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Increase in Hepatic Content of Oleic Acid Induced by Dehydroepiandrosterone in the Rat

Koichi Imai, Matajirou Koyama,*† Naomi Kudo,‡ Akira Shirahata‡ and Yoichi Kawashima‡§*

*Department of Pharmacy, Saitama Cardiovascular and Respiratory Center, Saitama 360-0105, Japan; and ‡Faculty of Pharmaceutical Sciences, Josai University, Saitama 350-0295, Japan

ABSTRACT. The effects of dehydroepiandrosterone (DHEA) on the acyl composition of lipids in rat liver were studied. The content of oleic acid (18:1) in hepatic lipids was increased markedly by feeding rats a diet containing 0.5% (w/w) DHEA for 14 days. Treatment of rats with DHEA caused an increase in the activity of the terminal desaturase of the stearoyl-CoA desaturation system, without changing either the activity of NADH-cytochrome b_5 reductase or the microsomal content of cytochrome b_5. Among the changes observed in hepatic lipids, the increase in 18:1 content in phosphatidylcholine (PtdCho) was the most prominent; an approximately 2.5-fold increase in the proportion of 18:1 was induced at position 2, but not at position 1, by DHEA. This selective elevation of 18:1 at position 2 of PtdCho seems to be produced by the concerted actions of the induced 1-acylglycerophosphocholine (1-acyl-GPC) acyltransferase and the induced stearoyl-CoA desaturase. The content of 18:1 in serum lipids was unchanged by DHEA treatment, suggesting that secretion of lipids containing 18:1 into the circulation was not affected by DHEA. These results suggest that the elevation of hepatic content of 18:1 caused by DHEA treatment is mainly due to the induction of stearoyl-CoA desaturase. BIOCHEM PHARMACOL **58**;6:925–933, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. dehydroepiandrosterone; acyl composition; stearoyl-CoA desaturase; oleic acid; rat liver

DHEA‖ is a naturally occurring C_{19} steroid that is secreted from the adrenal glands and is found in the peripheral circulation of mammals. Although the physiologic role of DHEA has not been defined conclusively, reduction in the serum concentration of DHEA is associated with a variety of pathophysiological conditions including obesity [1], diabetes mellitus [2], cardiovascular disease [3, 4], Alzheimer's disease [5], and cancers [6–8] in humans. In animal models, DHEA is effective in preventing diabetes [9], obesity [10, 11], hypercholesterolemia [12], atherosclerosis [13], cancers [14, 15], autoimmune disorders [16], and impairment of immune function following thermal injury [17].

Among these pathophysiological conditions, obesity and diabetes are associated particularly with energy metabolism, namely fatty acid metabolism. Moreover, DHEA-treated animals have significantly lower body weight gain and heavier liver weights compared with the control, without suppressing food intake [18, 19]. Treatment of animals with DHEA has been shown to decrease the serum level of triacylglycerol significantly [20]. In this context, extensive attempts have been made to determine the effects of DHEA on the enzymes that participate in lipolysis and lipogenesis. DHEA has been reported to be an inhibitor of glucose-6-phosphate dehydrogenase, one of the enzymes that supply the NADPH required for fatty acid synthesis [21–23]. Subsequently, DHEA was shown to change the activities of enzymes that are involved in energy metabolism and fatty acid metabolism, such as malic enzyme [22, 23], fatty acid synthetase [22, 23], carnitine acetyltransferase [24], long-chain acyl-CoA hydrolase [25, 26], lipoprotein lipase [23], fatty acyl-CoA oxidase [27], and fatty acid synthase [26]. Moreover, serum insulin levels in obese Zucker rats are lowered by DHEA treatment [23, 28]. These findings imply that DHEA alters fatty acid modification, resulting in changes in the fatty acid composition in tissues of animals. In fact, a few studies have reported that feeding of DHEA changes the acyl composition of phospholipids in livers and plasma [22, 29], although the information was obtained from mice with immunological abnormalities [22] and obese Zucker rats [29].

Thus, little information is available about the effects of DHEA on the metabolic modification of fatty acids in normal animals. The present work was undertaken to study the response of the livers of normal rats to DHEA

† Present address: Department of Pharmacy, Saitama Cancer Treatment Center, 818 Komuro, Ina, Kita-adachi, Saitama 362–0806, Japan.

§ Corresponding author: Yoichi Kawashima, Ph.D., Faculty of Pharmaceutical Sciences, Josai University, 1–1 Keyakidai, Sakado, Saitama 350-0295, Japan. FAX (81) 492-71-7984; E-mail: ykawash@josai.ac.jp

‖ *Abbreviations*: DHEA, dehydroepiandrosterone; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; and GPC, glycerophosphocholine.

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with regard to the biosynthesis of oleic acid (18:1)* and the acyl composition of lipids. Upon treatment of rats with DHEA, stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase were induced in the liver, leading to an increase in the content of 18:1 in hepatic lipids, especially PtdCho. We report the results herein.

