Labeled acetate turnover after oral administration in rat tissue

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Summary

The time courses for lipid synthesis in the liver and epididymal adipose tissue were investigated using labeled acetate. After the oral administration of labeled acetate to rats fed a fat free or 10% corn oil diet, the acetate incorporation were followed for 10 days (d). The lipid synthesis from acetate abundantly increased in the triacylglycerols of the liver, reached a maximum at 2 hours (h) after the administration and then quickly decreased. In the epididymal adipose tissue, the lipid synthesis reached a maximum after 8 h, and began to decrease slowly after 2 d. The acetate incorporation into the lipids was markedly lower in the liver, plasma and epididymal adipose tissue of rats fed the corn oil diet than in those fed the fat-free diet. However, the half-lives of esterified fatty acids were similar in the both dietary groups. The half-lives of esterified palmitic acid and oleic acid in the decreasing phase were 5.4 h and 8.9 h, respectively, in the liver, and 4.3 d and 5.6 d, in the epididymal adipose tissue. The time courses for incorporation into plasma lipids were parallel to those in the liver. Thus the fatty acids synthesized in the liver appeared to be transported to epididymal adipose tissues and to stay there longer. Moreover, it is remarkable that the 30% of acetate radioactivities administered were found after 2 h in the whole liver: the 75% of products from the acetate at the maximum were lipids and the 61% of lipids, triacylglycerols. It appeared that the major products from acetate in the liver were lipids.

Introduction

Studies on regulation of lipogenic enzyme gene expression have been conducted at the gene, cell and animal levels. The nutritional and hormonal regulation of lipogenic enzyme gene expressions and enzyme activities in the liver has been studied by many investigators using animals (1- 4). We previously reported that the incorporation of $^{3}H_2O$ into the fatty acid fraction was already high 1 h after the intraperitoneal injection to rats (5). When linolenic acid was considered to be an index of exogenous polyunsaturated fatty acid (PUFA), linolenic acid was

incorporated into the liver lipids within only 1 h of intubation, and quickly (within 2 h) suppressed lipogenic enzyme mRNA gene expressions (6). In total lipids of the liver, the linolenic acid was detected only 1 h after intubation and quickly disappeared in 48 h. Therefore, it appeared that the triacylglycerols-fatty acids were quickly synthesized from substrates, and exogenous triacylglycerols-fatty acids were quickly incorporated into liver lipids and disappeared. On the other hand, in the epididymal adipose tissue, the turnover of triacylglycerols is not so obvious as in the liver.

In early studies, it was shown that, under conditions that promote rapid lipogenic rates, the liver may synthesize as much as 30-50% of the body's fatty acids, in mice and rats (7-10). It is likely that most of these newly made fatty acids are subsequently transported to the extrahepatic tissues for storage and use with conflicting results (8). Borensztain and Getz (9) estimated that about 50% of the ^{14}C-labeled triacylglycerols fatty acids in epididymal adipose tissue had been synthesized by the liver after injection of labeled glucose and then transported to the epididymal adipose tissue. However, Baker et al. (8) concluded that, after intravenous injection of [U-^{14}C] glucose in mice, virtually all of the radioactivity found in the epididymal adipose tissue of the mice was actually synthesized by the epididymal adipose tissue itself.

We have found that a small part of labeled exogenous triolein orally given to rat stayed intact for a long time in epididymal adipose tissue, whereas the labeled triolein was only slightly incorporated into lipids and quickly metabolized in the liver (11). Thus it appeared that non-essential fatty acids might be scarcely exogenous and almost all endogenous in the liver, whereas the fatty acids might be a little exogenous and mostly endogenous in epididymal adipose tissue.

The aim of our studies including ref. 11 is to trace exogenous and endogenous triacylglycerols turnover after oral intake of triacylglycerols or its substrates. In the present experiment, the time courses for lipid synthesis and elimination after oral intubations of radioactive acetate to rats were examined in the liver and epididymal adipose tissue.

MATERIALS AND METHODS

Chemicals. [1-^{14}C] Acetic acid, sodium salt (37-111 MBq/mmol) was purchased from Moravek Biochemicals Inc. (Brea, CA). Reagents were obtained mostly from Wako Pure Chemical Industries (Osaka, Japan) and Sigma (St. Louis, MO).

