Effects of 8-2 Fluorotelomer Alcohol on Oleic Acid Formation in the Liver of Rats

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Effects of 8-2 fluorotelomer alcohol on fatty acid composition of lipid in the liver of rats were investigated. Feeding of male rats with a diet that contained 8-2 fluorotelomer alcohol at concentrations of 0.2, 0.4 and 0.8% (w/w) for 14 d caused a significant increase in proportion and content of oleic acid (18 : 1 (n-9)) in the liver. The treatment of rats with 8-2 fluorotelomer alcohol increased activities of palmitoyl-CoA chain elongase (PCE) and stearoyl-CoA desaturase (SCD) and mRNA expressions for rat fatty acid elongase 2 (rELO2) and stearoyl-CoA desaturase 1 (SCD1), but neither rat fatty acid elongase 1 (rELO1) or stearoyl-CoA desaturase 2 (SCD2), in the liver in dose-dependent manners. Multiple regression analyses, which were performed to estimate relative contribution of PCE and SCD for determination of the proportion or the content of 18 : 1 (n-9), revealed that the three parameters were significantly correlated and that standardized partial regression coefficient of PCE was higher than that of SCD. These results suggest that 8-2 fluorotelomer alcohol caused considerable changes in the composition and the content of fatty acid, especially 18 : 1 (n-9), in the liver by inducing PCE and SCD, and that PCE plays a crucial role in the increased proportion of 18 : 1 (n-9) in the liver of the rats given fluorotelomer alcohol.

Key words 8-2 fluorotelomer alcohol; oleic acid; palmitoyl-CoA chain elongase; stearoyl-CoA desaturase; liver; rat

Perfluorinated organic chemicals are widely used as various applications, because of their unique properties by the carbon-fluorine bonds.[1—3] 1*H*, 1*H*, 2*H*, 2*H*-Perfluorodecanol (8-2 fluorotelomer alcohol) has perfluorinated alkyl chain with 8 carbons adjacent to ethanol, and used to manufacture polymer and surfactant, which take advantage of low surface energy of the fluoroalkyl chain, great stability, hydrophobicity and oleophobicity.[4] Owing to the superior properties, 8-2 fluorotelomer alcohol is used as precursor compounds in the production of fluorinated polymers used in paper and carpet treatments and in manufacture of wide range of products such as paints, adhesives, waxes, polishes, metals and electronics.[5] These applications are similar to those of perfluorooctane sulfate (PFOS)-based products.[5] Since PFOS is persistent and widely dispersed in the environment and has been identified in human, wildlife, and environmental media samples,[6—15] a number of studies were conducted to investigate biological effects of PFOS. This compound has been revealed to posses undesirable toxic characteristics such as weight loss, hepatocellular hypertrophy and lipid vacuolation, reduction of serum cholesterol and thyroid hormones, increase in neonatal mortality, and disturbance of neuroendocrine.[16—19] On the other hand, 8-2 fluorotelomer alcohol is widely utilized, and the global production of 8-2 fluorotelomer alcohol is estimated to be 5×10^6 kg/year during the period 2000—2002.[20] This may make one suppose the increase in the risk of human exposure to this chemical. However, information about toxicological effects of this chemical is just becoming available.[21—23] A study on one-generation reproductive toxicity of fluoroalkylethanol mixture having fully fluorinated alkyl chain with six to twelve carbons showed no harmful effect on reproduction in rats.[21] Another study on subchronic toxicity of fluoroalkylethanol mixture demonstrated tooth alterations, hepatocellular hypertrophy, elevation of liver weight and thyroid follicular hypertrophy in rats.[22] The subsequent study characterized fluorotelomer alcohols as xenoestrogens *in vitro*.[23] Thus, information concerning toxicological effects of 8-2 fluorotelomer alcohol is very limited. Therefore, further investigations with regard to biological effects of 8-2 fluorotelomer alcohol are required to provide information for the safe use of this compound.

Several perfluorinated organic chemicals have been shown to cause alterations in hepatic lipid, in particular fatty acid composition.[24,25] Therefore, these compounds may affect human or animal health through alterations in energy metabolism since oleic acid is a major fatty acid synthesized *via de novo* pathway and enriched in triglyceride. However, information is lacking about the effect of 8-2 fluorotelomer alcohol on fatty acid composition in the liver of animals. The present study has two aims. The first is to confirm whether exposure of animals to 8-2 fluorotelomer alcohol causes alterations of fatty acid composition of hepatic lipid. The second is to confirm effects of 8-2 fluorotelomer alcohol on the activities of the enzymes responsible for the changes in fatty acid composition and on mRNA expression for the enzymes, if the compound causes hepatic lipid alterations.

