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Effects of dehydroepiandrosterone on oleic acid accumulation in rat liver

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Abstract

The purpose of the present study was to determine whether dehydroepiandrosterone (DHEA) affects *de novo* fatty acid synthesis, oleic acid formation, fatty acid oxidation, and very low density lipoprotein (VLDL) secretion, in relation to the accumulation of lipid containing oleic acid, in rat liver. The rates of hepatic *de novo* synthesis of both fatty acid and monounsaturated fatty acid, determined by incorporation of ^{3}H from $^{3}H_2O$ into fatty acid, were increased markedly when rats were fed a diet containing 0.5% (w/w) DHEA for 14 days. The treatment of rats with DHEA also enhanced the conversion of [^{14}C]stearic acid into oleic acid in the liver *in vivo*. DHEA did not suppress fatty acid degradation in the liver. Namely, mitochondrial palmitic acid oxidation in liver homogenates and isolated hepatocytes was increased approximately 1.9- and 5-fold, respectively, in DHEA-treated rats. Peroxisomal palmitic acid oxidation in isolated hepatocytes from rats treated with DHEA, however, was not significantly different from that of the control, despite the fact that peroxisomal degradation of palmitic acid in the liver homogenates was increased markedly. The rate of hepatic VLDL secretion in DHEA-treated rats was decreased markedly. These results indicate that the elevation of the hepatic fatty acid content, especially oleic acid, by DHEA feeding is due to an increase in both *de novo* fatty acid synthesis and the formation of oleic acid and to a decrease in the rate of hepatic VLDL secretion. Mitochondrial and peroxisomal fatty acid degradation does not appear to play a significant role in the accumulation of hepatic lipids.

Keywords: Dehydroepiandrosterone; *De novo* fatty acid synthesis; Fatty acid oxidation; VLDL secretion; Oleic acid; Liver

1. Introduction

DHEA, a naturally occurring C_{19} steroid that is secreted from the adrenal glands, is found in the peripheral circulation of mammals [1]. Although the exact physiological role of DHEA is not understood fully, it appears to help prevent obesity [2,3], atherosclerosis [4,5], cancer [6,7], diabetes [8,9], hypertension [10,11], and autoimmune disorders [12,13] in rodent models. Moreover, treatment of rats and mice with DHEA is known to induce enzymes involved in peroxisomal β-oxidation in the liver [14–16]. These previous findings suggest that fatty acid metabolism is possibly regulated by DHEA.

A recent study of ours demonstrated that administration of DHEA to rats markedly enhances the activities of enzymes that participate in oleic acid formation in the liver and significantly increases the hepatic content of oleic acid [17]. Oleic acid is a unique unsaturated fatty acid that animals are capable of synthesizing from *de novo* synthesized palmitic acid and from dietary palmitic or stearic acids by palmitoyl-CoA elongation and stearoyl-CoA desaturation [18–20]. Regulation of the formation of unsaturated fatty acids is important because they play a role in cellular activity, metabolism, and nuclear events that govern gene transcription [21]. The ratio of stearic acid to oleic acid is one of the factors influencing cell membrane fluidity. Alteration of this ratio is physiologically important in various diseases such as aging, cancer, diabetes, obesity, and hypertension [22,23]. Among these pathophysiological conditions, obesity and diabetes in particular are associated with energy metabolism, namely fatty acid metabolism. It is plausible, therefore, that the preventive effects of DHEA on obesity and diabetes are due to an increase in the proportion of oleic acid in hepatic lipids. However, a comprehensive understanding of the mechanism by which DHEA causes

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Abbreviations: DHEA, dehydroepiandrosterone; VLDL, very low density lipoprotein.

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the elevation of the hepatic content of oleic acid *in vivo* is still lacking.

The present study examined the question of whether DHEA affects fatty acid metabolism: *de novo* fatty acid synthesis, oleic acid formation, fatty acid oxidation, and VLDL secretion. The results obtained are discussed in relation to the increase caused by DHEA in the hepatic content of oleic acid.

2. Materials and methods

2.1. Materials

^3H_2O (1 mCi/mL) was obtained from Moravek Biochemicals Inc.; and [1-^{14}C]stearic acid (55 Ci/mol) and [1-^{14}C]palmitic acid (55 Ci/mol) were obtained from American Radiolabeled Chemicals Inc. DHEA was from the Tokyo Kasei Kogyo Co.; BSA (essentially fatty acid free) and 1 M Hyamine hydroxide in methanol were from the Sigma Chemical Co. Triton WR-1339 was obtained from Nacalai Tesque; triheptadecanoin from Nu-Chek Prep.; and standard diet (CE-2) from Clea Japan Inc. $AgNO_3$-impregnated TLC analysis was performed on precoated silica gel G TLC plates (Merck) that had been immersed in 10% (w/v) $AgNO_3$ in acetonitrile, dried horizontally, and activated at 110° for 1 hr. All other chemicals used were of analytical grade.