Animals. Male Wistar rats (Japan SLC Co., Hamamatsu), 4.5-wk old (75-80 g body weight), fed on a commercially available non-purified diet (No. MF, Oriental Shiryou Co., Osaka, Japan) were food-deprived overnight and then fed a fat-free diet or a 10 g/100 g corn oil diet for 3 d. The

composition of the fat-free diet was (g/100 g) sucrose, 76.3; casein, 20; cellulose, 5; salt mixture (12), 4.0; choline chloride, 0.1; vitamin mixture (12), 0.1. Corn oil replaced sucrose for the corn oil diet. The major fatty acid compositions of corn oil used were (% by weight) C16:0, 9.47; C18:0, 1.93; C18:1, 33.8 and C18:2, 52.9. Rats were individually housed in wire-bottomed cages in a temperature-controlled room (24℃) under an automatic lighting schedule (08:00 h to 20:00 h). The animals were allowed to consume diet and water ad libitum.

The rats were orally given 3.7 MBq[1-^{14}C] acetate in 5 ml of 50 mmol/L sodium acetate per kg body weight by a stomach tube at 09:00 h. The rats were sacrificed 1, 2, 4, 8, 16 h, and 1, 2, 4, 7, 10 d after intubation under diethyl ether anesthesia. Before sacrificing the rats, the blood was taken using a heparinized syringe from the inferior vena cava while under diethyl ether anesthesia. Plasma was obtained by centrifuging of heparinized blood at 4℃ for 20 min at 1200 x g. The liver, plasma and epididymal adipose tissue were immediately frozen in liquid nitrogen, and stored at -80℃ to extract total lipids as described below. Care and treatment of experimental animals were in accordance with the Guide for the Care and Use of Laboratory Animals (13).

Lipid extraction, fractionation and analysis. Total lipids of liver, plasma and epididymal adipose tissue were extracted according to the method of Folch et al. (14). The fractions of triacylglycerols were separated by thin-layer chromatography on silica gel H (Merck, Darmstadt) with a solvent of chloroform/methanol/water (65:25:4, by volume). The silica gel zone corresponding to triacylglycerols was identified by comparison with the authentic standard, which was visualized by exposure to iodine vapor. The silica gel zones were scraped and the lipids were extracted with chloroform/methanol (1:1 by volume). The recovery of triacylglycerols from the zones was over 90%. Each triacylglycerols amount was corrected by the recovery. After saponification of the lipids with 1.79 mol/L KOH ethanol at 60℃ for 1 h, the aqueous phase was washed with petroleum ether and acidified. The fractions were extracted with petroleum ether.

To measure the quantity of fatty acids, the fatty acids were methylated with *m*-trifluoromethylphenyltrimethylammonium hydroxide and applied onto a Shimadzu 9A gas chromatograph equipped with a hydrogen flame detector. A capillary column of CBP-M20-025 (Shimadzu, Japan), poly (ethylene glycol) coated in a tube 0.25 mm x 25 m was programmed to increase from 60℃ to 230℃ at 6℃/min and finally to maintain at 230℃ for 20 min. The carrier gas was nitrogen. The fatty acid concentrations were expressed as mg per mg protein. The protein was estimated by the method of Lowry et al. (15). The radioactivities were measured using a scintillation counter (Aloka, Japan). The tissue radioactivities were measured after the

tissues were solubilized with tissue solubilizer, NCS-II（Amersham Canada Limited）.

HPLC analysis. Major endogenous fatty acids were separated and collected by HPLC. The specific activity of each was calculated by dpm/fatty acid quantity. HPLC analysis was performed with a Waters Assocites（Milford, U.S.A）model 515 pump with the detection of a Waters 410 differential refractometer. A Simapack CLC-ODS reverse-phase preparative column was used for separation. The mobile phase was acetonitoril/water（85 : 15 by volume）.

Analyses. Plasma and liver triacylglycerols concentrations were measured by the method of Fletcher（16）. Acetyl-CoA carboxylase activity was assayed by the $H^{14}CO_3$-fixation method（17）. Fatty acid synthase activity was assayed according to Hsu et al.（18）. The enzyme activities in the 105,000 x g supernatant of the liver or epididymal adipose tissue homogenates are shown as mU/mg protein, when 1 mU is amount catalyzing the formation of 1 nmolof product /min at 37℃.

Statistical analysis. Two-way ANOVA was followed by an inspection of all difference between pairs of means using the least significant difference test（19）. Differences were considered significant at $P<0.05$.

