto rats (WKY), SHR, stroke-prone spontaneously hypertensive rats (SHRSP) and SHR/NDmcr-cp (+/+) rats (SHR/ND+) . Hierarchical clustering analysis revealed that SHR/NDcp and the other four strains and/or substrains of rats were clearly disparate in fatty acid profile of hepatic lipid and that the disparity observed was due to the drastic increases in the mass of monounsaturated fatty acids, especially palmitoleic acid and oleic acid, in the liver of SHR/NDcp. Activities of stearoyl-CoA desaturase (SCD) and palmitoyl-CoA chain elongase in hepatic microsomes of SHR/NDcp were markedly higher than those of WKY, SHR, SHRSP and SHR/ND+. Activities of palmitoleoyl-CoA chain elongase in the liver of SHR/NDcp were also higher, but to a lesser extent. mRNA levels of SCD1 and elongation of very long-chain fatty acids (Elovl6), but not Elovl5, in the liver of SHR/NDcp were remarkably higher than those of the other four groups of rats. These results suggest that the enhanced expressions of SCD1 and Elovl6 induced abnormalities in fatty acid profile in the liver of SHR/NDcp.

Key words fatty acid profile; palmitoleic acid; oleic acid; liver; SHR/NDmcr-cp (*cp*/*cp*) rat

Obesity, hypertension, hyperlipidemia and hyperglycemia are risk factors for cardiovascular diseases. Patients who have two or more of these risk factors are susceptible to cardiovascular diseases; these states are called the metabolic syndrome. To explore protection against and treatment of the metabolic syndrome, studies on the pathophysiology and metabolic consequences of the metabolic syndrome are required using pertinent animal models. Spontaneously hypertensive rats (SHR)/NDmcr-cp (*cp*/*cp*) rats (SHR/NDcp) spontaneously develop obesity and hypertension, and display dyslipidemia and hyperglycemia, the values of body weight, systolic blood pressure, serum triglyceride and blood glucose in SHR/NDcp being 1.43, 1.65, 25.4 and 1.25 times, respectively, compared with those in Wistar Kyoto rats (WKY) (control rats) at 19 or 20 weeks of age.[1] Thus, they provide a useful model of the metabolic syndrome.[2,3] Hypertensive rats that spontaneously developed as a colony from normotensive WKY, were named SHR.[4] By crossing SHR with Sprague-Dawley (SD) rats, obese spontaneously hypertensive rats, Koletsky strain, were obtained; this strain of rats is heterozygous for the *cp* gene.[5] To eliminate the noncorpulent genes of the Koletsky strain, the rats were mated with SHR, and a congenic rat strain (SHR/National Institutes of Health (NIH)-corpulent) was developed.[6] SHR/NDcp are a substrain of SHR/NIH-corpulent rats. With regard to metabolic abnormalities as to hypertension, hyperinsulinemia and hyperglycemia in SHR/NDcp, many studies have been conducted. On the other hand, information on dyslipidemia in SHR/NDcp is very limited.[1,7] To our knowledge, no information is available on fatty acid metabolism in the liver, in particular. To establish SHR/NDcp as a useful model of the metabolic syndrome, it is necessary to document characteristics of fatty acid metabolism in the liver, because hepatic fatty acid composition is considered to be a determinant of insulin sensitivity that acts independently of cellular energy balance and stress.[8] The aims of this study are (i) to characterize the fatty acid profile in the liver of SHR/NDcp and (ii) to demonstrate the mechanism underlying the abnormalities in hepatic fatty acid composition.

MATERIALS AND METHODS

Chemicals The following materials were obtained from the indicated commercial sources: [2-^{14}C]malonyl-CoA (56.0 Ci/mol) (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, U.S.A.); palmitoyl-CoA, palmitoleoyl-CoA, stearoyl-CoA, malonyl-CoA and bovine serum albumin (essentially fatty acid-free for enzyme assays and fraction V for protein assay) (Sigma Aldrich Japan, Tokyo, Japan); nicotinamide adenine dinucleotide reduced (NADH) (Oriental Yeast Co., Tokyo, Japan); nonadecanoic acid (Nu-Chek-Prep Inc., Elysian, MN, U.S.A.). All other chemicals used were of analytical grade.

Animals Fifteen-week-old male WKY, SHR, stroke-prone spontaneously hypertensive rats (SHRSP), SHR/NDcp and nonobese hypertensive littermates of SHR/NDcp (SHR/NDmcr-cp (+/+) rats (SHR/ND+)) 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. After acclimatization, rats were killed at the age of 22 weeks under diethyl ether anesthesia, and livers were excised. One part of the liver was frozen in liquid nitrogen and then stored at −80°C for determination of mRNA. The other part of the liver was perfused with ice-cold 0.9% NaCl. Liver was homogenized with 4 volumes of 0.25 M sucrose/1 mM ethylenediaminetetraacetic acid (EDTA)/10 mM Tris–HCl (pH 7.4) in a Potter glass-Teflon homogenizer. An aliquot of the homogenates was frozen in liquid nitrogen and stored at −80°C for analyses of fatty acids. The remaining

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Table 1. Sequences of Primers Used for Real-Time PCR