MATERIALS AND METHODS

Materials 1*H*, 1*H*, 2*H*, 2*H*-Perfluorodecanol (8-2 fluorotelomer alcohol) was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Stearoyl-CoA, palmitoyl-CoA, malonyl-CoA, and bovine serum albumin were purchased from Sigma (St. Louis, MO, U.S.A.). Nonadecanoic acid was from Nu-Chek Prep. Inc. (Elysian, MN, U.S.A.); NADH and NADPH were from Oriental Yeast Co. (Tokyo, Japan). All other chemicals used were of analytical grade.

Treatments of Animals and Preparation of Microsomes Male Wistar rats aged 6 weeks were purchased from SLC (Hamamatsu, Japan). Diets containing 8-2 fluorotelomer al-

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cohol were prepared as follows: Ten grams of 8-2 fluorotelomer alcohol was dissolved in 200 ml of acetone, mixed with 90 g of powdered laboratory chow (CE-2, Clea Japan, Tokyo, Japan) and then dried at room temperature overnight (10% (w/w) 8-2 fluorotelomer alcohol-containing diet). Next, 10% (w/w) diet was diluted with powdered laboratory chow in a mortar to obtain the diet containing 0.2, 0.4 or 0.8% (w/w) diet. After acclimatization for 7 d, the rats were fed *ad libitum* either a control diet or a diet containing 8-2 fluorotelomer alcohol at concentrations of 0.2, 0.4, and 0.8% (w/w) for 14 d. Blood was collected from the descending *vena cava* under diethyl ether anesthesia. Serum was prepared from the blood by centrifugation. Livers were quickly excised, and perfused with ice-cold 0.9% (w/v) NaCl. Part of the livers was frozen in liquid nitrogen and stored at −80°C for preparation of total RNA, and another part of the livers was homogenized in four volumes of 0.25 M sucrose/1 mM 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 −30 °C for lipid analyses. The other parts of homogenates were centrifuged at 18000×***g*** for 20 min, and the supernatant was recentrifuged under the same conditions. The resulting supernatant was centrifuged at 105000×***g*** for 60 min. The 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 the operations mentioned above were carried out at 0—4 °C. Protein concentrations were determined by the method of Lowry *et al*.[26] using bovine serum albumin as a standard. All animals studied complied with institutional board for animal study, Josai University.

Lipid Analyses After the addition of a known amount of nonadecanoic acid as an internal standard, total lipid was extracted from liver homogenates according to the methods of Bligh and Dyer.[27] After the solvent of lipid extract was evaporated, 1 ml of 10% (w/v) KOH/90% (v/v) methanol was added to the residue obtained, and then the mixture was heated at 80 °C for 60 min for saponification. Non-saponified lipids were removed three times by the extraction with *n*-hexane. After the addition of 1 ml of 6 M HCl, free fatty acids were extracted with *n*-hexane. The extract was taken to dryness, and to the residue was added 1 ml of 14% (w/v) BF_3/methanol. The mixture was heated at 100 °C for 10 min under nitrogen. Fatty acid methyl esters formed were extracted with *n*-hexane and subjected to gas-liquid chromatographic analyses. The amounts and compositions of the fatty acid methyl esters were determined by GC (Shimadzu GC-14A) equipped with a flame ionization detector by using a 0.32 mm×30 m fused silica capillary column (Supelcowax 10) at 230 °C with helium as a carrier gas.

Enzyme Assays Stearoyl-CoA desaturase (SCD) was assayed by the method of Oshino *et al*.[28] as the rate of stearoyl-CoA-stimulated reoxidation of NADH-reduced cytochrome b_5. The rate of cytochrome b_5 oxidation was measured by recording the changes in absorbance between 424 nm and 409 nm at 30 °C. The initial incubation mixture contained 1.2 mg of microsomal protein and 100 mM Tris–HCl buffer (pH 7.4) in a final volume of 3 ml. Cytochrome b_5 was reduced by adding 2 nmol of NADH, and then the rate of reoxidation was recorded. When the reoxidation was completed, 20 nmol of stearoyl-CoA was added, and cytochrome b_5 was reduced again by 2 nmol of NADH. The first order rate constant for the reoxidation of NADH-reduced cytochrome b_5 was calculated as described by Oshino and Sato.[29] The rate constant for the reoxidation of cytochrome b_5 was measured in the presence (k) and in the absence (k^-) of stearoyl-CoA; the rate constant for stearoyl-CoA desaturation was given by $k^+=k-k^-$.[30] The activities of NADH-cytochrome *c* reductase and NADH-ferricyanide reductase were assayed by the methods of Rogers and Strittmatter,[31] and Oshino and Sato,[32] respectively. The content of cytochrome b_5 in hepatic microsomes was estimated by the method of Omura and Sato.[33]

Palmitoyl-CoA chain elongase (PCE) was assayed spectrophotometically by the method of Nagi *et al*.[34] The assay was performed by recording the changes in absorbance between 303 nm and 350 nm at 37 °C. The reaction mixture for PCE contained 40 nmol of bovine serum albumin (essentially fatty acid free), 40 nmol of palmitoyl-CoA, 100 mM Tris–HCl buffer (pH 7.4), and 250 μg of microsomal protein in a final volume of 1 ml. The reaction was started by the addition of 60 nmol of malonyl-CoA and the increase in absorbance resulting from the formation of the β-ketopalmitoyl-CoA-bovine serum albumin complex was recorded over a period of time. The value, which was obtained from the incubation without malonyl-CoA, was subtracted to give the net condensation rate for the palmitoyl-CoA.