MATERIALS AND METHODS

Materials

DHEA was purchased from the Tokyo Kasei Kogyo Co. Stearoyl-CoA, palmitoyl-CoA, oleoyl-CoA, cytochrome *c*, BSA, and snake venom (*Crotalus adamanteus*) were obtained from the Sigma Chemical Co.; and triheptadecanoin and methyl heptadecanoate were from Nu-Chek Prep. NADH was from the Oriental Yeast Co., and 1-acyl-GPC (from egg PtdCho), from Avanti Polar Lipid. Lipase from *Mucor javanicus* was from the Amano Pharmaceutical Co. 2-Acyl-GPC was prepared from egg PtdCho by the action of lipase of M. *javanicus* as described by Ishihara *et al.* [30] just before use. Briefly, a mixture that contained 0.6 μmol of PtdCho, 1.2 mL of diethyl ether, 18.0 mg of lipase, and 6.0 mL of 0.1 M sodium borate buffer (pH 5.8) was incubated at 37° for 60 min. After the incubation, the fatty acids released were extracted with 30 mL of petroleum ether seven times from the incubation mixture, and then 2-acyl-GPC was extracted from the water phase by the method of Bligh and Dyer [31]. The 2-acyl-GPC obtained was dissolved in 0.05 M sodium borate (pH 5.5) and used within 4 hr to avoid migration of the acyl moiety of 2-acyl-GPC. All other chemicals used were of analytical grade.

Treatments of Animals and Preparation of Microsomes

Male Wistar rats (5 weeks old) were obtained from SLC. After acclimatization for 1 week, the rats (4 or 5/group) were fed *ad lib.* either a commercial diet (CE-2, Clea) or a diet containing 0.5% (w/w) DHEA for 7 or 14 days. Blood was collected from the descending vena cava under diethyl ether anesthesia, and then the rats were decapitated and their livers removed. Serum was obtained from the blood by centrifugation. The livers were perfused with ice-cold 0.9% (w/v) NaCl and rinsed in cold 0.25 M sucrose/1 mM EDTA/10 mM Tris–HCl buffer (pH 7.4). Each liver was cut into two pieces. One of them was frozen in liquid nitrogen and stored at -80° until used for the lipid analysis. The other piece was homogenized in 4 vol. of cold 0.25 M sucrose/1 mM EDTA/10 mM Tris–HCl (pH 7.4). The homogenates were centrifuged at 18,000 *g* for 20 min, and the supernatant was recentrifuged under the same conditions. The resulting supernatant was centrifuged at 105,000 *g* for 60 min. The pellet was resuspended in 0.25 M sucrose/10 mM Tris–HCl buffer (pH 7.4). The suspension was recentrifuged under the same conditions. The microsomal pellet obtained was resuspended in a small volume of 0.25 M sucrose/10 mM Tris–HCl buffer (pH 7.4) and used as an enzyme source. All operations were carried out at 0–4°.

Protein concentrations were determined by the method of Lowry *et al.* [32] with BSA as a standard.

Enzyme Assays

Terminal Δ^9 desaturase activity was assayed spectrophotometrically by the method of Oshino *et al.* [33] as the stearoyl-CoA-stimulated re-oxidation of NADH-reduced cytochrome b_5. The rate of cytochrome b_5 oxidation was measured with a Shimadzu UV-300 spectrophotometer by recording the changes in absorbance between 424 and 409 nm at 30°. The cuvette contained 0.9 mg of microsomal protein and 300 μmol of Tris–HCl buffer (pH 7.4) in a final volume of 3.0 mL. Microsomal cytochrome b_5 was reduced by 2 nmol of NADH, and re-oxidation was recorded. When the re-oxidation was completed, 20 nmol of stearoyl-CoA was added, and cytochrome b_5 was reduced again by 2 nmol of NADH. The first-order constant for the re-oxidation of NADH-reduced cytochrome b_5 was calculated as described by Oshino and Sato [34]. The rate constant for the re-oxidation of NADH-reduced cytochrome b_5 was measured in the presence (κ) and in the absence (κ^-) of stearoyl-CoA; the rate constant for Δ^9 desaturase was given by $\kappa^+ = \kappa - \kappa^-$ [35]. NADH-ferricyanide reductase and NADH-cytochrome *c* reductase were assayed by the methods of Rogers and Strittmatter [36] and Oshino *et al.* [37], respectively. NADH-ferricyanide reductase activity was determined by measuring the decrease in extinction at 420 nm. The reaction mixture contained 100 μmol of Tris–HCl buffer (pH 7.4), 0.22 μmol of potassium ferricyanide, 1 μmol of KCN, 0.1 μmol of NADH, and 20 μg of microsomal protein in a final volume of 1.0 mL. Reaction rates were calculated using an extinction coefficient of 1.02 cm^{-1} mM^{-1}. NADH-cytochrome *c* reductase was assayed by measuring the increase in optical density at 550 nm. The reaction mixture contained 100 μmol of Tris–HCl buffer (pH 7.4), 30 nmol of cytochrome *c*, 1 μmol of KCN, 0.1 μmol of NADH, and 5 μg of microsomal protein in a final volume of 1.0 mL. Reaction rates were calculated using an extinction coefficient of 21.1 cm^{-1} M^{-1}. The content of cytochrome b_5 in hepatic microsomes was estimated by the method of Omura and Sato [38] except that an extinction coefficient of 112 cm^{-1} mM^{-1} was used for reduced minus oxidized between 490 and 424 nm. 1-Acyl-GPC acyltransferase and 2-acyl-GPC acyltransferase were assayed essentially according to Lands and Hart [39]. The reaction mixture for 1-acyl-GPC acyltransferase contained 100 μmol of Tris–HCl buffer (pH 7.4), 20–30 nmol of oleoyl-CoA, 150 nmol of 1-acyl-GPC, 1 μmol of 5,5′-dithiobis(2-