	Primer (5′—3′)	Accession No.
SCD1	F: TCACCTTGAGAGAAGAATTAGCA	J02585
	R: TTCCCATTCCCTTCACTCTGA	
SCD2	F: TGCACCCCCAGACACTTGTAA	AB032243
	R: GGATGCATGGAAACGCCATA	
Fads1	F: AACTGGTTTGTGTGGGTGACG	NM_053445
	R: GAGACCCAGTCCACATTCCG	
Fads2	F: GCCACTTAAAGGGTGCCTCC	BC081776
	R: TGCAGGCTCTTTATGTCGGG	
Elovl1	F: AGCACTTCGGATGGTTCGAGT	BC085795
	R: GAGTGGTGGAAGACATGGAGGA	
Elovl2	F: TATTCTTGCTTGCCCGTGAGA	NM_001109118
	R: CTGCCATTGTTGATCTGCCA	
Elovl5	F: ACCACCATGCCACTATGCTCA	AB071985
	R: GGACGTGGATGAAGCTGTTGA	
Elovl6	F: AGAACACGTAGCGACTCCGAA	AB071986
	R: CAAACGCGTAAGCCCAGAAT	
β-Actin	F: TGCAGAAGGAGATTACTGCC	V01217
	R: CGCAGCTCAGTAACAGTCC	

homogenates were centrifuged at 18000×***g*** for 20 min, and the supernatant was centrifuged under the same conditions. The resulting supernatant was centrifuged at 105000×***g*** for 60 min. The obtained pellet was resuspended in 0.25 M sucrose/0.1 mM EDTA/10 mM Tris–HCl (pH 7.4) and recentrifuged under the same conditions. The resulting pellet was resuspended in a small volume of 0.25 M sucrose/0.1 mM EDTA/10 mM Tris–HCl (pH 7.4) and used as microsomes. All operations were carried out at 0—4°C. Protein concentrations were determined by the method of Lowry *et al.*[9] using bovine serum albumin as a standard. All animal studies complied with the regulations of the institutional board for animal studies, Josai University.

Quantitative Fatty Acid Profiling After the addition of a known amount of nonadecanoic acid as an internal standard, total lipid was extracted from liver homogenates according to the method of Bligh and Dyer.[10] After the solvent of lipid extract was evaporated, 1 L of 10% (w/v) KOH/90% methanol was added to the obtained residue, and then the mixture was heated at 80°C for 60 min for saponification under a nitrogen atmosphere. After the addition of 4 mL of water, non-saponified materials were removed three times by the extraction with *n*-hexane. The aqueous phase was acidified by the addition of 6 M HCl, and free fatty acids were extracted three times with *n*-hexane. The extract was taken to dryness, and to the residue was added 1 mL of 14% (w/v) boron trifluoride/methanol. The mixture was heated at 100°C for 10 min under a nitrogen atmosphere. After the addition of 4 mL of water, fatty acid methyl esters were extracted three times with *n*-hexane. All solvents used contained 0.005% (w/v) butylated hydroxytoluene. The mass and proportion of the fatty acid methyl esters were determined by GLC [Shimadzu GC-2014 (Kyoto, Japan)], equipped with a flame ionization detector and Supelcowax 10 column (0.53 mm×30 m).

Enzyme Assays Stearoyl-CoA desaturase (SCD) activity was assayed spectrophotometrically by the method of Oshino *et al.*[11] as the stearoyl-CoA stimulated re-oxidation of NADH-reduced cytochrome b_5. The rate of cytochrome b_5 oxidation was measured by recording the changes in absorbance between 424 and 409 nm at 30°C. The cuvette contained 1.2 mg of microsomal protein and 100 mM Tris–HCl (pH 7.4) in a final volume of 3 mL. Cytochrome b_5 was reduced by adding 2 nmol NADH and the re-oxidation was recorded. When the re-oxidation was completed, 20 nmol stearoyl-CoA was added, and cytochrome b_5 was reduced again using 2 nmol NADH. The first-order rate constant for the re-oxidation of NADH-reduced cytochrome b_5 was calculated as described by Oshino and Sato.[12] The rate constant for the re-oxidation of cytochrome b_5 was measured in the presence (k) and in the absence (k^-) of stearoyl-CoA; the rate constant for SCD was given by $k^+=k-k^-$.[13]

Palmitoyl-CoA chain elongase (PCE) and palmitoleoyl-CoA chain elongase (POCE) were assayed as the activities of microsomal condensation of palmitoyl-CoA or palmitoleoyl-CoA with malonyl-CoA by measuring the incorporation of [2-^{14}C]malonyl-CoA into the exogenous acyl-CoAs essentially according to a method reported previously.[14] The assay mixture contained 15 nmol palmitoyl-CoA or palmitoleoyl-CoA, 100 nmol [2-^{14}C]malonyl-CoA (20 nCi), 12 nmol bovine serum albumin, 0.5 μmol KCN, 250 μg of microsomal protein and 100 mM Tris–HCl (pH 7.4) in a total volume of 0.5 mL. The mixture was incubated under a nitrogen atmosphere at 37°C for 4 min, and the reaction was stopped by adding 1 mL of 10% KOH/90% methanol. The mixture was heated at 80°C for 30 min under a nitrogen atmosphere and then acidified by adding 3 mL of 4 M HCl. The condensation products were extracted four times with 2 mL of *n*-hexane. The combined *n*-hexane extract was washed with 4 mL of acidic water, transferred to a counting vial, and dried under nitrogen. The residue was dissolved in toluene-based scintillator, and the radioactivity was measured using a liquid scintillation counter. The control value, which was obtained from the incubation without acyl-CoA, was subtracted to give the net condensation rate for palmitoyl-CoA or palmitoleoyl-CoA.

RNA Extraction and Quantification of Gene Expression Total RNA was isolated from liver tissues using QIAzol reagent and RNeasy kit (QIAGEN, Hilden, Germany). cDNA was synthesized from 500 ng of total RNA with avian myeloblastosis virus reverse transcriptase (Takara, Shiga, Japan). Polymerase chain reaction (PCR) amplification was carried out using SYBR Premix EX Taq (2x) (Perfect Real Time, Takara, Shiga, Japan). The amplification and detection were performed with Applied Biosystems 7500 real time PCR system (Life Technologies Corp., Carlsbad, CA, U.S.A.). The thermal cycling program was as follows: 10 s denaturation step at 95°C followed by 50 cycles of 5 s denaturation at 95°C, and 34 s annealing/extension at 60°C. Melting curve analysis was performed to confirm the real-time PCR products. Changes in gene expression were calculated by using the comparative threshold cycle (C_t) method. C_t values were first normalized by subtracting the C_t value obtained from β-actin (control). The sequences of primers used in this study are listed in Table 1.