RESULTS

Animal profiles

The rats were fed a fat free or 10 % corn oil diet for 3d（at the start of oral intubation）. Lipogenic enzyme activities were markedly lower in epididymal adipose tissue than in liver. In rat fed a 10% corn oil diet, the enzyme activities of acetyl-CoA carboxylase and fatty acid synthase were reduced to 50-60% of those of the fat free diet, but they were reduced to 20-40% in the liver（Table 1）. The triacylglycerols concentrations in the liver were markedly lowered in rats given the 10% corn oil diet for 3 d than the fat free diet. In the epididymal adipose tissue, however, the triacylglycerols of concentrations were significantly increased by corn oil, while that of plasma was decreased.

Time courses for incorporation of labeled acetate into total lipids in liver and epididymal adipose tissue after oral administration. After the oral administration of labeled acetate to rats fed the fat-free diet, the incorporation into total lipids of the liver reached the maximum at 2 h, then quickly decreased to 11% of the maximum in 1 d and almost disappeared in 2 d（Fig. 1）. In the rats fed the corn oil diet, the incorporation in the liver was 31% of the level of rats fed the fat-free diet at the maximum during 2h after the administration.

Table 1. Effects of dietary fat on lipogenic enzyme activities and triacylglycerol levels.

	fat-free diet	corn oil diet
Enzyme activity (mU/mg protein)		
Acetyl-CoA carboxylase		
Liver	17.3 ± 3.35	9.16 ± 1.22*
Epididymal adipose tissue	10.2 ± 2.23	5.16 ± 1.78*
Fatty acid synthase		
Liver	3.01 ± 0.70	1.53 ± 0.27*
Epididymal adipose tissue	2.42 ± 0.56	2.16 ± 0.44
Triacylglycerol levels		
Liver (mg/g)	46.0 ± 6.74	13.2 ± 2.21*
Epididymal adipose tissue (mg/g)	653 ± 49.1	824 ± 44.8*
Plasma (mg/ml)	1.21 ± 0.23	0.89 ± 0.13*

Rats were food-deprived overnight and refed a fat-free or 10% corn oil diet for 3 d.
* Significantly different from the fat-free diet by Student t-test ($p<0.05$). Mean ± SD (n=4).

The time courses for incorporation into plasma lipids were similar to those in the liver.

In the epididymal adipose tissue, however, the labeled acetate incorporation into total lipids after oral administration to rats fed the fat-free diet was still low at 1-2 h, considerably elevated at 4 h, reached the maximum at 8 h, then decreased to 88% of the maximum level in 1 d, and remained at 28% even after 10 d (Fig. 1).

The acetate incorporation into total lipids was markedly lower in the liver, plasma and epididymal adipose tissue of rats fed the corn oil diet than in those fed the fat-free diet. In the rats fed the corn oil diet, the incorporation in the liver, plasma and epididymal adipose tissue was 31%, 50% and 26%, respectively, of the level of rats fed the fat-free diet at each maximum.

Incorporation of labeled acetate into total lipids and triacylglycerols of liver, plasma and epididymal adipose tissue after oral administration. After oral administration of labeled acetate to rats, the time courses of acetate incorporation into total radioactivities, total lipids and triacylglycerols for 16 h are shown in Figure 2. The acetate incorporation into total lipids at each maximum was about 75%, 60%, and 89%, respectively, of the total radioactivities in