RNA Extraction and Real-Time Quantitative PCR Total RNA was prepared from the livers of rats using an RNeasy kit (Qiagen, Hilden, Germany). RNA was quantified spectrophotometrically based on the absorption at 260 nm. cDNA was prepared from the total RNA with avian myeloblastosis virus reverse transcriptase (Takara, Tokyo, Japan). The specific primer sets for SCD 1 and rat fatty acid elongase2 (rELO2) were designed using Primer Express 2.0 software (Applied Biosystems, Foster City, CA, U.S.A.). The specific primer set for β-actin was used as described by Zhang *et al*.[35] PCR amplification was carried out using QuantiTect SYBER Green PCR master mix (Qiagen) with 300 nM primers and the following primer sequences: rELO1 (forward, 5′-GCTTCATCCACGTCCTCATGT-3′; reverse, 5′-TCAGCACAAACTGGACCAGCT-3′), rELO2 (forward, 5′-AGAACACGTAGCGACTCCGAA-3′; reverse, 5′-CAAACGCGTAAGCCCAGAAT-3′), SCD1 (forward, 5′-AAAGTTTCTAAGGCCGCTG-3′; reverse, 5′-GTCTGAGCCAGCAATCTCAA-3′), SCD2 (forward, 5′-TGCACCCCCAGACACTTGTAA-3′; reverse, 5′-GGATGCATGGAAACGCCATA-3′) and β-actin (forward, 5′-TGCAGAAGGAGATTACTGCC-3′; reverse, 5′-CGCAGCTCAGTAACAGTCC-3′).

The amplification and detection were performed with iCycler IQ real-time detection system (Bio-Rad, Richmond, CA, U.S.A.). PCR conditions were a 15 min denaturation step at 95 °C followed by 40 cycles of 15 s denaturation at 94 °C, 30 s annealing at 57 °C, and 30 s extension at 72 °C. Reverse transcriptase-PCR products were analyzed by electrophoresis on ethidium bromide-stained agarose to ensure that a single amplicon of the extracted size was indeed obtained. To measure PCR efficiency, serial dilutions of reverse transcribed RNA (0.1 to 100 ng) were amplified, and a line

was obtained by plotting cycle threshold (C_T) values as a function of the starting reverse-transcribed RNA the slope of which was used for efficiency calculation using the formula $E=10^{|(1/\text{slope})|-1}$.[36] The quantity of any given gene, expressed as the value relative to the amount in the control rats was calculated after determination of the difference between the C_T of the given gene A and that of the calibrator gene B (β-actin) in 8-2 fluorotelomer alcohol-treated rats ($\Delta C_{T1}=C_{1A}-C_{1B}$) and control rats ($\Delta C_{T0}=C_{0A}-C_{0B}$) using the $2^{-\Delta\Delta C_T(1-0)}$ formula.[36]

Statistics of Analyses Analysis of variance was used to test the significance of the differences from the control group. Where differences were significant, the statistical significance between any two means was determined using Schéffe's multiple range test. Linear or multiple regression analyses were performed to evaluate the correlation between two or three parameters, respectively.[37]

RESULTS

Male Wistar rats were fed on a control diet or a diet containing 8-2 fluorotelomer alcohol for 14 d. The treatment with 8-2 fluorotelomer alcohol did not significantly lower body weight (data not shown). There was no significant effect following the administration of 8-2 fluorotelomer alcohol on the overall health of the rats. Liver weights were increased by the treatment of rats with 8-2 fluorotelomer alcohol in a dose-dependent manner (Fig. 1).

Effects of 8-2 Fluorotelomer Alcohol on Fatty Acid Composition of Hepatic Lipid Table 1 shows effects of the treatment with 8-2 fluorotelomer alcohol on fatty acid composition of total lipid in the liver. The administration of 8-2 fluorotelomer alcohol to rats considerably increased proportions of oleic acid (18 : 1 (n-9)) and arachidonic acid (20 : 4 (n-6)). Proportion of linoleic acid (18 : 2 (n-6)) markedly decreased. Proportions of palmitic acid (16 : 0), eicosapentaenoic acid (20 : 5 (n-3)) and docosahexaenoic acid (22 : 6 (n-3)) were decreased to a lesser extent. Proportions of palmitoleic acid (16 : 1), stearic acid (18 : 0), *cis*-vaccenic acid and 6, 9, 12-eicosatrienoic acid (20 : 3 (n-6)) were not changed. Among the fatty acid synthesized from 16 : 0, the proportion