* In the short notation of fatty acids, the first number indicates the chain length and the second number represents the number of double bonds: 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3 (n-3), α-linoleic acid; 20:3 (n-9), 5,8,11-eicosatrienoic acid; 20:3 (n-6), 6,9,12-eicosatrienoic acid; 20:4 (n-6), arachidonic acid; 20:5 (n-3), eicosapentaenoic acid; 22:5 (n-3), docosapentaenoic acid; and 22:6 (n-3), docosahexaenoic acid.

nitrobenzoic acid), and 50–75 μg of microsomal protein in a final volume of 1.0 mL. The reaction mixture for 2-acyl-GPC acyltransferase consisted of 100 μmol of Tris–HCl buffer (pH 7.4), 15 nmol of palmitoyl-CoA, 75 nmol of 2-acyl-GPC, 1 μmol of 5,5′-dithiobis(2-nitrobenzoic acid), and 100 μg of microsomal protein in a final volume of 1.0 mL. After preincubation in the absence of acyl-CoA, the incubation was initiated by the addition of acyl-CoA, and the increase in absorbance at 412 nm was followed at 30°. Control values without acyl-GPC were subtracted to give net acyl transfer rates.

Lipid Analyses

After the addition of a known amount of triheptadecanoin as an internal standard, total lipids were extracted from livers and serum by the method of Bligh and Dyer [31]. Triacylglycerol, cholesterol ester, and phospholipids were separated by TLC on silica gel G plates (Merck), which were developed with *n*-hexane:diethyl ether:acetic acid (80:30:1, by vol.). PtdCho, PtdEtn, PtdIns, and PtdSer were isolated by TLC on silica gel G plates as described by Holub and Skeaff [40]. After visualizing by spraying 0.001% (w/v) primuline in acetone, the regions on each plate that corresponded to specific lipids were scraped off and transferred to tubes. To the tubes, except for the triacylglycerol assay tube, was added a known amount of methyl heptadecanoate as an internal standard. Next, 10 mL of chloroform:methanol:0.1 M HCl (4:4:1, by vol.) was added to the tubes. After being kept at 4° overnight under nitrogen, the tubes were sonicated for 15 min with a bath-type sonicator. Finally, the lipid was extracted from the silica gel, and the extract was washed with 3 mL of 0.1 M HCl. For analysis of acyl composition, methyl esters of fatty acids were prepared from each extract using sodium methoxide or boron trifluoride in methanol. To analyze the acyl composition in positions 1 and 2 of PtdCho, an aliquot of PtdCho, which was isolated by TLC, was hydrolyzed by phospholipase A_2 from snake venom according to Lands and Merkl [41]. Free fatty acids and 1-acyl-GPC formed by the hydrolysis were separated by TLC and were converted to methyl esters of fatty acids as mentioned above. The amounts and compositions of the fatty acid methyl esters were determined by GLC (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° with helium as a carrier gas.

Statistical Analysis

The statistical significance of the difference between two means was estimated by Student's *t*-test.

RESULTS

Weight gain was suppressed significantly by treatment with DHEA for 14 days, and the final body weights of rats after the DHEA treatment were 88% of the control ($P < 0.001$) (199.0 ± 3.5 vs 175.4 ± 6.2 g). On the other hand, DHEA significantly increased liver weights by 67% ($P < 0.001$) (9.6 ± 0.3 vs 16.0 ± 1.1 g). No difference was found in cumulative food intake between the two groups.

Effects of DHEA on 18:1 Content and Stearoyl-CoA Desaturation in the Liver

Table 1 shows the fatty acid composition of total lipids in the livers of control rats and rats that had been treated with DHEA for 14 days. The proportion of 18:1 was increased considerably by the administration of DHEA. The increase compensated for a marked reduction in the proportion of linoleic acid (18:2). The proportion of 5,8,11-eicosatrienoic acid (20:3, n-9) increased considerably as well, and slight increases in the proportions of 6,9,12-eicosatrienoic acid (20:3, n-6) and arachidonic acid (20:4, n-6) also were observed. The treatment with DHEA caused a 2-fold increase in the total fatty acid content of whole liver. The increase was due mainly to the increase in the amounts of palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), 18:1, and 20:4; the amount of 18:2 was not changed. It should be noted that the extent of the increase in the content of 18:1 was the greatest among these fatty acids. Figure 1 shows the time course of the changes in the content of 18:1 in liver lipids. The amount of 18:1 in hepatic lipids increased gradually after the initiation of the DHEA treatment. The 18:1 content in hepatic lipids of rats that had been fed a diet containing DHEA for 14 days was approximately 3.6-fold greater than the control.