Statistical Analyses Homogeneity of variance was established using one-way analysis of variance. When a difference was significant ($p<0.05$), Scheffé's multiple range test was used as a *post-hoc* test. The results were considered to be significant if the value of p was <0.05. Hierarchical clustering analysis was performed to evaluate where differences existed in the fatty acid profiles by using JMP 5.1 (SAS Institute Japan, Tokyo, Japan).

Table 2. Fatty Acid Profile of Hepatic Lipid of WKY, SHR, SHRSP, SHR/ND+ and SHR/NDcp

	WKY	SHR	SHRSP	SHR/ND+	SHR/NDcp
			(μmol/g liver)		
16:0	21.73±2.75[a]	23.98±0.99[a]	23.33±1.63[a]	25.01±1.22[a]	72.90±26.68[b]
16:1n-7	0.84±0.39[a]	1.12±0.14[a]	0.82±0.26[a]	1.16±0.22[a]	21.15±7.63[b]
18:0	14.27±1.08	13.75±0.94	15.49±1.81	14.62±0.79	13.24±1.25
18:1n-9	6.36±1.67[a]	7.42±0.60[a]	5.17±0.70[a]	8.00±0.82[a]	57.79±24.76[b]
18:1n-7	3.75±0.47[a]	4.36±0.38[a]	2.36±0.33[a]	3.70±0.12[a]	12.56±3.78[b]
18:2n-6	17.17±1.34[a]	21.50±1.51[a]	16.45±1.27[a]	20.00±2.85[a]	39.14±15.21[b]
18:3n-3	0.16±0.03[a]	0.40±0.07[a]	0.25±0.06[a]	0.53±0.29[a]	1.57±0.58[b]
20:3n-9	0.04±0.02	0.02±0.01	0.08±0.07	0.20±0.22	0.26±0.11
20:3n-6	0.60±0.03[a]	0.74±0.18[a]	0.57±0.15[a]	0.63±0.08[a]	3.30±1.11[b]
20:4n-6	16.41±1.75[ab]	15.15±1.11[ab]	13.94±2.44[ab]	17.31±2.60[a]	12.79±1.05[b]
20:5n-3	0.76±0.35[ab]	0.89±0.22[ab]	0.93±0.46[ab]	0.61±0.08[a]	1.59±0.58[b]
22:5n-3	1.23±0.34	2.00±1.13	1.25±0.30	1.39±0.05	2.35±0.94
22:6n-3	4.28±0.52	4.32±0.48	5.08±0.48	4.42±0.36	5.22±1.05
Total	87.58±6.87[a]	95.65±3.88[a]	85.72±3.20[a]	97.57±7.61[a]	243.84±83.25[b]
Total (μmol/liver)	1247.13±268.36[a]	1439.66±121.08[a]	1089.95±80.93[a]	1517.02±113.12[a]	7707.14±3742.40[b]

Values represent mean±S.D. for 4 or 5 rats. Differences in horizontal means without a common superscript (a, b) are significant ($p<0.05$). If no superscript appears, the differences in the means are not siginificant ($p>0.05$). The fatty acids are designated by the numbers of carbon atoms and double bonds; palmitic acid, 16:0; palmitoleic acid, 16:1n-7; stearic acid, 18:0; oleic acid, 18:1n-9; *cis*-vaccenic acid, 18:1n-7; linoleic acid, 18:2n-6; α-linolenic acid, 18:3n-3; 8,11,14-eicosatrienoic acid, 20:3n-6; 5,8,11-eicosatrienoic acid, 20:3n-9; arachidonic acid, 20:4n-6; 5,8,11,14,17-eicosapentaenoic acid, 20:5n-3; 7,10,13,16,19-docosapentaenoic acid, 22:5n-3; 4,7,10,13,16,19-docosahexaenoic acid, 22:6n-3.