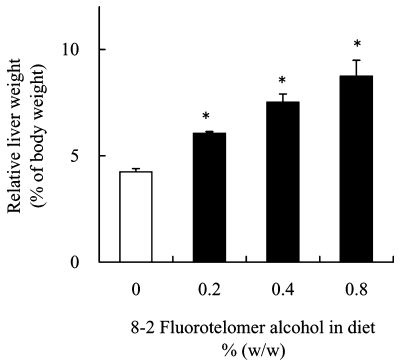

Fig. 1. Effects of 8-2 Fluorotelomer Alcohol on Liver Weight

Rats were fed on a control diet or a diet containing 8-2 fluorotelomer alcohol at the concentrations of 0.2, 0.4, and 0.8% (w/w) for 14 d. Each value represents % (w/w) of body weight and the mean±S.D. for four rats. * Significantly different from control at $p<0.05$.

Table 1. Effects of 8-2 Fluorotelomer Alcohol Feeding on Fatty Acid Composition of Hepatic Lipid

Fatty acid	8-2 Fluorotelomer alcohol in diet % (w/w)			
	0	0.2	0.4	0.8
			(mol%)	
16 : 0	25.8±1.3	21.9±0.7*	23.6±1.7**	21.4±1.4**
16 : 1	1.8±0.9	1.6±0.3	1.5±0.4	1.5±0.1
18 : 0	19.5±0.8	22.6±1.0	22.2±2.6	24.2±1.8*
18 : 1 (n-9)	7.8±0.7	9.0±0.7	11.3±2.4*	14.7±1.5**
18 : 1 (n-7)	3.4±0.2	2.5±0.4	2.5±0.3	2.3±0.5
18 : 2 (n-6)	17.7±1.8	12.4±1.2	7.4±5.0**	7.7±0.8**
20 : 3 (n-9)	0.4±0.2	0.2±0.0	0.3±0.1	0.8±0.1**
20 : 3 (n-6)	1.0±0.5	2.0±0.6	2.0±0.5	1.7±0.4
20 : 4 (n-6)	17.5±2.0	23.0±1.4	24.1±2.3**	23.3±1.3**
22 : 5 (n-3)	1.1±0.3	0.5±0.1	0.4±0.1*	0.4±0.1*
22 : 6 (n-3)	4.2±0.7	3.1±0.4	2.7±0.8*	1.8±0.3**
Total (μmol/g liver)	77.1±3.1	80.1±3.6	93.4±10.3	83.1±11.4
			(μmol/whole liver)	
16 : 0	209.9±17.8	266.0±17.1	408.6±57.8**	373.9±48.0**
16 : 1	14.2±6.7	19.3±3.7	26.2±7.5	26.4±3.6
18 : 0	158.6±16.1	275.6±26.7	382.6±30.6**	424.0±68.4**
18 : 1 (n-9)	70.6±8.1	133.8±3.8	227.4±70.0**	307.4±55.2**
18 : 1 (n-7)	31.2±4.7	37.6±5.7	45.9±9.1	45.0±11.2
18 : 2 (n-6)	143.2±11.5	150.9±12.1	158.1±39.8	133.8±13.2
20 : 3 (n-9)	3.4±1.5	2.8±0.6	6.1±2.5	13.5±1.1**
20 : 3 (n-6)	7.7±3.5	24.8±7.8	35.1±7.6**	29.4±7.6**
20 : 4 (n-6)	143.2±24.3	280.6±29.7	414.4±19.3**	406.7±40.1**
22 : 5 (n-3)	9.2±2.6	16.1±1.4	7.3±0.8	4.6±1.7*
22 : 6 (n-3)	34.3±7.7	28.5±15.8	45.3±11.3	31.7±4.3
Total (μmol/liver)	823.5±61.8	1218.3±83.2	1727.7±124.3**	1747.9±192.4**

Rats were fed on a control diet or a diet containing 8-2 fluorotelomer alcohol at the concentrations of 0.2, 0.4 and 0.8% (w/w) for 14 d. Each value represents the mean±S.D. for four rats. *, ** Significantly different from the control at $p<0.05$ (*) and $p<0.01$ (**), respectively. The fatty acid are designated by the number of carbon atoms and double bond; palmitic acid, 16 : 0; palmitoleic acid, 16 : 1; stearic acid, 18 : 0; oleic acid, 18 : 1 (n-9); *cis*-vaccenic acid, 18 : 1 (n-7); linoleic acid 18 : 2 (n-6); eicosatrienoic acid, 20 : 3 (n-9) and 20 : 3 (n-6); arachidonic acid, 20 : 4 (n-6); eicosapentaenoic acid, 20 : 5 (n-3); docosahexaenoic acid, 22 : 6 (n-3).

of 20 : 3 (n-9) increased while 16 : 1 remained unchanged. On the basis of g liver, the amounts of 18 : 1 (n-9) were calculated to be 6.0, 7.2, 10.6 and 12.2 μg/g liver in control, 0.2%, 0.4% and 0.8% 8-2 fluorotelomer-containing diet group, respectively. On the basis of whole liver, hepatic content of fatty acid was increased in a dose-dependent manner, and the content in rats that were fed on a diet containing 0.8% (w/w) of 8-2 fluorotelomer alcohol was approximately 2.1 times over the control.