Since 18:1 is produced by the stearoyl-CoA desaturation system, and the system in the liver consists of three components, NADH-cytochrome b_5 reductase, cytochrome b_5, and cyanide-sensitive factor (terminal desaturase) [42, 43], we examined which of the three components were affected by the administration of DHEA. To measure the activity of NADH-cytochrome b_5 reductase, the non-physiological electron acceptors ferricyanide and cytochrome *c* were used. The activity of terminal desaturase was assayed as the rate constant for the stearoyl-CoA-stimulated re-oxidation reaction of NADH-reduced cytochrome b_5. The activity of NADH-cytochrome b_5 reductase, when measured as the activities of NADH-ferricyanide reductase and NADH-cytochrome *c* reductase, was not increased considerably by the administration of DHEA (Fig. 2, A and B); the content of cytochrome b_5 in hepatic microsomes was affected slightly by DHEA (Fig. 2C). In contrast to the two components of the desaturation system, the activity of terminal desaturase was enhanced markedly by the administration of DHEA, and the activity in the rats treated with DHEA for 14 days was approximately 4 times that of the control (Fig. 2D).

Effects of DHEA on Acyl Composition of Hepatic Lipids

To estimate the contribution of individual lipids to the change in the fatty acid composition of total hepatic lipids,

TABLE 1. Effects of DHEA on fatty acid composition of hepatic lipids

Fatty acid	DHEA	Fatty acid		
		mol%	μmol/g liter	μmol/liter
16:0	−	27.07 ± 1.63	22.69 ± 1.54	218.0 ± 15.7
	+	25.10 ± 2.43	25.82 ± 1.22*	414.3 ± 41.5†
16:1	−	2.41 ± 0.69	2.00 ± 0.45	19.1 ± 3.9
	+	3.52 ± 0.64‡	3.61 ± 0.43*	58.2 ± 10.7†
18:0	−	15.87 ± 1.18	13.28 ± 1.77	127.9 ± 19.7
	+	17.08 ± 1.10	17.71 ± 2.36‡	282.1 ± 23.1†
18:1	−	9.51 ± 0.41	7.97 ± 0.49	76.7 ± 6.8
	+	16.70 ± 0.85†	17.28 ± 1.80*	276.2 ± 24.2†
18:2	−	20.47 ± 0.44	17.19 ± 1.41	165.3 ± 16.9
	+	10.03 ± 0.44†	10.36 ± 0.66†	165.6 ± 7.5
18:3 (n–3)	−	0.44 ± 0.08	0.37 ± 0.04	3.5 ± 0.4
	+	0.07 ± 0.00*	0.08 ± 0.00†	1.2 ± 0.1†
20:3 (n–9)	−	0.11 ± 0.07	0.09 ± 0.05	0.9 ± 0.4
	+	0.78 ± 0.14†	0.82 ± 0.18*	13.0 ± 2.3†
20:3 (n–6)	−	0.62 ± 0.04	0.52 ± 0.03	5.0 ± 0.3
	+	1.70 ± 0.16†	1.77 ± 0.27*	28.1 ± 2.8†
20:4 (n–6)	−	16.05 ± 0.92	13.49 ± 1.52	129.8 ± 15.9
	+	19.50 ± 1.35*	20.20 ± 2.53*	321.8 ± 21.7†
20:5 (n–3)	−	1.10 ± 0.15	0.92 ± 0.11	8.8 ± 1.1
	+	0.83 ± 0.17‡	0.87 ± 0.21	13.7 ± 2.5*
22:5 (n–3)	−	1.34 ± 0.06	1.13 ± 0.12	10.8 ± 1.3
	+	0.61 ± 0.11†	0.64 ± 0.15*	10.1 ± 2.0
22:6 (n–3)	−	5.11 ± 0.31	4.29 ± 0.39	41.3 ± 4.1
	+	4.06 ± 0.39*	4.21 ± 0.62	67.0 ± 6.4†
Total	−		83.92 ± 5.83	807.0 ± 69.3
	+		103.35 ± 7.58*	1651.1 ± 65.5†

Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days.
Each value represents the mean ± SD for four rats.
*–‡Significant difference from control: *$P < 0.01$, †$P < 0.001$, and ‡$P < 0.05$.

the hepatic lipids were separated by TLC into individual lipids and the acyl compositions of the lipids were analyzed. As shown in Table 2, a considerable increase in the proportion of 18:1 and a marked decrease in the proportion of 18:2 were observed in PtdCho. In addition to the changes in 18:1 and 18:2, DHEA caused a significant increase in the proportions of 16:0, 16:1, and 20:4 in PtdCho. In triacylglycerol and cholesterol ester, a substantial increase in the proportion of 18:1 and a marked decrease in the proportion of 18:2 were brought about, as well. Considering the large proportion of PtdCho in hepatic lipids, the increase in the proportion of 18:1 in PtdCho is primarily responsible for the increase in 18:1 content in the liver. The proportion of 18:1 changed to a lesser extent in PtdEtn, PtdIns, and PtdSer. It should be noted that the proportions of 20:4 in PtdCho and PtdEtn were increased significantly by the administration of DHEA: by 20 and 34%, respectively. In contrast to the cases of PtdCho and PtdEtn, the proportions of 20:4 in cholesterol ester, triacylglycerol, PtdIns, and PtdSer were not increased.