2.2. Treatments of animals

Male Wistar rats (5-weeks-old) were obtained from Japan SLC Inc. After acclimatization for 1 week, the animals were fed *ad libitum* either a standard diet or a diet containing 0.5% (w/w) DHEA. Blood was collected from the descending vena cava under diethyl ether anesthesia, and then the animals were decapitated and their livers removed. Serum was obtained from the blood by centrifugation.

2.3. Incorporation of ^3H from ^3H_2O into hepatic fatty acids in vivo

De novo fatty acid synthesis in the liver was assayed essentially according to Lakshmanan and Veech [24]. Rats were injected intraperitoneally with ^3H_2O at a dose of 4 mCi/kg body weight. One hour after the injection, blood was collected in lightly heparinized tubes, and the livers were isolated. The livers were then perfused with ice-cold saline through a vein to remove the blood, and then were rinsed with ice-cold saline. The major portion of the liver (2 g) was homogenized with 40 mL of chloroform:methanol (2:1, v/v). The homogenates were filtered and evaporated under a stream of nitrogen. The residue was dissolved in 2 mL of ethanol and saponified with 4 mL of 5 M NaOH at 80° for 3 hr under a nitrogen atmosphere. The unsaponifiable fraction was extracted five times with 6 mL of petroleum ether. To the aqueous phase was added 2 mL of 6 M HCl. Fatty acids were extracted three times with 4 mL of *n*-hexane, and the *n*-hexane extracts were combined. After washing with 6 mL of water, the *n*-hexane phase was transferred to a counting vial. After evaporation under a stream of nitrogen, the fatty acids obtained were dissolved in scintillation fluid, and radioactivity was measured by liquid scintillation analysis. The ^3H radioactivity in a suitable aliquot of the plasma was determined as well. The water content of the plasma was determined by finding the dry weight of the plasma (the average dry weight was 8.5%). This water content value was employed to calculate the specific radioactivity of body water.

2.4. Incorporation of ^3H from ^3H_2O into monounsaturated fatty acid

The rate of *de novo* synthesis of monounsaturated fatty acid in the liver was determined by the incorporation of ^3H from ^3H_2O into monounsaturated fatty acid. Lipid was extracted from the livers of rats treated with tritiated water by the method of Bligh and Dyer [25]. The lipid was saponified with 5% (w/v) KOH in 90% methanol at 80° for 1 hr under a nitrogen atmosphere. Following the addition of 4 mL of water, the aqueous mixture was washed out three times with 4 mL of *n*-hexane to remove unsaponified lipid. After acidification with 6 M HCl, fatty acids were extracted twice with 4 mL of *n*-hexane. Following evaporation of the combined extract under a stream of nitrogen, the fatty acids obtained were methylated with boron trifluoride in methanol. Saturated and monounsaturated fatty acid esters were separated by TLC on $AgNO_3$-impregnated silica gel G, which was developed with *n*-hexane:diethyl ether (9:1, v/v). The spots were detected under UV light after spraying with 0.05% (w/v) Rhodamine B in 95% ethanol. The areas corresponding to authentic methyl saturated and methyl monounsaturated fatty acids were scraped off the plate. The methyl esters obtained were extracted with toluene and dissolved in scintillation fluid. The radioactivities in the saturated and monounsaturated fatty acid esters were measured by liquid scintillation analysis.

2.5. Desaturation of stearic acid in vivo

The [1-^{14}C]stearic acid solution to be injected was prepared essentially according to Kawashima *et al.* [26]. After evaporation of [1-^{14}C]stearic acid under a stream of nitrogen, the residue was dissolved in a very small volume of ethanol to which an equivalent amount of NaOH as a 10.9 mM solution was added. To this solution was added 12 vol. of rat serum that had been filtered through a Millipore filter (0.22 μm), and the solution was mixed and allowed to stand at room temperature for 10 min. Under

light diethyl ether anesthesia, 0.2 mL of the injection solution containing 10 μCi of [1-^{14}C]stearic acid was injected into the exposed right jugular vein of rats that had been fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. Ten minutes after the injection, the rats were decapitated and their livers isolated. The livers were first perfused with ice-cold saline through a vein to remove the blood, and then were rinsed with ice-cold saline. Hepatic lipid was extracted and saponified. Fatty acids were extracted and converted to methyl esters to be separated into saturated and monounsaturated fatty acid esters by TLC on $AgNO_3$-impregnated silica gel G plates as described above. The radioactivities in the saturated and monounsaturated fatty acid esters were measured as described above.