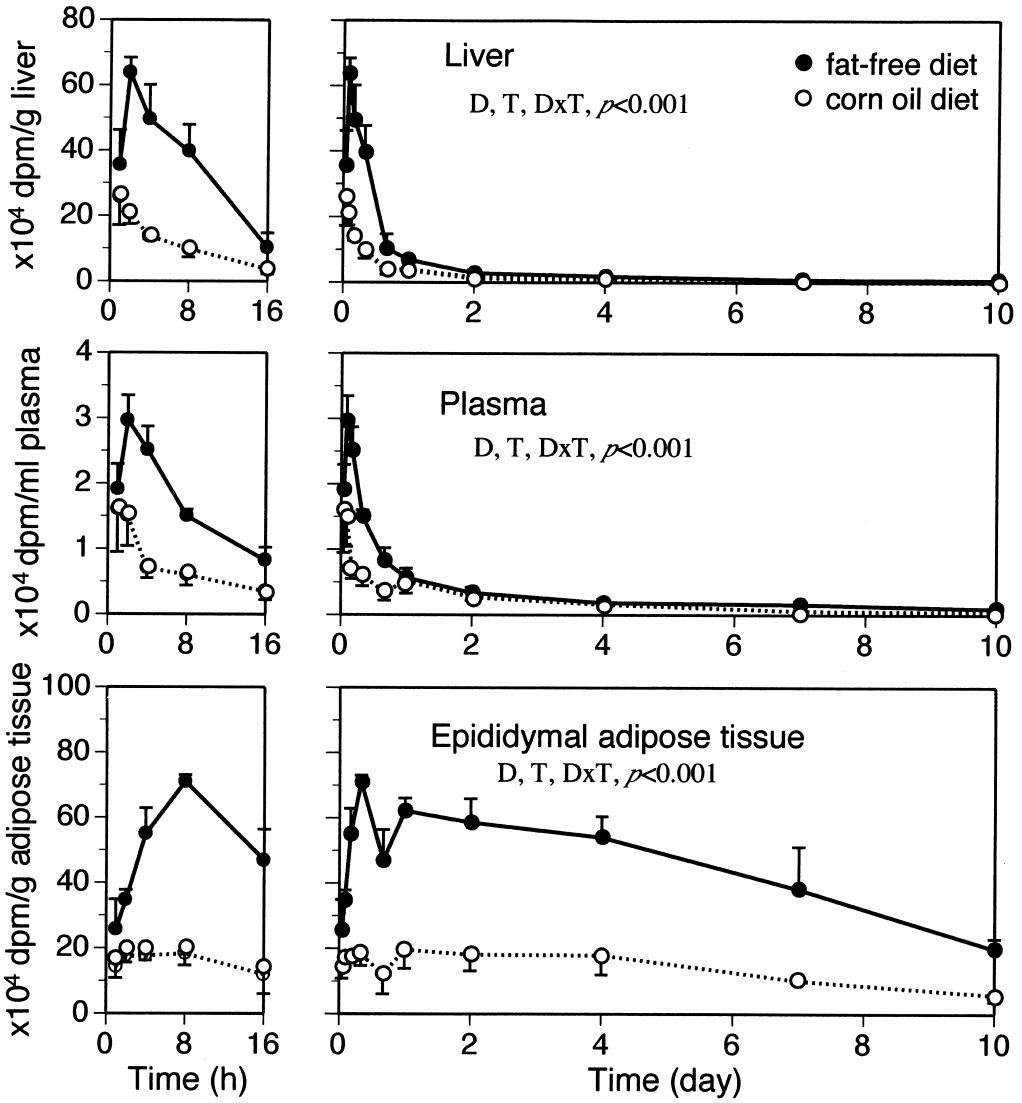

Fig. 1. Time courses for total lipid radioactivity in liver, plasma and epididymal adipose tissues for 10 d after oral administration of labeled acetate to rats. Rats were food-deprived overnight and refed a fat-free or 10% corn oil diet for 3 d. Then rats were orally given 3.7 MBq［1-^{14}C］acetic acid, sodium salt. Mean ± SD(n=4). Two-way ANOVA for diet（D）and time（T）are shown in figures.

the liver, plasma, and epididymal adipose tissue of rats fed the fat-free diet (Fig. 2). The incorporations into triacylglycerols in the liver and plasma showed change almost parallel to those into total lipids in rats. In the epididymal adipose tissue, almost all radioactivities were incorporated into lipids, and almost all lipid radioactivities, triacylglycerols. In rats fed the corn oil diet, the acetate incorporations into total lipids at each maximum were 30% of those in the