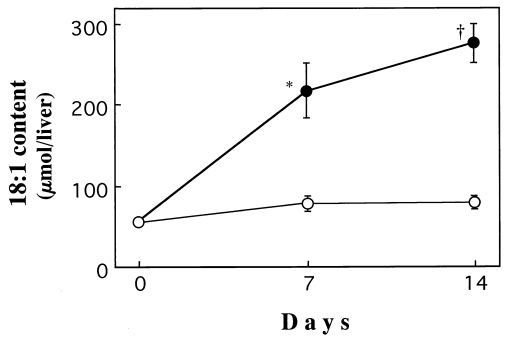

FIG. 1. Effects of treatment with DHEA on 18:1 content in hepatic lipids. Rats were fed either a control diet (○) or a diet containing 0.5% (w/w) DHEA (●) for 7 or 14 days. Each value represents the mean ± SD for 4 rats. Key: significantly different from control at (*) $P < 0.01$, and (†) $P < 0.001$.

Among the changes observed in the hepatic lipids, the increase in 18:1 content in PtdCho was the most evident. Since 18:1 is known to be distributed to both position 1 and position 2 of PtdCho, the acyl compositions of positions 1 and 2 of hepatic PtdCho were analyzed separately. A 2.5-fold increase in the proportion of 18:1 was induced at position 2, but not at position 1, by the administration of DHEA to rats (Table 3).

The effects of DHEA treatment on microsomal 1-acyl-

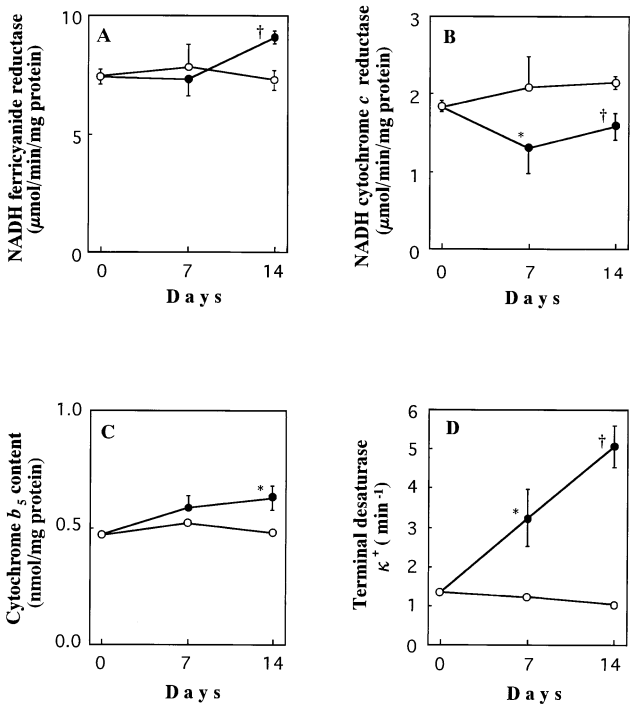

FIG. 2. Effects of treatment with DHEA on the stearoyl-CoA desaturation system. Rats were fed either a control diet (○) or a diet containing 0.5% (w/w) DHEA (●) for 7 or 14 days. A, NADH ferricyanide reductase; B, NADH cytochrome c reductase; C, cytochrome b5; D, terminal desaturase. Each value represents the mean ± SD for 4 or 5 rats. Key: significantly different from control at (*) $P < 0.01$, and (†) $P < 0.001$.

GPC acyltransferase and 2-acyl-GPC acyltransferase were examined (Table 4). The activity of 1-acyl-GPC acyltransferase was increased 2.5 times as much as the control, following the administration of DHEA for 14 days. Compared to the marked increase in the activity of 1-acyl-GPC acyltransferase, DHEA little affected the activity of 2-acyl-GPC acyltransferase.

Effects of DHEA on 18:1 Content in Serum

The effects of DHEA on the acyl compositions of lipids in serum were examined. As shown in Table 5, the proportions of 18:1 in cholesterol ester, triacylglycerol, and phospholipid were increased markedly following challenge with DHEA; marked decreases of the 18:2 proportion in these three lipids were brought about. Upon the treatment, the serum level of triacylglycerol was lowered by 48%, whereas the content of 18:1 in triacylglycerol was suppressed slightly, to 81% of the control. On the other hand, total cholesterol ester and phospholipid in serum were not changed significantly, but the contents of 18:1 in cholesterol ester and phospholipid were 1.4- and 1.6-fold over the control levels, respectively.

DISCUSSION

The present study showed that the administration of DHEA to male normal Wistar rats resulted in marked increases in the proportions and the contents (on the basis of g liver) of 18:1 and 20:3 (n-9), but not 16:0, in hepatic lipids. The present results are, in part, consistent with previously reported findings [22, 29]. Miller *et al.* [22] investigated in detail the effect of DHEA feeding on the acyl composition of phospholipids in livers of New Zealand Black/New Zealand White F_1 female mice and showed that DHEA increased the proportions of 16:0 and 18:1, but decreased the proportion of 18:0. Mohan and Cleary [29] reported that DHEA feeding elevated the proportion of 18:1 in PtdCho and cardiolipin of liver mitochondria of female obese Zucker rats. Although there is a small discrepancy between our present results and the previous findings [22, 29], this may be due to the different experimental conditions, such as sex, species, and abnormalities of animals used.