It is noteworthy that the extent of the increase in hepatic content of 18 : 1 (n-9) was the greatest among fatty acids. Contents of 18 : 1 (n-9) on the basis of whole liver in the rats that were fed on a diet containing 0.8% (w/w) of 8-2 fluorotelomer alcohol was approximately 4.3 times as great as that of the control.

Effect of 8-2 Fluorotelomer Alcohol on Palmitoyl-CoA Chain Elongase and Stearoyl-CoA Desaturase and mRNA Expression in the Liver Activity of PCE in hepatic microsomes was significantly increased in a dose-dependent manner by the feeding of rats with a diet that contained 8-2 fluorotelomer alcohol (Fig. 2). Feeding a diet containing 0.8% (w/w) of 8-2 fluorotelomer alcohol increased 2.7 times the activity. The administration of 0.4% (w/w) and 0.8% (w/w) of 8-2 fluorotelomer alcohol increased 2.1 and 4.7 times, respectively, the activity of SCD (Table 2). 8-2 Fluorotelomer alcohol did not affect activities of either NADH-cytochrome *c* reductase or NADH-ferricyanide reductase and cytochrome b_5 content. The treatment of rats with 8-2 fluorotelomer alcohol increased levels of mRNA for rELO2 and SCD1 10.5 and 3.6 times, respectively (Figs. 3B, C). However, there were no significant changes in the expression of mRNA for either rELO1 or SCD2 (Figs. 3A, D).

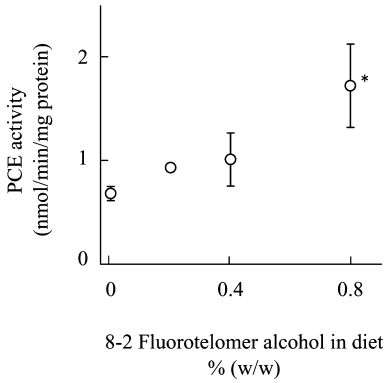

Fig. 2. Effect of 8-2 Fluorotelomer Alcohol on the Activity of PCE in Hepatic Microsomes

Rats were fed on a control diet or a diet containing 8-2 fluorotelomer alcohol at the concentrations of 0.2, 0.4, and 0.8% (w/w) for 14 d. Each value represents the mean±S.D. for four rats. * Significantly different from control at $p<0.05$.

To examine the relationships between the activity of PCE and mRNA expression of rELO2 and between the activity of SCD and mRNA expression of SCD1, linear regression analyses were carried out. One linear regression line was obtained for 4 sets of mean data between the activity of PCE and the mRNA expression of rELO2, with a high correlation being seen between the two parameters ($r^2=0.794$, $p<0.05$) (Fig. 4A). Similarly, a high correlation was observed between the activity of SCD and the mRNA expression of SCD1 ($r^2=0.984$, $p<0.05$) (Fig. 4B), whereas no correlation was seen between the activity of PCE and the mRNA expression of rELO1 ($r^2=0.189$, $p>0.05$), and between the activity of SCD and the mRNA expression of SCD2 ($r^2=0.245$, $p>0.05$).

Relationship among Palmitoyl-CoA Chain Elongase Activity, Stearoyl-CoA Desaturase Activity and Hepatic Content of 18 : 1 (n-9) Linear regression analyses were carried out to examine the relationship between the proportion of 18 : 1 (n-9) and the activity of either PCE or SCD and between the content of 18 : 1 (n-9) and the activity of either PCE and SCD. One regression line was obtained for 4 sets of mean data between the proportion of 18 : 1 (n-9) and the activity of PCE from Table 1 and Fig. 2, with a high correlation being seen between the two parameters ($r^2=0.937$, $p<0.05$) (Fig. 5A). Similarly, high correlations were observed between the proportion of 18 : 1 (n-9) and the activity of SCD from Tables 1 and 2 ($r^2=0.963$, $p<0.05$) (Fig. 5B), between the content of 18 : 1 (n-9) on the basis of g liver and the activity of PCE ($r^2=0.761$, $p<0.05$) (Fig. 5C), and between the content of 18 : 1 (n-9) on the basis of g liver and the activity of SCD ($r^2=0.766$, $p<0.05$) (Fig. 5D).