2.6. Mitochondrial and peroxisomal fatty acid oxidation in homogenates

Mitochondrial and peroxisomal fatty acid oxidation was measured essentially according to Mannaerts *et al.* [27]. Liver homogenates (5%, w/v) in 0.25 M sucrose containing 0.1% ethanol were prepared in a Dounce homogenizer with six strokes each of a loose and a tight-fitting plunger. A modified Krebs–Henseleit bicarbonate buffer [28], containing no calcium, twice the normal amount of $MgSO_4$, 4 vol. of 0.154 M NaCl, and 100 vol. of 0.154 M KCl per 130 vol. of buffer, was used in the assays.

Mitochondrial fatty acid oxidation was measured in a final volume of 2.5 mL that contained 2 mL of modified Krebs–Henseleit bicarbonate buffer (pH 7.4); 0.2 mM sodium [1-^{14}C]palmitate (sp. act. 1 μCi/μmol), bound to 7.2 mg/mL of defatted albumin (molar ratio of substrate:albumin = 1.67); 4 mM ATP; 0.5 mM L-carnitine; 0.05 mM CoA; 2 mM dithiothreitol; and 0.5 mL of 5% (w/v) liver homogenate in sucrose/ethanol. Fatty acid oxidation was determined in the presence and absence of 2 mM KCN, and the cyanide-sensitive part of the oxidation was taken as mitochondrial oxidation.

Peroxisomal fatty acid oxidation was measured in a final volume of 2.5 mL containing 2 mL of modified Krebs–Henseleit bicarbonate buffer (pH 7.4); 0.2 mM sodium [1-^{14}C]palmitate (sp. act. 1 μCi/μmol); 4 mM ATP; 0.5 mM CoA; 2 mM NAD^+; 2 mM KCN; 2 mM dithiothreitol; and 0.5 mL of a 5% (w/v) liver homogenate in 0.25 M sucrose containing 0.1% ethanol. Peroxisomal oxidation was routinely measured in the absence of albumin and also in the presence of 7.2 mg/mL of albumin, the concentration employed being optimum to the assay for mitochondrial fatty acid oxidation.

Incubations were carried out in siliconized glass vials that initially contained the buffer, the labeled substrate, and the appropriate components listed above. The vials were gassed with O_2:CO_2 (95:5), capped with rubber stoppers from which were suspended plastic center-wells, and shaken at 37° with 80–90 oscillations/min. Reactions were started with the addition of homogenates, and the vials were incubated at 37° for 4 min with shaking. The incubation was terminated by the injection of 0.4 mL of 5% $HClO_4$ into the vial, and 0.4 mL of 1 M Hyamine hydroxide in methanol was injected into the center well. The reaction vials were shaken for another 45 min at room temperature to trap labeled CO_2 into Hyamine hydroxide [28]. Then the contents of the center well were transferred to a counting vial and was mixed with scintillation fluid. The radioactivity was measured by liquid scintillation analysis. The acidified content of the vial was transferred to a tube and mixed with 0.3 mL of 70% $HClO_4$; after centrifugation (1600 *g* for 10 min at room temperature), the supernatant was transferred to a tube and was neutralized with 0.8 mL of 5 M KOH. After the addition of 0.7 mL of 3 M acetate buffer (pH 4.0), the reaction mixture was extracted four times with 5 mL of petroleum ether to remove traces of the [1-^{14}C]palmitic acid. An aliquot of the aqueous phase was mixed with scintillation fluid, and the radioactivity was counted (acid-soluble labeled oxidation products).

Peroxisomal palmitoyl-CoA oxidation in homogenates was assayed spectrophotometrically by measuring the cyanide-insensitive formation of NADH, according to the method of Lazarow and de Duve [29] with some minor modifications [30]. Protein concentrations were determined by the method of Lowry *et al.* [31] with BSA as a standard.