18:1 is a main product of stearoyl-CoA desaturase, and 20:3 (n-9) is a typical product derived from 18:1 by further desaturation and elongation when animals are under essential fatty acid-deficient conditions [44, 45]. Moreover, hormones such as insulin and hydrocortisone have been shown to stimulate the activity of stearoyl-CoA desaturase in both animals and cultured tissues [46, 47], resulting in the increase in the proportion of 18:1 in lipid. These facts strongly imply that the increase in the contents of these fatty acids in the liver induced by DHEA is caused by the increase in the activity of hepatic Δ^9 desaturation. In the present study, we made attempts to clarify the enzymatic mechanism responsible for the increase in the 18:1 level. As expected, the present study demonstrated that the administration of DHEA to rats increased the activity of stearoyl-CoA desaturase in the liver. These results are consistent with a hypothesis presented by Miller *et al.* [22], who assumed an increase in the rate of desaturation of 18:0 to 18:1 in the liver, based on analysis of the acyl composition of hepatic phospholipids of immunologically altered mice.

It has been established that the stearoyl-CoA desaturation system consists of three components, NADH-cytochrome b_5 reductase, cytochrome b_5, and a terminal desaturase [42, 43]. The present study showed that DHEA increased only the terminal desaturase activity without affecting either NADH-cytochrome b_5 reductase activity or cytochrome b_5 content. It is known that the microsomal stearoyl-CoA desaturation activity is affected profoundly by various physiological conditions of animals, such as age, hormonal status, diabetes, and dietary conditions [42, 43]. The activity of Δ^9 desaturation in rat liver is decreased by food deprivation and increased by re-feeding after the deprivation; Oshino and Sato [48] presented evidence that the activity of the terminal desaturase changes in parallel to the changes in the overall activity of the desaturation. In contrast to the terminal desaturase, the other components, NADH-cytochrome b_5 reductase and cytochrome b_5, were not affected significantly by the food deprivation and re-feeding [34]. Prasad and Joshi [49] also showed that only the terminal enzyme activity in the stearoyl-CoA desatu-

TABLE 2. Effects of DHEA on acyl composition of various hepatic lipids

Fatty acid	DHEA	Cholesterol ester	Triacylglycerol	PtdCho (mol%)	PtdEtn	PtdIns	PtdSer
16:0	−	43.1 ± 3.2	33.0 ± 2.1	26.7 ± 0.7	26.4 ± 1.0	11.1 ± 0.9	7.2 ± 1.7
	+	37.2 ± 4.7	33.5 ± 1.3	28.5 ± 0.7*	22.0 ± 1.0†	11.8 ± 0.4	8.5 ± 1.0
16:1	−	2.7 ± 0.0	5.4 ± 1.6	1.4 ± 0.2	0.7 ± 0.2	0.3 ± 0.1	0.5 ± 0.1
	+	4.8 ± 0.6*	7.2 ± 0.8	2.1 ± 0.2‡	0.9 ± 0.1	0.8 ± 0.1‡	1.0 ± 0.2‡
18:0	−	39.4 ± 5.3	1.8 ± 0.2	19.7 ± 0.3	21.4 ± 0.4	42.1 ± 1.1	44.2 ± 1.7
	+	38.6 ± 3.2	1.7 ± 0.1	19.5 ± 0.9	25.7 ± 1.3‡	39.6 ± 1.4*	43.2 ± 3.7
18:1	−	6.7 ± 2.3	24.9 ± 0.4	7.6 ± 0.3	5.6 ± 0.1	2.0 ± 0.1	3.7 ± 0.3
	+	13.3 ± 0.5*	45.9 ± 1.2†	12.2 ± 0.9‡	7.2 ± 0.8*	4.2 ± 0.6‡	5.1 ± 0.7*
18:2	−	4.3 ± 1.2	26.6 ± 2.0	19.2 ± 0.6	8.7 ± 0.8	5.2 ± 0.9	5.2 ± 0.8
	+	2.3 ± 0.2*	8.7 ± 0.7†	8.8 ± 0.4†	3.6 ± 0.1‡	3.1 ± 0.1*	4.9 ± 1.0
18:3 (n–3)	−	ND§	1.4 ± 0.1	ND	ND	ND	ND
	+	ND	0.2 ± 0.0†	ND	ND	ND	ND
20:3 (n–9)	−	0.5 ± 0.5	ND	0.1 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
	+	0.5 ± 0.5	ND	0.7 ± 0.1‡	0.4 ± 0.1	3.7 ± 0.6‡	0.7 ± 0.1
20:3 (n–6)	−	ND	0.3 ± 0.0	0.8 ± 0.1	0.4 ± 0.0	1.9 ± 0.3	0.6 ± 0.1
	+	ND	0.3 ± 0.0	2.2 ± 0.1‡	0.8 ± 0.0†	3.3 ± 0.2‡	2.1 ± 0.2†
20:4 (n–6)	−	1.4 ± 0.4	1.6 ± 0.2	17.9 ± 0.5	21.9 ± 0.6	33.3 ± 1.6	23.5 ± 0.9
	+	1.3 ± 0.2	1.5 ± 0.2	21.5 ± 0.7†	29.4 ± 0.6†	30.6 ± 1.2*	20.2 ± 0.8‡
20:5 (n–3)	−	1.8 ± 0.9	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.1	0.2 ± 0.1	0.9 ± 0.1
	+	2.0 ± 1.8	0.4 ± 0.1‡	0.6 ± 0.1*	0.5 ± 0.1†	ND	0.7 ± 0.5
22:5 (n–3)	−	ND	1.7 ± 0.5	0.9 ± 0.1	2.5 ± 0.2	1.2 ± 0.2	1.9 ± 0.2
	+	ND	0.2 ± 0.1*	0.4 ± 0.1†	1.0 ± 0.1†	0.7 ± 0.1‡	1.4 ± 0.3*
22:6 (n–3)	−	ND	2.4 ± 0.8	4.8 ± 0.2	10.8 ± 0.6	1.5 ± 0.5	11.5 ± 0.4
	+	ND	0.6 ± 0.1*	3.6 ± 0.1†	8.4 ± 0.3†	2.3 ± 0.3*	12.3 ± 0.7
Total (μmol/g liver)	−	3.7 ± 0.4	5.6 ± 0.6	32.3 ± 2.6	13.4 ± 2.3	5.5 ± 0.5	2.3 ± 0.2
	+	3.0 ± 0.1*	6.4 ± 1.2	41.4 ± 4.1‡	15.9 ± 2.0	7.5 ± 0.7‡	2.1 ± 0.2
Total (μmol/liver)	−	36.3 ± 4.7	53.6 ± 6.0	310.7 ± 30.5	128.8 ± 23.8	52.7 ± 6.0	21.8 ± 1.9
	+	49.5 ± 2.3‡	102.4 ± 21.5*	659.6 ± 26.5†	253.4 ± 18.4†	119.8 ± 5.2†	34.2 ± 3.0†

Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. Each value represents the mean ± SD for four rats.

*–‡Significant difference from control: *$P < 0.05$, †$P < 0.001$., and ‡$P < 0.01$.

§ND: less than 0.1% of the total fatty acids.

ration system is responsible for the decrease in the overall desaturation activity in diabetic rats and for the recovery of the decreased activity by the administration of insulin or the feeding of fructose. Consequently, it seems to be a generally accepted concept that the changes in Δ^9 desaturation activity are due mainly to changes in terminal desaturase activity, but not to changes in electron flow rate from NADH to cytochrome b_5 via NADH-cytochrome b_5 reductase. Our present results strongly support this concept of the regulation of Δ^9 desaturation.

The present study revealed that DHEA caused a marked increase in the proportion of 18:1 in hepatic lipids, particularly in PtdCho, and that the change in the proportion of 18:1 in PtdCho was due mainly to the increase in 18:1

TABLE 3. Effects of DHEA on fatty acyl composition of C-1 and C-2 positions of phosphatidylcholine in the liver

	Position 1		Position 2	
Fatty acid	Control	DHEA	Control	DHEA
	(mol%)			
16:0	49.2 ± 0.4	51.5 ± 2.0	4.2 ± 0.5	3.3 ± 1.0
18:0	39.3 ± 1.3	39.6 ± 1.7	3.5 ± 0.7	3.6 ± 1.6
18:1	7.7 ± 0.7	5.1 ± 0.3*	7.5 ± 0.2	19.1 ± 1.4*
18:2	2.0 ± 1.1	1.4 ± 0.2	33.7 ± 1.3	15.2 ± 0.7*
20:4 (n–6)	0.3 ± 0.1	0.5 ± 0.1†	34.7 ± 1.2	42.2 ± 1.6*
22:6 (n–3)	ND‡	ND	9.7 ± 0.6	7.3 ± 0.3*

Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. Each value represents the means ± SD for four rats. Only major fatty acids of PtdCho are shown.

*,†Significant difference from control: *$P < 0.001$, and †$P < 0.01$.

‡ND: less than 0.1% of the total fatty acids.

TABLE 4. Effects of DHEA on 1-acyl-GPC acyltransferase and 2-acyl-GPC acyltransferase in the liver

Treatments	1-Acyl-GPC acyltransferase	2-Acyl-GPC acyltransferase
	(nmol/min/mg protein)	
Control	90.2 ± 4.5	39.9 ± 2.2
DHEA	224.3 ± 16.7*	43.8 ± 2.1†

Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. 1-Acyl-GPC acyltransferase and 2-acyl-GPC acyltransferase were assayed with oleoyl-CoA and palmitoyl-CoA, respectively, as substrates. Each value represents the mean ± SD for four rats.

*,†Significant difference form control: *$P < 0.01$ (0.01), and †$P < 0.05$.