The relative contributions of PCE and SCD to the increase in the proportion or the content of 18 : 1 (n-9) were determined by a multiple linear regression analysis (Table 3). The 4 sets of mean data from Tables 1, 2 and Fig. 2 revealed a significant correlation among the three parameters. One regression line was obtained for the relationship among the activity of PCE, the activity of SCD and the proportion of 18 : 1 (n-9) ($r^2=0.823$, $p<0.01$), and among the activity of PCE, the activity of SCD and the content of 18 : 1 (n-9) on the basis of g liver ($r^2=0.741$, $p<0.01$). The standardized partial regression coefficient between the proportion of 18 : 1 (n-9) and the activity of PCE was 0.748 ($p<0.01$), whereas that between the proportion of 18 : 1 (n-9) and the activity of SCD was 0.205 ($p>0.05$). The standardized partial regression coefficient between the content of 18 : 1 (n-9) and the activity of PCE, and between the content of 18 : 1 (n-9) per g liver and the activity of SCD were 0.695 ($p<0.01$) and 0.241 ($p>0.05$), respectively.

Table 2. Effects of 8-2 Fluorotelomer Alcohol Feeding on the Component Activity of Stearoyl-CoA Desaturation System in Hepatic Microsomes

8-2 Fluorotelomer alcohol in diet % (w/w)	NADH-cytochrome *c* reductase (μmol/min/mg protein)	NADH-ferricyanide reductase (μmol/min/mg protein)	Cytochrome b_5 content (nmol/min/mg protein)	Desaturase (k^+) (min^{-1})
0	1.88±0.36	0.55±0.15	0.45±0.27	0.94±0.15
0.2	1.94±0.27	0.72±0.06	0.67±0.02	1.21±0.20
0.4	1.70±0.23	0.65±0.06	0.58±0.01	1.97±0.50
0.8	1.99±0.43	0.67±0.09	0.58±0.04	4.41±1.09*

Rats were fed on a control diet or a diet containing 8-2 fluorotelomer alcohol at the indicated concentrations for 14 d. Each value represents the mean±S.D. for four rats. The rate constant for the reoxidation of NADH-reduced cytochrome b_5 was measured with stearoyl-CoA (k) and without stearoyl-CoA (k^-), the rate constant (k^+) for desaturation was given by $k^+=k-k^-$. * Significantly different from control at $p<0.01$.

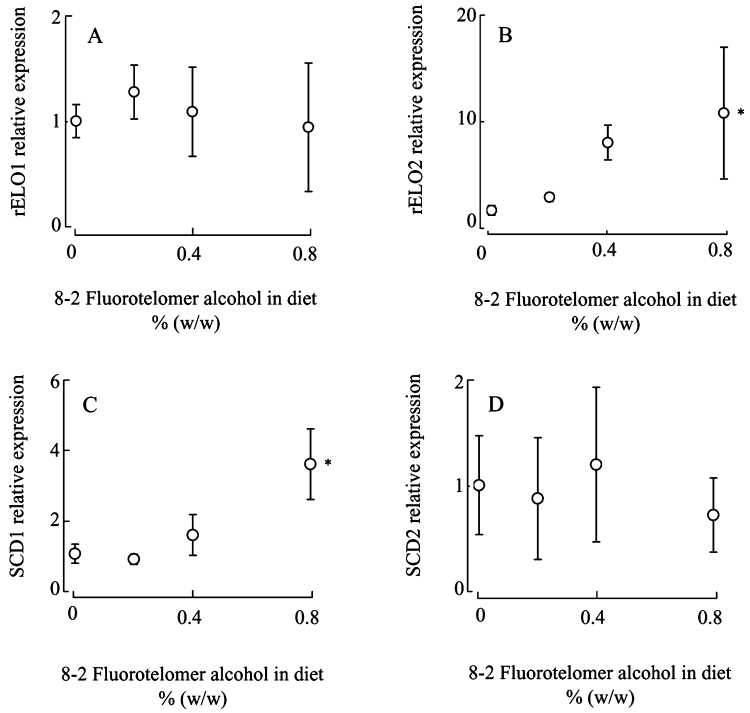

Fig. 3. Effects of 8-2 Fluorotelomer Alcohol on the mRNA Expression in the Liver

Rats were fed on a control diet or a diet containing 8-2 fluorotelomer alcohol at the concentrations of 0.2, 0.4, and 0.8% (w/w) for 14 d. A, rELO1; B, rELO2; C, SCD1; D, SCD2. mRNA levels of SCD1, SCD2, rELO1, and rELO2 were normalized by the expression of β-actin. The data are expressed as the relative mRNA level compared with the average expression level in the rats feeding on a control diet (=1). * Significantly different from control at $p<0.05$.