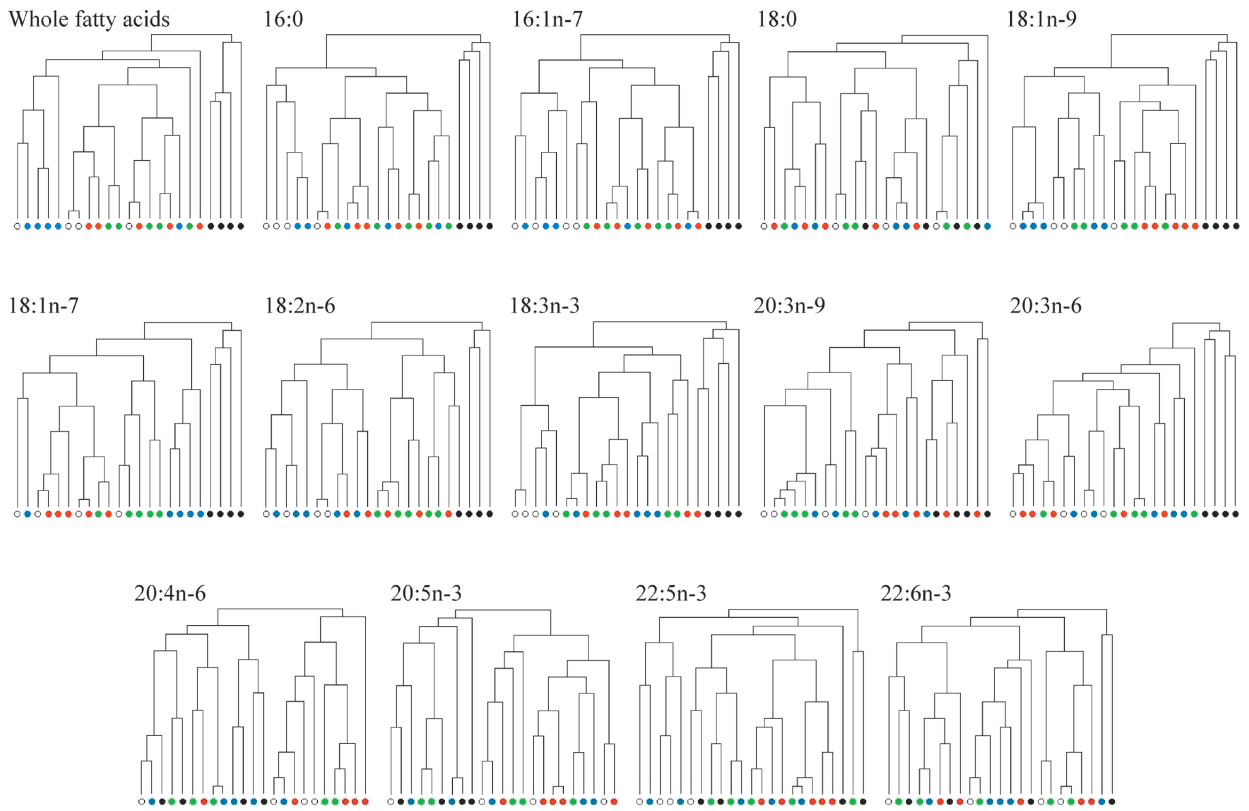

Fig. 1. Hepatic Fatty Acid Profiling

Dendrograms of hierarchical clustering of fatty acids were calculated on the bases of fatty acid profiles of Table 2. ○, WKY; ●, SHR; ●, SHRSP; ●, SHR/ND+; ●, SHR/NDcp.

RESULTS

Characteristic Profile of Fatty Acid in the Liver of SHR/NDcp Table 2 shows the fatty acid profile of hepatic lipid of WKY, SHR, SHRSP, SHR/ND+ and SHR/NDcp. On the basis of the data of Table 2, hierarchical clustering was used to evaluate where the largest differences existed in the fatty acid profiles (Fig. 1). The results show that the lower on the tree the branch is, the more similar the group; alternatively, the higher the branch is, the more disparate the groups. Hierarchical clustering for all kinds of fatty acid species as variables showed that SHR/NDcp and the other four groups

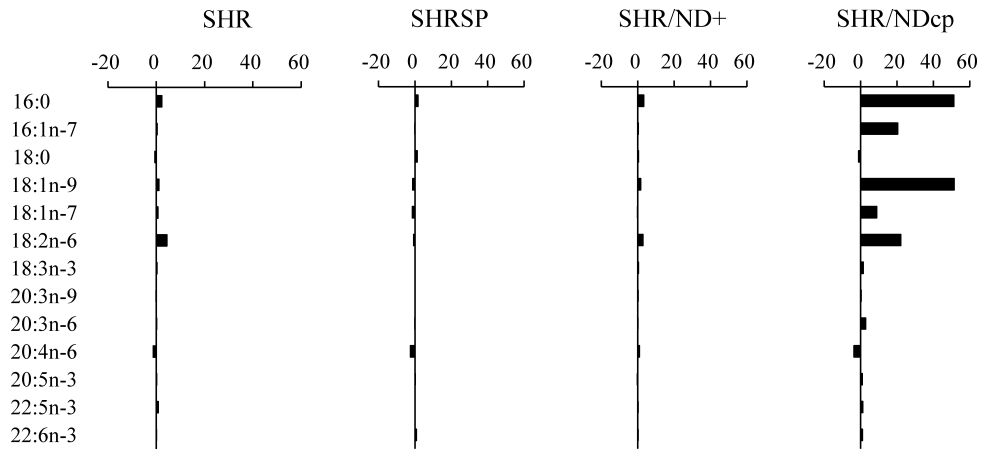

Fig. 2. Difference of Fatty Acids in Hepatic Lipid between WKY and the Other Four Groups, SHR, SHRSP, SHR/ND+ and SHR/NDcp

With regard to each fatty acid, differences in hepatic content (μmol/g liver) between the means of WKY and those of the other four groups of rats were calculated from the data of Table 2.

(WKY, SHR, SHRSP and SHR/ND+) were clearly disparate in profile among all kinds of fatty acids in the liver. Moreover, there was no disparity in profile of all kinds of fatty acids in the liver among WKY, SHR, SHRSP and SHR/ND+. Among the individual fatty acids examined, 16 : 0, 16 : 1 n-7, 18 : 1 n-9, 18 : 1 n-7 and 20 : 3 n-6 were responsible for the disparity in profile of all kinds of fatty acids between SHR/NDcp and the other four groups of rats. Striking enrichments of particular fatty acids, 16 : 1 n-7 and 18 : 1 n-9, were observed in the liver of SHR/NDcp (Fig. 2). Body weights and liver weights of SHR/NDcp were 1.4 and 2.1 times, respectively, greater than those of WKY, but no notable difference was observed in body weights and liver weights among WKY, SHR, SHRSP and SHR/ND+ (unpublished data), so that total masses (mol/whole liver) of these monounsaturated fatty acids (MUFAs) were additionally higher. There were no fatty acids of which contents (mol/g liver) in the liver of SHR/NDcp were lower than those of WKY, SHR, SHRSP and SHR/ND+.