2.7. Fatty acid oxidation in isolated hepatocytes

Hepatocytes were prepared by the method of Tanaka *et al.* [32]. Cells (5×10^6) were incubated in duplicate in siliconized scintillation counting vials in a final volume of 2 mL of Krebs–Henseleit bicarbonate buffer (pH 7.4), which contained 0.3 mM sodium [1-^{14}C]palmitate (sp. act. 1.3×10^6 dpm) and 23 mg of defatted albumin per mL [33]. The vials were gassed with O_2:CO_2 (95:5) and were incubated at 37° for 15 min with shaking. Incubations were terminated by the addition of 0.4 mL of 5% $HClO_4$, and labeled CO_2 and acid-soluble labeled products were measured as described above.

2.8. VLDL secretion rate

Rats that had been starved for 24 hr were injected intravenously with 20% (w/v) Triton WR-1339 in saline at a dose of 300 mg/kg body weight. Three hours after the injection, the animals were anesthesized with diethyl ether, and blood from the descending vena cava was collected into a tube containing EDTA (final concentration of 1 mg/mL). Serum was obtained from the blood by centrifugation (1600 *g* for 15 min at 4°). The VLDL secretion rate was determined by measuring the change in serum triacylglycerol levels [34]. The amount of total blood was calculated as one-twelfth of the body weight. The

Table 1
Effects of DHEA feeding on the rate of fatty acid and monounsaturated fatty acid syntheses in the liver

Treatment	Liver weight (g)	Fatty acid synthetic rate		Monounsaturated fatty acid synthetic rate	
		(μmol/g liver/hr)	(μmol/liver/hr)	(μmol/g liver/hr)	(μmol/liver/hr)
Control	10.08 ± 0.62	10.1 ± 2.8	123.2 ± 29.2	1.8 ± 0.3	18.4 ± 3.1
DHEA	16.72 ± 2.30*	22.8 ± 4.9**	387.8 ± 129.1***	5.6 ± 1.8***	95.8 ± 43.7***

Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. They were then injected with ^3H_2O (4 mCi/kg body wt). Total fatty acid was purified from the livers, and the radioactivity was determined. The rate of incorporation was calculated on the basis of dilution of ^3H_2O by total body water space which was estimated with the plasma radioactivity of each rat, on the assumption that water constitutes 91.5% of plasma. Each value represents the mean ± SD for four rats.

*,**,*** Significantly different from the control: $^*P < 0.001$; $^{**}P < 0.01$; and $^{***}P < 0.05$.

amount of serum triacylglycerol was determined as described previously [17].

2.9. Statistical analyses

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

3. Results

Food intake was decreased slightly in DHEA-treated rats compared with the control rats. However, there was no significant difference in the cumulative food intake for 14 days between control and DHEA-fed rats (209.0 ± 24.8 vs. 191.7 ± 8.2, $P > 0.13$). The total amount of DHEA consumed by the rats fed the DHEA diet for 14 days was 4.99 ± 0.14 (g/kg body wt/14 days) when calculated from the cumulative food intake.

3.1. Effect of DHEA feeding on de novo synthesis of fatty acid in the liver

To examine the effects of DHEA on the rate of hepatic *de novo* synthesis of fatty acid, the incorporation of ^3H from tritiated water into fatty acid and monounsaturated fatty acid in the liver was measured (Table 1). The administration of DHEA to rats increased the rate of fatty acid synthesis approximately 2.3 and 3.1 times on the basis of g liver and of whole liver, respectively. Similarly, the rates of *de novo* synthesis of monounsaturated fatty acid in the liver of DHEA-treated rats were enhanced approximately 3.1 and 5.2 times over that of the control rats on the basis of g liver and of whole liver, respectively. The liver weight of DHEA-treated rats was approximately 1.7-fold greater than that of the controls.

3.2. Effect of DHEA feeding on the formation of oleic acid in vivo

To examine whether DHEA enhances stearic acid desaturation *in vivo*, [^{14}C]stearic acid was injected intravenously into control and DHEA-treated rats, and the formation of radiolabeled oleic acid was measured 10 min after the injection (Table 2). The conversion of radioactive stearic acid to oleic acid was increased approximately 2.1-fold by the treatment of rats with DHEA, whereas there was no difference in total radioactivity incorporated into hepatic lipid between control and DHEA-fed rats. Consequently, the formation of oleic acid in the liver of DHEA-treated rats was approximately 2.2-fold greater than that in the controls.