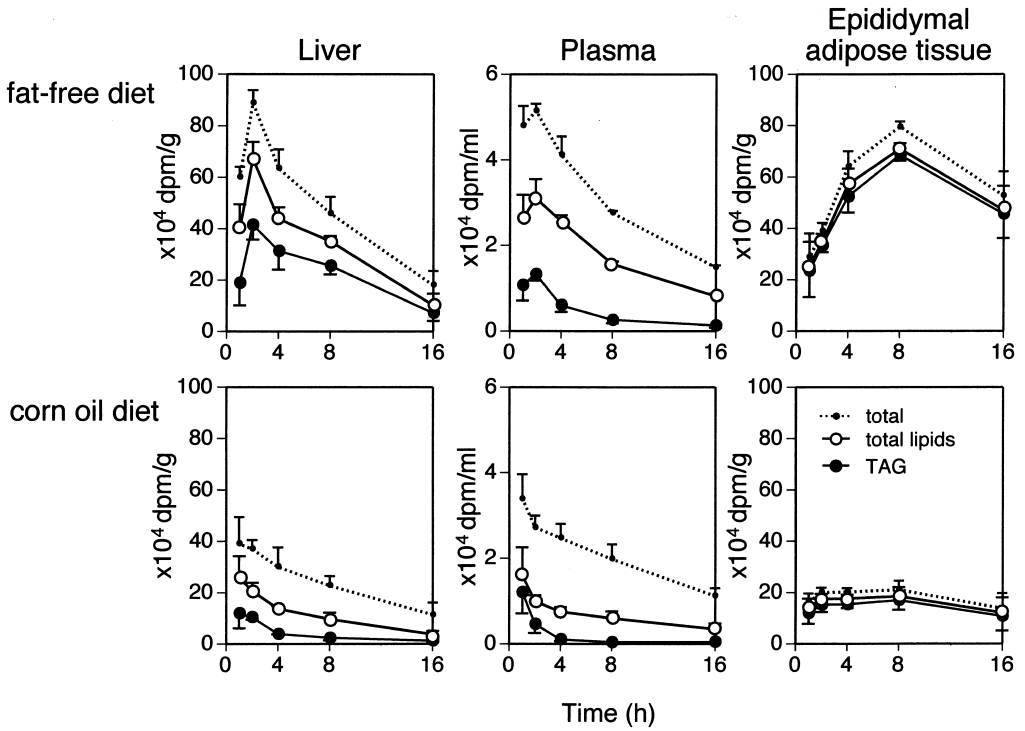

Fig. 2. Labeled acetate incorporation into total, total lipids and triacylglycerols in liver, plasma and epididymal adipose tissue for 16 h after oral administration to rats. Rats were the same to those shown in the legend of Fig. 1. Mean ± SD (n=4). Two-way ANOVA for diet (D) and time (T) in total, total lipids and triacylglycerols of liver, plasma and adipose tissue : D, T, $p<0.001$, DxT (except plasma triacylglycerols), $p<0.05$.

liver of rats fed the fat-free diet and 26% of those in the epididymal adipose tissue.

The percent incorporations of the acetate in whole liver per total administration to whole body are about 30%. The incorporations of total acetate administration into total lipids and triacylglycerols in the liver were 21% and 14%, respectively. It was remarkable that, the relative incorporations at the maximum, 2 h after the labeled acetate administration are the 75% of products from the acetate at the maximum were lipids in the liver, and the 61% of the lipids was triacylglycerols. Thus, the major products from acetate in the liver were lipids. The acetate incorporations were markedly suppressed by dietary PUFA, mostly due to the decrease in the incorporations into lipids and triacylglycerols.

HPLC analysis. The time courses for labeled acetate incorporations (dpm/g or ml) into esterified fatty acid (synthesized from acetate) in the liver, plasma and epididymal adipose tissue