Activities of Enzymes Involved in Formation of MUFA Species in the Liver Microsomal activities of SCD, PCE and POCE in the livers were determined (Fig. 3). SCD activities in the liver of SHR/NDcp were 4.6 times higher than those of WKY, and there was no significant difference in SCD activities among WKY, SHR, SHRSP and SHR/ND+ (Fig. 3A). The activity of PCE in microsomes of the liver of SHR/NDcp was 5.7 times greater than that of WKY, and no significant differences were observed in the activities of PCE among the other four groups of rats (Fig. 3B). As for POCE, the activity in hepatic microsomes of SHR/NDcp was 1.8 times higher than that of WKY, no difference being observed in the activities among WKY, SHR and SHR/ND+ (Fig. 3C). The activity of POCE in the liver of SHRSP was 0.44 times lower than that of WKY.

Expression of Genes Encoding Fatty Acid Desaturases and Elongases in the Liver The levels of mRNA encoding enzymes that are involved in desaturation and chain elongation of fatty acids in the liver of rats, were determined by real-time PCR (Figs. 4, 5). With regard to the expression of genes encoding desaturases, the levels of SCD1 mRNA of which translation product catalyzes conversion of 16 : 0 and 18 : 0 to 16 : 1 n-7 and 18 : 1 n-9, respectively, in the liver of SHR/NDcp were markedly higher than those of WKY, SHR, SHRSP and SHR/ND+, and the extent of the enhancement of expression was 5.5 times the level in WKY (Fig. 4A). There was no notable difference in the expression of SCD1 gene among WKY, SHR, SHRSP and SHR/ND+ (Fig. 4A). In contrast, the mRNA levels of SCD2 in the liver were slightly, but significantly, lower in SHR/NDcp than in WKY and SHR/ND+ (Fig. 4B). The levels of fatty acid desaturase (Fads)1 mRNA encoding Δ5 desaturase, which converts 20 : 3 n-6 to 20 : 4 n-6, in SHR/NDcp were lower than those in the other four groups of rats, the value being 0.46 times lower than that of WKY (Fig. 4C). There was no significant difference in the expression of Fads2 mRNA of which product is Δ6 desaturase that converts 18 : 2 n-6 to 18 : 3 n-6 between WKY and SHR/NDcp, whereas the levels of Fads2 mRNA in SHR was 0.39 times lower than that in WKY (Fig. 4D). Of the seven elongases identified in rats, four elongases are demonstrated to be expressed in the liver of rats; elongation of very long-chain fatty acids (Elovl)5 mRNA appears to be expressed in a high abundance, whereas the relative abundances of mRNAs encoding Elovl1, Elovl2 and Elovl6 are far lower than that of Elovl5.[15] The differences are reported in substrate preference of these elongase subtypes.[16] Namely, translation product of Elovl1 elongates a broad array of saturated and monounsaturated fatty acids; Elovl2 protein has a narrow substrate preference for polyunsaturated fatty acids with carbon chain length of 20—22; Elovl5 product converts 16 : 1 n-7, but not 16 : 0, to 18 : 1 n-7 as well as 18 : 3 n-6 to 20 : 3 n-6; Elovl6 protein has a narrow substrate preference for saturated and monounsaturated fatty acids with carbon chain length of 12—16. With regard to the expression of genes encoding elongases, the level of mRNA encoding Elovl1 in SHR/NDcp was significantly lower than those in WKY and SHR/ND+ (Fig. 5A). The mRNA levels of Elovl2 in SHR, SHR/ND+ and SHR/NDcp were significantly lower than that in WKY (Fig. 5B). The mRNA level of Elovl5 in the liver of SHRSP was slightly, but significantly, higher than that of WKY; there was no significant difference in the expression of the gene encoding Elovl5 among the other four groups of rats (Fig. 5C). The mRNA level of Elovl6 in the liver of SHR/NDcp was 81 times higher than that in WKY, and, on the other hand, no significant difference was observed in the levels among the other four groups of rats (Fig. 5D).

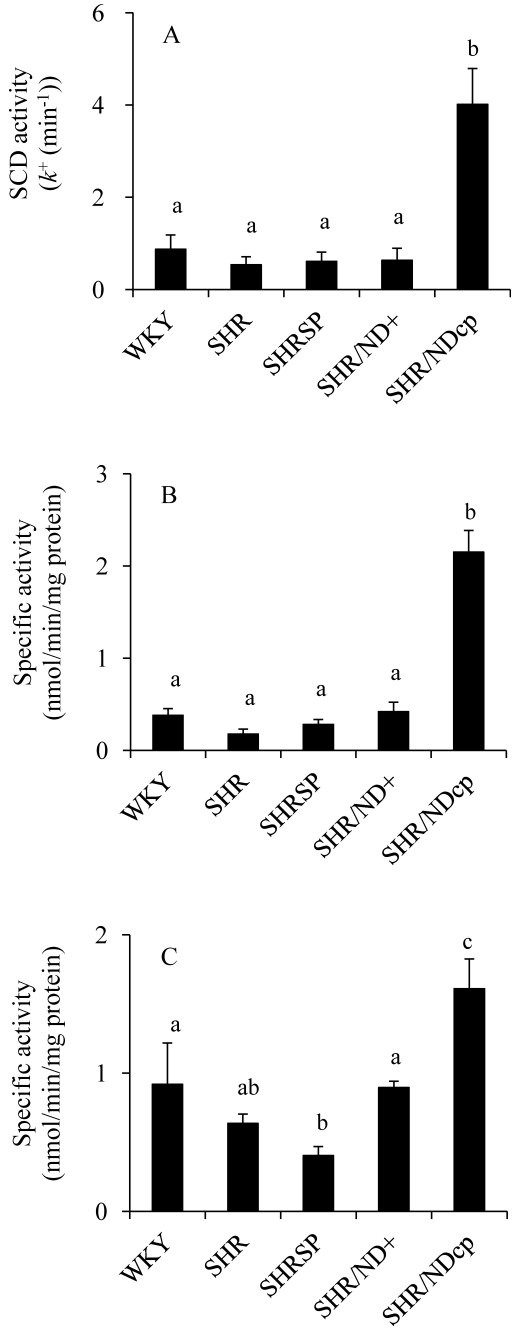

Fig. 3. Activities of SCD, PCE and POCE in Hepatic Microsomes

A, SCD; B, PCE; C, POCE. Values represent mean±S.D. for 4 or 7 rats. [a—c]Differences without a common superscript are statistically significant ($p<0.05$).