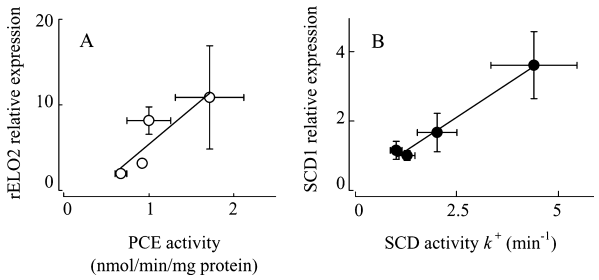

Fig. 4. Relationship between the Activities of PCE or SCD and mRNA of rELO2 or SCD1

Regression analyses were performed on 4 sets of mean data from Fig. 2 and Fig. 3B and on 4 sets of mean data from Table 2 and Fig. 3C. A, the activity of PCE *versus* mRNA level of rELO2 ($Y=8.640X-3.835$, $r^2=0.794$, $p<0.05$); B, the activity of SCD *versus* mRNA level of SCD1 ($Y=0.797X+0.046$, $r^2=0.9839$, $p<0.05$).

DISCUSSION

The present study clearly showed that the administration of 8-2 fluorotelomer alcohol to male rats markedly changed fatty acid composition of hepatic lipid and increased the content of fatty acids on the basis of both g liver and whole liver. The effects were not accompanied by a significant weight loss, therefore, thought to be very specific effects of this chemical on the liver. Among the fatty acids, the most striking increase in the proportion was observed with 18 : 1 (n-9). On the basis of g liver, the amount of 18 : 1 (n-9) was increased by 8-2 fluorotelomer alcohol in a dose-dependent manner. Since the treatment of rats with 8-2 fluorotelomer alcohol enlarged livers, the content of 18 : 1 (n-9) on the basis of whole liver was increased strikingly. Toxicological implications of 8-2 fluorotelomer alcohol-induced increase in 18 : 1 (n-9) are not fully elucidated. It is possible that 8-2 fluorotelomer alcohol extensively alters energy metabolism and causes a detrimental effects on health.

The present results of the increase by 8-2 fluorotelomer alcohol in the proportion and the content of 18 : 1 (n-9) in the liver strongly suggest that the administration of the compound induces the activities of hepatic PCE or SCD or both, because 18 : 1 (n-9) is known to be formed from 16 : 0 *via* 18 : 0 by the sequential actions of PCE and SCD.[38] The present study demonstrated that the administration of 8-2 fluorotelomer alcohol to rats increased the activities of both PCE and SCD in dose-dependent manners in the liver. It has been established that the stearoyl-CoA desaturation system consists of three components, NADH-cytochrome b_5 reductase, cytochrome b_5, and terminal desaturase.[39] The present study showed that the increase in the activity of SCD by the administration of 8-2 fluorotelomer alcohol was due mainly to changes in the activity of SCD itself, but not to changes in other components responsible for electron flow from NADH to cytochrome b_5 *via* NADH-cytochrome b_5 reductase. The multiple regression analyses among the activities of PCE and SCD and the proportion or the content of 18 : 1 (n-9) revealed that the standardized partial regression coefficient of PCE was higher than that of SCD. These results strongly suggest that the contribution of PCE to control the proportion and the content of 18 : 1 (n-9) in the liver is greater than that of SCD. This conclusion agrees with that of the previous study by Kudo *et al.*,[40] who showed that PCE play an important role in the formation of 18 : 1 (n-9) in the liver. This is also supported by the fact that 8-2 fluorotelomer alcohol increased the proportion of 20 : 3 (n-9), which synthesized from 18 : 1 (n-9) while it did not increase 16 : 1 synthesized

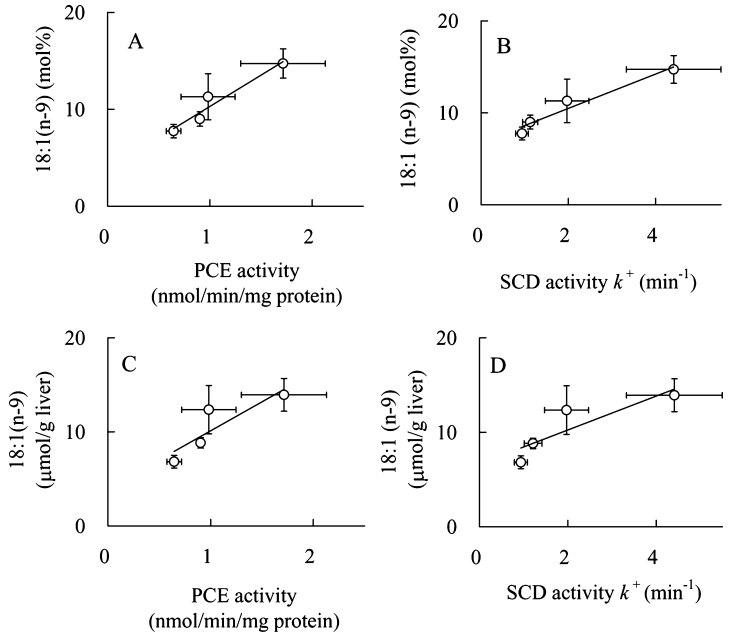

Fig. 5. Relationship between Either the Proportion or the Content of 18 : 1 (n-9) and the Activity of Either PCE or SCD