3.3. Effects of DHEA feeding on mitochondrial and peroxisomal [^{14}C]palmitic acid oxidation

The effect of DHEA feeding on the rate of [^{14}C]palmitic acid oxidation in the liver homogenates was examined (Fig. 1). In the present study, the rates of mitochondrial and peroxisomal palmitic acid oxidation were measured in liver homogenates, rather than in purified mitochondrial and peroxisomal fractions in order to avoid possible damage to the organelles during the centrifugations. Mitochondrial oxidation was measured in the presence of albumin (substrate:albumin ratio, $v = 1.67$), and peroxisomal oxidation was assayed in the presence ($v = 1.67$)

Table 2
Effects of DHEA feeding on hepatic formation of [^{14}C]oleic acid from [^{14}C]stearic acid *in vivo*

Treatment	^{14}C Incorporated into hepatic lipid (10^6 dpm/liver)	Conversion of stearic acid to oleic acid (%)	Oleic acid formed (10^6 dpm/liver)
Control	1.76 ± 0.15	9.53 ± 1.90	0.17 ± 0.03
DHEA	1.88 ± 0.36	20.35 ± 5.32*	0.37 ± 0.07**

Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. They were then injected intravenously with 10 μCi of [^{14}C]stearic acid, and the livers were isolated 10 min after the injection. Each value represents the mean ± SD for four rats.

*,** Significantly different from the control: $^*P < 0.01$ and $^{**}P < 0.001$.

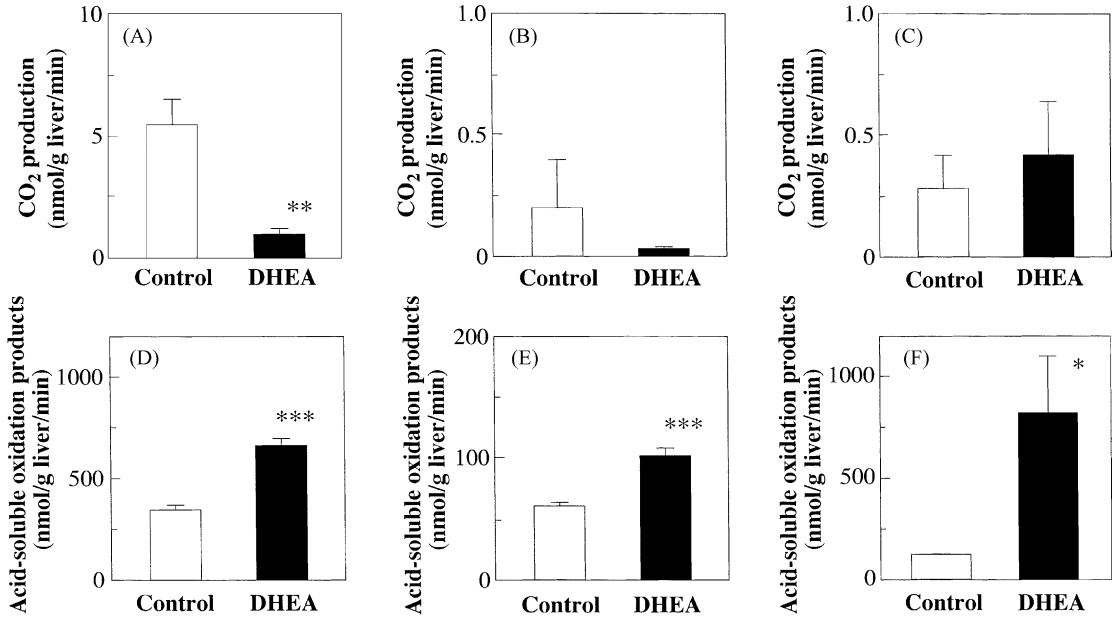

Fig. 1. Effects of DHEA feeding on mitochondrial and peroxisomal oxidation of palmitic acid in livser homogenates. Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. Liver homogenates were incubated with 0.2 mM sodium [^{14}C]palmitate at 37° for 4 min. Mitochondrial oxidation was measured in the presence of albumin (substrate:albumin ratio, $v = 1.67$) and peroxisomal oxidation was measured in the presence ($v = 1.67$) and absence ($v = \infty$) of albumin. The $^{14}CO_2$ produced was trapped with Hyamine hydroxide, and acid-soluble oxidation products were extracted. A and D, mitochondrial oxidation; B, C, E, and F, peroxisomal oxidation; A–C, CO_2 production; D–F, formation of acid-soluble oxidation products; A, B, D, and E, in the presence of albumin; C and F, in the absence of albumin. Open columns (□), control rats; closed columns (■), DHEA-treated rats. Each value represents the mean ± SD for three rats. Significant differences from the control are indicated by an asterisk(s): (*) $P < 0.05$; (**) $P < 0.01$; and (***) $P < 0.001$.