TABLE 5. Effects of DHAE on acyl composition of serum lipids

		Cholesterol ester		Triacylglycerol		Phospholipid	
Fatty acid	DHEA	mol%	μmol/mL	mol%	μmol/mL	mol%	μmol/mL
16:0	−	20.9 ± 2.0	0.22 ± 0.03	28.5 ± 0.3	0.41 ± 0.05	26.3 ± 0.6	0.59 ± 0.01
	+	19.3 ± 1.6	0.20 ± 0.03	29.4 ± 0.5*	0.22 ± 0.05†	27.7 ± 0.4*	0.65 ± 0.06
16:1	−	3.1 ± 0.7	0.03 ± 0.01	3.9 ± 0.6	0.06 ± 0.01	1.1 ± 0.2	0.02 ± 0.00
	+	5.4 ± 1.1‡	0.06 ± 0.02*	5.3 ± 0.4*	0.04 ± 0.01*	1.5 ± 0.0*	0.04 ± 0.00‡
18:0	−	19.1 ± 1.1	0.21 ± 0.01	1.9 ± 0.2	0.03 ± 0.01	20.2 ± 0.3	0.45 ± 0.02
	+	19.1 ± 3.6	0.20 ± 0.01	2.7 ± 0.1†	0.02 ± 0.00*	20.6 ± 0.5	0.48 ± 0.05
18:1	−	6.1 ± 0.3	0.07 ± 0.01	23.0 ± 0.5	0.33 ± 0.04	7.1 ± 0.2	0.16 ± 0.00
	+	11.8 ± 1.4*	0.12 ± 0.03*	36.4 ± 0.4†	0.27 ± 0.06	11.2 ± 0.2†	0.26 ± 0.03*
18:2	−	22.3 ± 1.2	0.24 ± 0.02	32.6 ± 0.8	0.47 ± 0.05	25.2 ± 0.8	0.57 ± 0.02
	+	11.2 ± 1.0†	0.12 ± 0.03‡	19.5 ± 1.0†	0.15 ± 0.03†	14.4 ± 0.1†	0.34 ± 0.03†
18:3 (n–3)	−	ND§	ND	2.0 ± 0.1	0.03 ± 0.00	ND	ND
	+	ND	ND	1.0 ± 0.1†	0.01 ± 0.00†	ND	ND
20:3 (n–9)	−	ND	ND	ND	ND	ND	ND
	+	0.7 ± 0.1	0.01 ± 0.00	ND	ND	0.8 ± 0.1‡	0.020 ± 0.003‡
20:3 (n–6)	−	ND	ND	0.2 ± 0.0	0.003 ± 0.00	0.8 ± 0.1	0.017 ± 0.002
	+	0.4 ± 0.0	0.01 ± 0.00	0.4 ± 0.1‡	0.003 ± 0.00	1.9 ± 0.0†	0.044 ± 0.004†
20:4 (n–6)	−	25.9 ± 1.2	0.28 ± 0.01	1.5 ± 0.1	0.02 ± 0.002	14.1 ± 0.3	0.32 ± 0.02
	+	30.2 ± 2.4*	0.32 ± 0.06	2.7 ± 0.4‡	0.02 ± 0.002	18.3 ± 0.2†	0.43 ± 0.05*
20:5 (n–3)	−	2.6 ± 1.0	0.03 ± 0.01	1.4 ± 0.2	0.02 ± 0.00	0.8 ± 0.1	0.02 ± 0.002
	+	1.9 ± 0.2	0.02 ± 0.00	0.9 ± 0.1‡	0.01 ± 0.00†	0.6 ± 0.1*	0.01 ± 0.003*
22:5 (n–3)	−	ND	ND	1.9 ± 0.2	0.03 ± 0.00	1.0 ± 0.1	0.02 ± 0.002
	+	ND	ND	0.4 ± 0.1†	0.003 ± 0.001†	0.4 ± 0.1†	0.01 ± 0.003†
22:6 (n–3)	−	ND	ND	3.0 ± 0.4	0.04 ± 0.004	3.4 ± 0.2	0.08 ± 0.01
	+	ND	ND	1.3 ± 0.2†	0.01 ± 0.001†	2.6 ± 0.1‡	0.06 ± 0.01‡
Total	−		1.07 ± 0.02		1.45 ± 0.16		2.25 ± 0.06
	+		1.05 ± 0.15		0.75 ± 0.15†		2.35 ± 0.23

Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. Each value represents the mean ± SD for four rats.

*–‡Significant difference from control: *$P < 0.05$, †$P < 0.001$, and ‡$P < 0.01$.

§ND less than 0.1% of the total fatty acids.

content at position 2 of PtdCho. Moreover, the present study showed that treatment of rats with DHEA caused a marked increase in the activity of 1-acyl-GPC acyltransferase, but not in 2-acyl-GPC acyltransferase, in the liver. Accordingly, it is likely that 1-acyl-GPC acyltransferase induced by DHEA increased the formation of PtdCho containing 18:1 in concert with the action of the induced stearoyl-CoA desaturase. Since this enzyme is known not to be influenced by physiological conditions of animals such as hormonal status and dietary conditions [50], it is interesting that DHEA, a steroid hormone, increases the activity of this enzyme.

In the present study, we showed that the serum triacylglycerol level was lowered by DHEA in accordance with previous findings [20]. On the contrary, there were no significant effects on serum levels of phospholipids and cholesterol ester. Consequently, despite the marked increase in the level of 18:1 in the liver, the level of 18:1 in serum was not changed by DHEA treatment. These results suggest that the increase of the hepatic 18:1 content was not due to the accumulation of lipids containing 18:1 as a result of reduced secretion of lipids containing 18:1 into the blood circulation.

Although the physiological significance of the altered fatty acid composition induced by DHEA has not been clarified, it is of interest to note that changes in fatty acid composition of membrane lipids may have many consequences: alterations in membrane properties and in membrane-bound enzyme activities.

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