DISCUSSION

To understand how dissimilar SHR/NDcp are in the fatty acid profile in hepatic lipid compared with WKY, SHR, SHRSP and SHR/ND+, hierarchical clustering was employed in the present study. Hierarchical clustering is a widely used method for cluster analysis which consists in grouping data objects into a tree of clusters based on their distance (or similarity). Visualizing this tree provides a useful summary of the data. The clustering analyses clearly showed that SHR/NDcp and the other four strains and/or substrains of rats, WKY, SHR, SHRSP and SHR/ND+, were apparently disparate in fatty acid profile in hepatic lipid and that the disparity was accounted for by the selective elevations of contents of 16 : 0, 16 : 1 n-7, 18 : 1 n-9, 18 : 1 n-7 and 20 : 3 n-6 in the liver of SHR/NDcp. Of these fatty acid species, the extents of the elevation of the individual fatty acids in SHR/NDcp *versus* WKY were predominant in 16 : 1 n-7 (20.3 μmol/g liver; 25.2-fold), followed by 18 : 1 n-9 (51.4 μmol/g liver; 9.1-fold) and 20 : 3 n-6 (2.7 μmol/g liver; 5.5-fold). Although 18 : 1 n-7 is one of the MUFAs that are capable of being synthesized in the liver, its elevation (8.8 μmol/g liver; 3.3-fold) in SHR/NDcp was far lower than that of 18 : 1 n-9. Thus, SHR/NDcp displayed abnormalities in fatty acid composition of the liver. Pathways of biosynthesis of the MUFAs in the liver are shown in Fig. 6. 18 : 1 n-9 is synthesized from 16 : 0 *via* 18 : 0. Namely, 16 : 0 is elongated by PCE[17,18] and subsequently desaturated by Δ9 desaturation to yield 18 : 1n-9.[19] The present study showed that the activities of PCE and SCD in hepatic microsomes of SHR/NDcp were much higher than those of WKY, SHR, SHRSP and SHR/ND+; PCE and SCD activities in SHR/NDcp were 5.7 and 4.6 times, respectively, higher than those in WKY. These results enable us to elucidate the preferential elevation of 18 : 1 n-9 in the liver of SHR/NDcp. It is considered that the enzyme that catalyzes Δ9 desaturation of 16 : 0 is also SCD,[19] and that 18 : 1 n-7 is synthesized from 16 : 0 by sequential Δ9 desaturation and palmitoleoyl-CoA chain elongation.[18,20] The present study showed that the POCE activity in hepatic microsomes of SHR/NDcp was 1.8 times higher than that of WKY. It is likely, therefore, that the enrichments of these MUFAs in the liver were mostly due to the increases in the activities of microsomal SCD, PCE and POCE in the liver of SHR/NDcp. These results well account for the facts that the elevation of hepatic content of 16 : 1 n-7 was striking and that of 18 : 1 n-7 was moderate in the liver of SHR/NDcp.

Two genes encoding SCD in the liver of rats have been cloned and characterized so far.[21] Of the SCD subtypes, SCD1 is expressed at a high level in the liver,[21] and its expression is regulated by a number of nutritional and hormonal factors.[22] SCD1 is considered to be important in lipid homeostasis and body weight regulation.[22—25] On the other hand, in the liver of rats, SCD2 is also expressed, but less than SCD1.[21] The present study demonstrated that gene expression of SCD1 in the liver of SHR/NDcp was 5.5 times higher than that of WKY, whereas there was no notable difference in the expression of the gene encoding SCD2 among the five groups of rats. With regard to fatty acid elongase, 7 isoforms (Elovl1 *ca*. 7) have been identified so far.[26,27] Of the 7 isoforms, rat liver expresses four isoforms: Elovl1, Elovl2, Elovl5 and Elovl6.[15] The expression of only Elovl6 was strikingly high in the liver of SHR/NDcp; on the other hand, no differences were observed in the expression of Elovl5 mRNA between SHR/NDcp and the other four groups of rats. The present results are in accordance with the previous findings that 16 : 0 is elongated by Elovl6 and desaturated by SCD1 to yield 18 : 1 n-9.[16,28] Moreover, Elovl6 plays a crucial role in the elongation of 16 : 1 n-7 to 18 : 1 n-7, as well as in that of 16 : 0 to 18 : 0.[8,16,28] Elovl5 is demonstrated to participate in POCE, but little to convert 16 : 0 to 18 : 0.[16,28] Interestingly, however, our present study showed that the increase in the activity of POCE in SHR/NDcp was 1.8 times compared to that of WKY whereas PCE activity in SHR/NDcp was 5.7 times, and the expression of Elovl5 was not enhanced in the liver of SHR/NDcp, despite