and the absence ($v = \infty$) of albumin. In the presence of albumin, the formation of acid-soluble products by mitochondrial and peroxisomal oxidation of [^{14}C]palmitic acid was increased approximately 1.9 and 1.7 times, respectively, by the treatment of rats with DHEA (Fig. 1D and E). On the other hand, the rate of $^{14}CO_2$ production by mitochondrial [^{14}C]palmitic acid oxidation in liver homogenate was decreased by 83% (Fig. 1A). However, the rate of $^{14}CO_2$ production by peroxisomal [^{14}C]palmitic acid oxidation was not changed significantly (Fig. 1B). In contrast with mitochondrial fatty acid oxidation, which was severely impaired when albumin was omitted from the incubation mixtures, the rate of peroxisomal oxidation has been found to be maximal in the absence of albumin [27]. The maximal rate of the formation of acid-soluble products, but not $^{14}CO_2$ production, by peroxisomal oxidation of [^{14}C]palmitic acid in liver homogenates was induced by the administration of DHEA to rats (Fig. 1C and F, respectively). Feeding DHEA to the rats for 7 days increased peroxisomal palmitoyl-CoA oxidation in liver homogenates approximately 8.4-fold over the control when assayed as the cyanide-insensitive palmitoyl-CoA-stimulated formation of NADH by the method of Lazarow and de Duve [29] (5.0 ± 0.3 vs. 42.2 ± 5.2 nmol/min/mg protein). In addition, the extent of the induction of peroxisomal palmitoyl-CoA oxidation after a 14-day treatment of rats with DHEA was essentially the same as that after a 7-day treatment with DHEA (data not shown).

The rates of [^{14}C]palmitic acid oxidation from control and DHEA-treated rats were compared in isolated hepatocytes, as well (Fig. 2). In agreement with the results derived from the study employing liver homogenates, the rate of formation of acid-soluble products was much greater than that of $^{14}CO_2$ production. Treatment of rats with DHEA increased the formation of acid-soluble products by mitochondrial oxidation approximately 5-fold (Fig. 2C). This differed from liver homogenates where the formation of acid-soluble products by peroxisomal [^{14}C]palmitic acid oxidation in isolated hepatocytes was not changed significantly by DHEA treatment (Fig. 2D). In isolated hepatocytes from DHEA-treated rats, the rate of $^{14}CO_2$ production by mitochondrial [^{14}C]palmitic acid oxidation was 45% of the control, while the rate of $^{14}CO_2$ production by peroxisomal [^{14}C]palmitic acid oxidation was not changed significantly (Fig. 2A and B).

3.4. Effect of DHEA feeding on hepatic VLDL secretion

Triton WR-1339 was utilized to determine whether DHEA affects the rate of hepatic VLDL secretion. Since Triton WR-1339 coats triacylglycerol-rich lipoproteins and prevents triacylglycerol from lipolysis by lipoprotein lipase, Triton WR-1339 has been used extensively for the quantitation of VLDL secretion rates in animals [35]. The