Regression analyses were performed on 4 set of mean data from Table 1 and Fig. 2 and on 4 set of mean data from Table 2. A, the proportion of 18 : 1 (n-9) *versus* the activity of PCE (Y=6.458X+3.8428, r^2=0.937, p<0.05); B, the proportion of 18 : 1 (n-9) *versus* the activity of SCD (Y=2.312X+6.105, r^2=0.963, p<0.05); C, the content of 18 : 1 (n-9) *versus* the activity of PCE (Y=6.153X−3.954, r^2=0.761, p<0.05); D, the content of 18 : 1 (n-9) *versus* the activity of SCD (Y=1.792X+6.659, r^2=0.766, p<0.05).

Table 3. Relative Contribution of PCE and SCD to the Proportion or the Content of 18 : 1 (n-9) in the liver[a)]

Independent variable	Standardized partial correlation coefficient	p
Proportion of 18 : 1 (n-9)		
PCE activity	0.748	<0.01
SCD activity	0.205	>0.05
Content of 18 : 1 (n-9) on the basis of g liver		
PCE activity	0.695	<0.01
SCD activity	0.241	>0.05

a) Multiple linear regression analyses were performed on the 4 sets of mean data from Fig. 2, Table 2 and Table 1. From the activity of PCE, the activity of SCD and the proportion or the content of 18 : 1 (n-9), two linear regression lines were obtained. Y=0.75X_1+0.21X_2. Y, proportion of 18 : 1 (n-9); X_1, the activity of PCE; X_2, the activity of SCD; r^2=0.823; p<0.01. Y=0.70X_1+0.24X_2; Y, content of 18 : 1 (n-9) on the basis of g liver; X_1, the activity of PCE; X_2, the activity of SCD; r^2=0.806; p<0.001.

from 16 : 0 by SCD alone.

In the liver of rats, there are two genes, rELO1 and rELO2, responsible for PCE and two genes, SCD1 and SCD2, responsible for SCD.[41)] SCD1 is predominant in the liver and its expression is altered by some drugs and nutritional states[42)] while SCD2 is predominant in brain but not in liver.[42)] The level of rELO2 mRNA is altered by the same conditions as was SCD1.[42)] The present study made attempts to clarify which genes are concerned in the increases in the activities of PCE and SCD. The treatment of rats with 8-2 fluorotelomer alcohol increased mRNA expression of rELO2 and SCD1, but not rELO1 or SCD2. Moreover, significant correlations are confirmed between PCE activity and rELO2 mRNA expression and between SCD activity and SCD1 mRNA expression. It seems likely, therefore, that the elevated expression of rELO2 and SCD1 is responsible for the elevated activities of PCE and SCD, respectively.

The mechanism by which 8-2 fluorotelomer alcohol causes an increase in the levels of rELO2 and SCD1 remains to be solved. Several studies have shown that PFOA is formed from 8-2 fluorotelomer alcohol as a metabolic product in mammals.[43,44)] It is possible that PFOA formed induces SCD and PCE since administration of rats with PFOA induces both enzymes[45)] and increases mRNA levels of SCD1 and rELO2 (Kudo *et al*., unpublished data). However, it cannot be excluded that 8-2 fluorotelomer alcohol itself and/or its metabolites induce these enzymes. In fact, the activities of acyl-CoA oxidase, a peroxisomal enzyme, was far greater than those expected by PFOA concentrations remained in the liver of 8-2 fluorotelomer alcohol-administered rats.[43)]

The present study showed that the administration of 8-2 fluorotelomer alcohol to rats decreased the proportion of 18 : 2 (n-6) and increased the proportion and the content of 20 : 3 (n-6) and 20 : 4 (n-6) in the liver. The biosynthesis of 20 : 4 (n-6) from 18 : 2 (n-6) involves sequential reactions catalyzed by Δ^6 desaturase, elongase for 18 : 3 (n-6) and Δ^5 desaturase.[46)] These facts may imply the increase in the activities of these elongase and desaturases, although enzymatic mechanism responsible for the increase in the proportion and the content of 20 : 4 (n-6) by 8-2 fluorotelomer alcohol remains to be elucidated.

In conclusion, 8-2 fluorotelomer alcohol caused great changes in fatty acid composition in the liver of rats. Especially, the proportion and the content of 18 : 1 (n-9) were markedly increased. These elevations of proportion and content of 18 : 1 (n-9) were caused by the induction of both PCE encoded by rELO2 and SCD encoded by SCD1, and, in particular, PCE plays a more important role in the increase in the proportion and content of 18 : 1 (n-9) in the liver than SCD does.

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