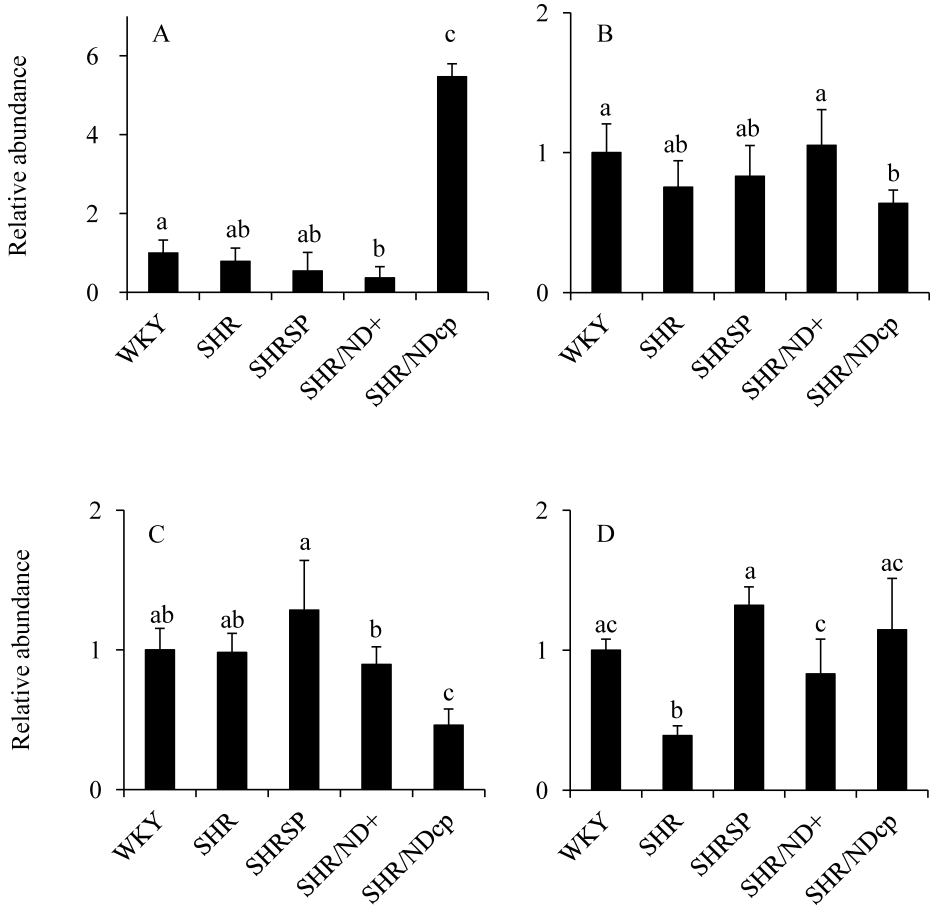

Fig. 4. Levels of mRNA Encoding Desaturases in the Liver

A, SCD1; B, SCD2; C, Fads1; D, Fads2. Values represent mean±S.D. for 4 or 9 rats. [a—c] Differences without a common superscript are statistically significant ($p<0.05$).

the fact that expression of Elovl6 was strikingly augmented. These results obtained from SHR/NDcp are distinct from the findings that obesity enhances expression of Elovl5 in the liver of obese mice (C57BL/6J-*Lepob*/*ob*) as well as Elovl6.[16] These findings, taken together, suggest that POCE in the liver of SHR/NDcp is composed of at least two elongases with a relatively large proportion of Elovl5 product and a relatively small proportion of Elovl6 product and that the elevated activity of POCE is due to the increase in Elovl6 product in the liver of SHR/NDcp. These present results demonstrated that coordinate alterations in the expression of genes encoding SCD, Elovl6 and Elovl5 produce the disordered balance in n-9 and n-7 MUFA species in the liver of SHR/NDcp.

20 : 3 n-6 is one of the intermediates of 20 : 4 n-6 formation; 20 : 4 n-6 is considered to be synthesized from 18 : 2 n-6 *via* the pathway of 18 : 2 n-6→6,9,12-octadecatrienoic acid (18 : 3 n-6) →20 : 3 n-6→20 : 4 n-6.[29] The process of 18 : 2 n-6 →18 : 3 n-6 is catalyzed by Δ6 desaturase, which is encoded by Fads2.[29,30] The elongase responsible for the conversion of 18 : 3 n-6 to 20 : 3 n-6 is encoded by Elovl5.[16] Δ5 Desaturase that catalyzes the final step of 20 : 4 n-6 formation is encoded by Fads1.[29] The process of 18 : 2 n-6 to 18 : 3 n-6 is considered to be the rate-limiting step of 20 : 4 n-6 synthesis in rat livers with physiologically normal states.[29] However, the difference between the activities of Δ6 desaturation (18 : 2 n-6→18 : 3 n-6) and Δ5 desaturation (20 : 3 n-6→20 : 4 n-6) is not significantly large, and the process of 18 : 3 n-6→20 : 3 n-6 is much faster than that of either Δ5 desaturation or Δ6 desaturation in rat livers.[29] The present study showed that the expression of Fads1 was 0.46 times lower in SHR/NDcp than in WKY, whereas there was no difference in the expressions of Fads2 and Elovl5 between SHR/NDcp and WKY. It seems likely, therefore, that the rate-limiting step of 20 : 4 n-6 formation was changed from the process of Δ6 desaturation to that of Δ5 desaturation, so that 20 : 3 n-6 was accumulated in hepatic lipid of SHR/NDcp.