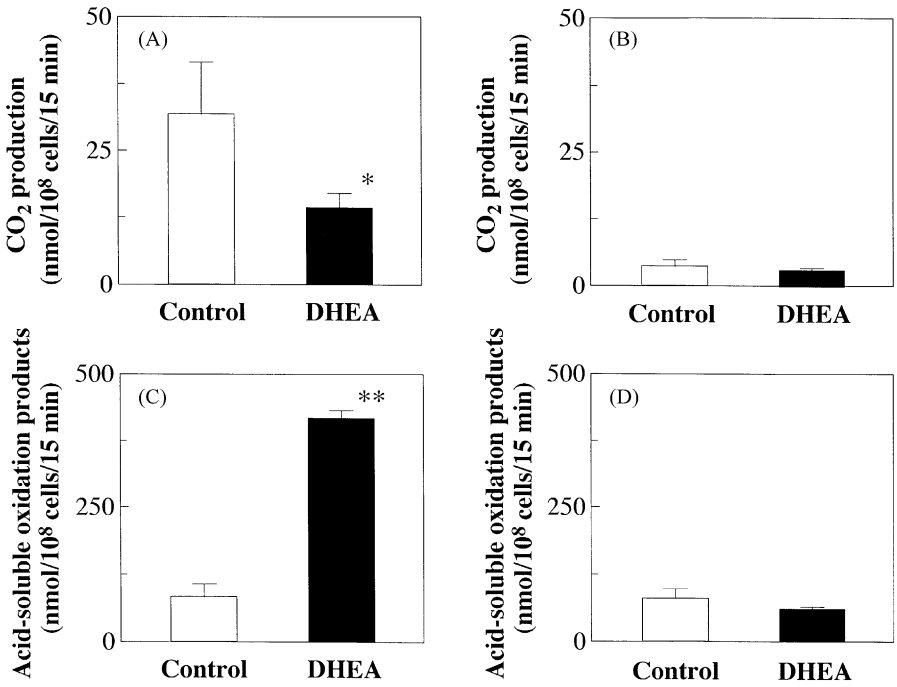

Fig. 2. Effects of DHEA feeding on mitochondrial and peroxisomal oxidation of palmitic acid in isolated hepatocytes. Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. Hepatocytes (5×10^6 cells) were incubated with 0.3 mM sodium [^{14}C]palmitate at 37° for 15 min. The $^{14}CO_2$ produced was trapped with Hyamine hydroxide, and acid-soluble oxidation products were extracted. A and C, mitochondrial oxidation; B and D, peroxisomal oxidation; A and B, CO_2 production; C and D, formation of acid-soluble oxidation products. Open columns (□), control rats; closed columns (■), DHEA-treated rats. Each value represents the mean ± SD for three rats. Significant differences from the control are indicated by an asterisk(s): (*) $P < 0.05$ and (**) $P < 0.001$.

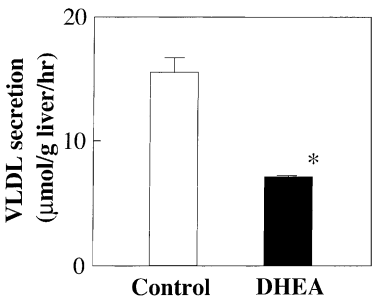

Fig. 3. Effects of DHEA feeding on the rate of hepatic VLDL secretion. Rats were fed either a control diet or a diet containing 0.5% (w/w) DHEA for 14 days. Rats that had been starved for 24 hr were injected intravenously with 20% (w/v) Triton WR-1339 at a dose of 300 mg/kg body weight. Open column (□), control rats; closed column (■), DHEA-treated rats. Each value represents the mean ± SD for four rats. Key: (*) significantly different from the control, $P < 0.001$.

rate of hepatic VLDL secretion in the DHEA-treated rats was 46% of the control (Fig. 3).

4. Discussion

Feeding DHEA to normal rats increases hepatic lipid containing oleic acid [17]. Moreover, Abadie *et al.* [36] demonstrated that DHEA treatment increases the proportion of hepatic oleic acid in Zucker rats. In the present study, we investigated the *in vivo* mechanism whereby DHEA regulates oleic acid in the liver of normal rats.

4.1. Effects of DHEA on lipogenesis

Treatment of rats with DHEA has been found to cause an increase in the hepatic content of fatty acids, in particular oleic acid, and the induction of palmitoyl-CoA elongase and stearoyl-CoA desaturase [37]. These previous findings strongly suggest that the induced enzymes produced an increase in the amount of oleic acid, resulting in an elevation of oleic acid in the liver. There is, however, no direct evidence showing that the synthesis of fatty acids, especially oleic acid, is increased by DHEA in the liver *in vivo*. In the present study, we examined the effects of DHEA on the *in vivo* formation of fatty acid and oleic acid in the liver. The incorporation of ^3H from ^3H_2O into lipids has been demonstrated to give accurate lipid synthetic rates, because the specific activity of body water can be correctly determined as the precursor pool does not change [38]. The present study showed that the incorporation of ^3H from ^3H_2O into hepatic fatty acid was increased by the administration of DHEA to rats, strongly indicating that DHEA causes an increase in *de novo* fatty acid synthesis. These results are consistent with the previous findings of Berdanier and McIntosh [39], who showed that feeding DHEA to BHT rats increased hepatic *de novo* fatty