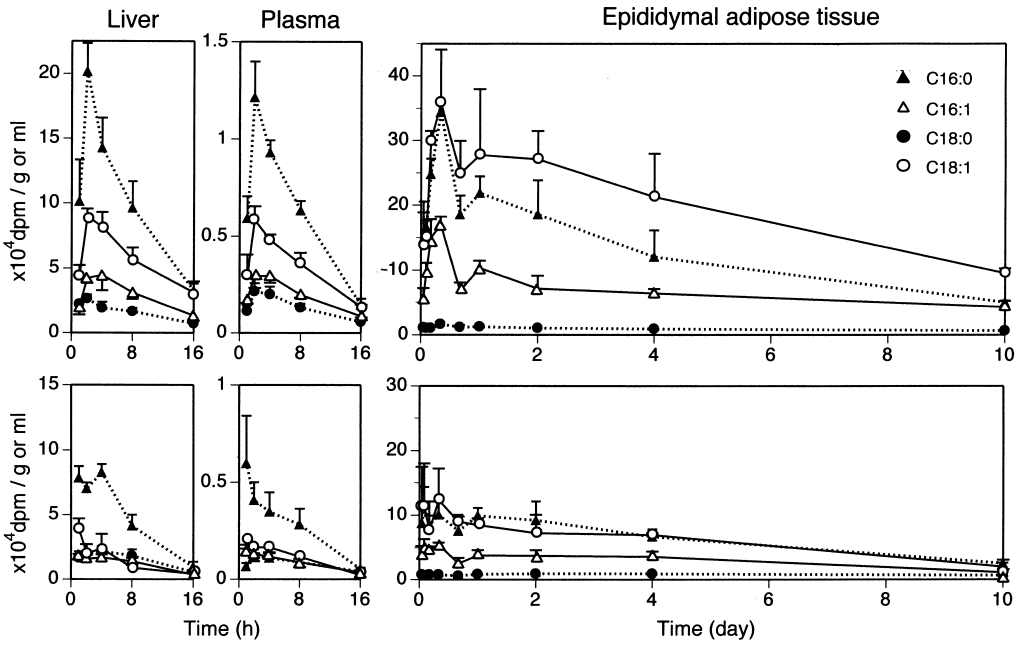

Fig. 3. Time courses for labeled acetate incorporation into esterified fatty acids in liver, plasma and epididymal adipose tissue after oral administration to rats. Rats were orally given the labeled acetate as described in the legend of Fig. 1. Mean ± SD (n=4). Two-way ANOVA for fatty acids (F) and time (T) in rats fed the fat-free diet (upper) for 0-16 h or 0-10 d : F, T, FxT, $p<0.001$ in liver, plasma and epididymal adipose tissues; in rats fed corn oil diet (lower) : F, T, FxT, $p<0.001$ in liver, F, T, $p<0.001$, FxT, $p<0.01$ in plasma, F, T, $p<0.001$ in epididymal adipose tissues. Two-way ANOVA for diet (D) and time courses (T) (upper and lower figures) in each fatty acid for 0-16 h or 0-10 d : for palmitic acid (C16 : 0), palmitoic acid (C16 : 1), oleic acid (C18 : 1), D, T, $p<0.001$, DxT, $p<0.01$ in liver, D, T, $p<0.001$, DxT, $p<0.05$ in plasma and epididymal adipose tissue; for stealic acid (C18 : 0), T, $p<0.001$ in liver, D, T, $p<0.001$ in plasma, D, $p<0.001$ in epididymal adipose tissue.

of rats fed the fat-free or corn oil diet are shown in Figure 3. The time courses in the epididymal adipose tissue for the 10 d after the acetate administration are shown. However, as the acetate incorporations into total lipids of the liver and plasma quickly decreased, those for the 16 h after the acetate administration are shown. The incorporation into each fatty acid reached a maximum at 2 h in the liver and plasma, and at 8 h in the epididymal adipose tissue, similarly to the incorporation into total lipids.

The major fatty acids synthesized from labeled acetate (dpm/g or ml) were in the higher order of palmitic acid(C16 : 0)>oleic acid(C18 : 1)>palmitoleic acid(C16 : 1)>stealic acid(C18 : 0) in the liver and plasma, and C18 : 1>C16 : 0>C16 : 1>C18 : 0 in the epididymal adipose tissue, with change about parallel to that of the triacylglycerol-fatty acid contents in the tissues. However, the specific activities of fatty acids (dpm/mg) reached a similar level at 16 h after the administration in