The physiological or pathological significance of the elevations of hepatic contents of 16 : 1 n-7 and 18 : 1 n-9 in SHR/NDcp has not been clarified yet. Two possibilities, however, are conceivable for the accumulation of 18 : 1 n-9. One possibility is that accumulation of 18 : 1 n-9 as intracellular lipid caused dyslipidemia such as hepatosteatosis, hypertriglyceridemia and obesity in SHR/NDcp. Intracellular accumulation of lipid in organs is considered to be one of the most likely causes of dysfunction of the organ with regards to insulin resistance.[31] 18 : 1 n-9, which is synthesized from 18 : 0 by SCD, is a major component of triglyceride, and deficiency of the SCD1 gene in mice protects against hypertriglyceridemia,[32] obesity,[33,34] and postprandial plasma insulin.[34,35] Moreover, mice deficient in Elovl6 showed marked protection from hyperinsulinemia, hyperglycemia and hyperleptinemia.[8] An alternative possibility is that the formation of 18 : 1 n-9 was increased in the liver of SHR/NDcp to prevent the function of the tissue from endoplasmic reticulum (ER) stress by saturated fatty acids such as 18 : 0 and 16 : 0, which are known

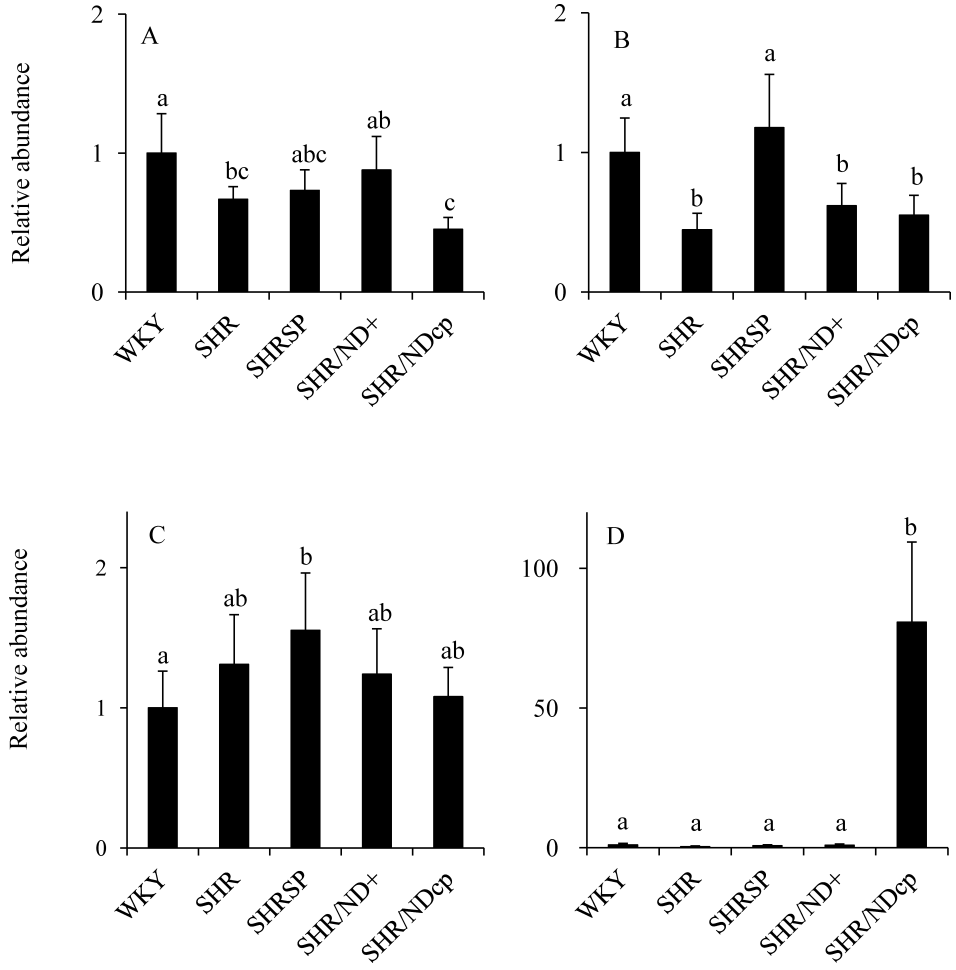

Fig. 5. Levels of mRNA Encoding Elongases in the Liver

A, Elovl1; B, Elovl2; C, Elovl5; D, Elovl6. Values represent mean±S.D. for 4 or 9 rats. [a—c]Differences without a common superscript are statistically significant ($p<0.05$).

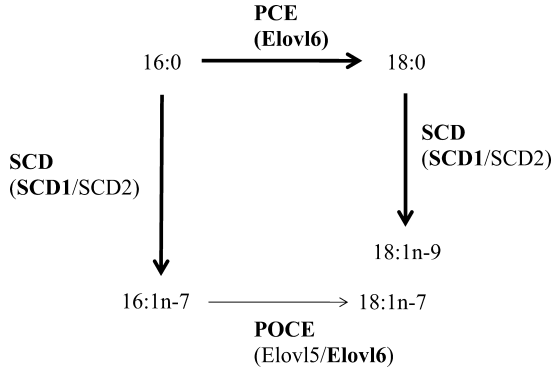

Fig. 6. Diagram of Enzymes Regulating Synthesis of 16 : 1 n-7, 18 : 1 n-7 and 18 : 1 n-9

to cause ER stress in the liver.[36,37] 18 : 1 n-9 is preferentially incorporated into triglyceride, and triglyceride is probably the least toxic form in which the surplus of lipid, in particular 18 : 0 and 16 : 0, can be sequestered and may, at least in the short term, actually protect against severe metabolic trauma.[38] With regard to 16 : 1 n-7, recent studies have suggested that this specific fatty acid species has significant impact on the insulin sensitivity of the liver and whole body.[8,39] In this regard, therefore, it is considered that hepatic fatty acid composition is likely to be a determinant of insulin sensitivity that acts independently of cellular energy balance and stress.[8]

In conclusion, SHR/NDcp provides an excellent example of defective liporegulation and is an interesting model to study the physiological or pathological significance of disturbances in fatty acid metabolism in metabolic syndrome. Currently, however, the detailed mechanism underlying this disorder in regulation of fatty acid synthesis remains to be resolved.

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