acid synthesis, determined by the incorporation of ^3H from ^3H_2O into fatty acids. Moreover, the present results showed that the administration of DHEA to rats markedly enhanced the incorporation of ^3H from ^3H_2O into monounsaturated fatty acid and increased the conversion *in vivo* of [^{14}C]stearic acid to oleic acid. To our knowledge, these results are the first evidence demonstrating that DHEA increases the formation of oleic acid in the liver *in vivo*. It is known that, in the liver, palmitic acid, which is synthesized *de novo* from acetyl-CoA, is elongated and then desaturated to form oleic acid [18–20]. Accordingly, the results obtained here are consistent with our previous findings that the treatment of rats with DHEA markedly induced palmitoyl-CoA chain elongase and stearoyl-CoA desaturase, which are considered to participate in oleic acid formation in the liver [17,37]. It may be concluded that the increase in the hepatic content of oleic acid by DHEA feeding, which was observed in our previous studies [17,37], is attributable to the DHEA-caused increase in the hepatic formation of palmitic acid and its conversion to oleic acid, but not to the increased utilization of dietary oleic acid, because no change was observed in food intake by DHEA treatment.

4.2. Effect of DHEA on fatty acid degradation

There is a possibility that DHEA causes hepatic accumulation of lipid by suppressing fatty acid degradation. However, DHEA is known to induce peroxisomal β-oxidation when the activity is assayed *in vitro* employing liver homogenates as an enzyme source [14]. Different from the case of peroxisomal β-oxidation, the effects of DHEA on mitochondrial β-oxidation have not been studied. Moreover, no information is available on the relationship *in vivo* between the effects of DHEA on mitochondrial and peroxisomal β-oxidation and on the accumulation of fatty acid in the liver. The present study showed that the treatment of rats with DHEA caused a considerable increase in mitochondrial formation of acid-soluble products from palmitic acid in both liver homogenates and isolated hepatocytes, although the CO_2 production was decreased, and that the formation of acid-soluble oxidation products was approximately 29 times greater than the CO_2 production in isolated hepatocytes from DHEA-treated rats. Contrary to the expectation, these results undoubtedly indicate that DHEA does not suppress, but rather induces mitochondrial β-oxidation.

It should be noted that peroxisomal palmitic acid oxidation in isolated hepatocytes was not changed significantly, despite the fact that the peroxisomal degradation of palmitic acid to acid-soluble products in liver homogenates was elevated markedly by the treatment of rats with DHEA. These results strongly indicate that peroxisomal β-oxidation induced by DHEA does not always function *in vivo* to degrade fatty acid. Namely, a fatty acid such as palmitic acid seems to be an endogenously poor substrate for peroxisomal β-oxidation in the liver of DHEA-treated rats. This conclusion is similar to that of Mannaerts *et al.* [27], who compared the effects of clofibrate on mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes.

Accordingly, it is not plausible that DHEA causes the accumulation of hepatic lipid by lowering the activities of either mitochondrial or peroxisomal β-oxidation of fatty acid.

4.3. Effect of DHEA on VLDL secretion

Miyazaki *et al.* [40] recently suggested that asebia mice homozygous for a natural mutation of the gene for stearoyl-CoA desaturase are deficient in VLDL secretion. Similarly, Legrand *et al.* [41] demonstrated that the inhibition by sterculic acid of stearoyl-CoA desaturase resulted in the impairment of VLDL secretion in cultured chicken hepatocytes. Based on these findings, the authors concluded that endogenous synthesis of oleic acid in the liver is crucial for the synthesis and secretion of VLDL. The present study showed, however, that the rate of hepatic VLDL secretion was decreased markedly by treatment with DHEA, despite the fact that the formation of oleic acid was enhanced considerably. Moreover, previous studies showed that the serum level of triacylglycerol was lowered by treating rats with DHEA [17,42,43], whereas the hepatic content of oleic acid present in triacylglycerol as an acyl moiety was increased [17]. Accordingly, it is suggested that the impairment of VLDL secretion by DHEA is not always related to the amount of oleic acid formed in the liver. The precise mechanism is now under investigation.

The content of fatty acids in the liver is considered to be regulated by lipogenesis, fatty acid degradation, and VLDL secretion. The present *in vivo* experiments demonstrated that the elevation by DHEA of the hepatic content of fatty acids, especially oleic acid, is due mainly to an increase in both *de novo* fatty acid synthesis and stearic acid desaturation and to a decrease in the rate of hepatic VLDL secretion. The induced activity of fatty acid degradation, especially the markedly induced peroxisomal β-oxidation, seems not to influence the accumulation of oleic acid in the liver.

In conclusion, our present study demonstrated that DHEA markedly affects fatty acid metabolism: *de novo* fatty acid synthesis, oleic acid formation, fatty acid degradation, and VLDL secretion in rat liver *in vivo*. Further study, however, is required to determine whether DHEA causes biochemical changes in humans that are similar to those observed in rodents.

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