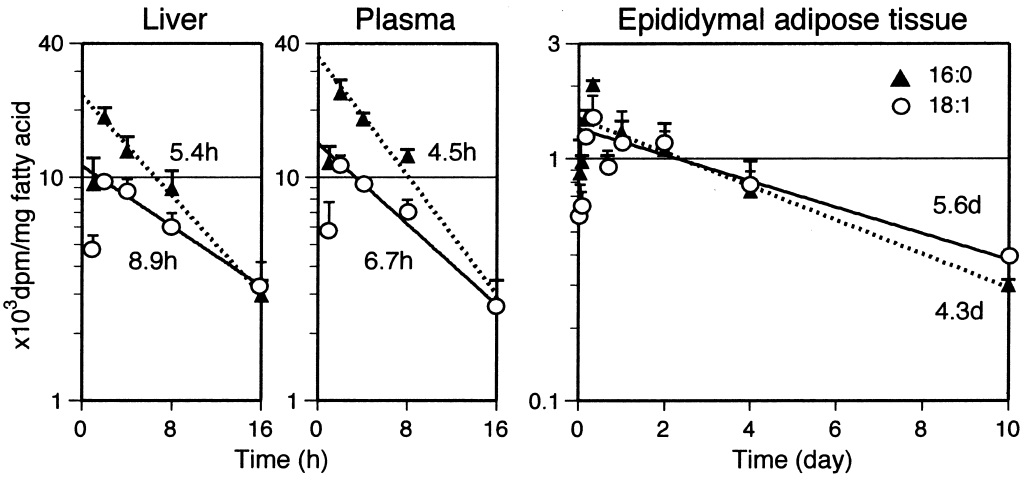

Fig. 4. Semi logarithmic plots of fatty acid specific radioactivities in liver, plasma and epididymal epididymal adipose tissue after oral administration of labeled acetate to rats. After the oral administration of labeled acetate, the specific activities (dpm/mg fatty acid) of palmitic acid (C16:0, ▲) and oleic acid (C18:1, ○) in liver, plasma and epididymal adipose tissue were plotted against time on semi logarithmic graphs. The specific activities were calculated from data shown in Fig. 3. The regression lines and half-lives were calculated by computer analysis. The half-lives are written on the graphs.

both dietary groups (not shown but calculated using data shown in Fig. 3). This means that fatty acid synthesis from labeled acetate should reach a steady state after 16 h. The acetate incorporation into stealic acid (C18:0) was very slight and less than that of the others.

Half-lives of endogenous fatty acids. Using data shown in Figure 3, the semilogarithmic plots of the specific radioactivity of individual fatty acids (dpm/mg fatty acid) against time during the decreasing phases are shown in Figure 4. The regression lines were calculated by computer analysis. The decay curves for the 16 h after acetate administration in the liver and plasma are shown, as the radioactivities decreased too rapidly to measure accurately after 1 d. However, those for the 10 d in the epididymal adipose tissue are shown, as the radioactivities decreased slowly. The half-lives of palmtic acid (C16:0) and oleic acid (C18:1) were 5.4 h and 8.9 h, respectively, in the liver of the rats fed the fat-free diet, 4.5 h and 6.7 h, respectively, in the plasma, and 4.3 d and 5.6 d, respectively, in the epididymal adipose tissue. The half-lives were similar in the liver and plasma, but much longer in the epididymal adipose tissue. The half-lives of oleic acid (C18:1) were slightly longer than those of palmtic acid (C16:0). In the rats fed the corn oil diet, the half-lives of palmtic acid (C16:0) and oleic acid (C18:1) were 4.5 h and 8.9 h,

respectively, in the liver, 4.7 h and 5.2 h, respectively, in the plasma, and 4.4 d and 4.8 d, respectively, in the epididymal adipose tissue. Thus the half-lives were similar in the epididymal adipose tissue of rats fed the fat-free diet and those fed the corn oil diet（figures not shown）.

DISCUSSION

The rats used in the present experiment were fed the fat-free or corn oil diet for 3 d after fasting overnight and then orally given the labeled acetate. Lipogenic enzyme activities and triacylglycerols concentrations in the liver were lowered in rats given the 10% corn oil diet than the fat free diet.

In the epididymal adipose tissue of rats, lipogenic enzyme activities（mU/mg protein）in a fed state were 30-50% of those in the liver, and the protein concentrations in the epididymal adipose tissue（mg/g tissue）were about 4% of those in the liver. Thus, fatty acid synthesis ability should be much lower in the epididymal adipose tissue than in the liver（Table 1, Ref. 20, 21）. Esterified fatty acids are synthesized abundantly in the liver, and the time courses for labeled acetate incorporation into plasma lipids showed change parallel to those in the liver. The lipids synthesized in the liver should be carried out to the plasma and transported to other tissues. Moreover, the increase in lipid synthesis from acetate was delayed in the epididymal adipose tissue compared to the liver after the administration of labeled acetate. Therefore, it is suggested that fatty acids are synthesized slightly in the epididymal adipose tissue, and a considerable amount of esterified fatty acids synthesized in the liver is transported into the epididymal adipose tissue.

After oral administration of labeled acetate to rats fed the fat-free diet, the acetate incorporation into total lipids quickly reached a high maximum level at 2 h in the liver and plasma, and at 8 h in the epididymal adipose tissue. The acetate incorporations into total lipids and triacylglycerols at the 2 h were 75% and 46%, respectively, of the total radioactivities in the liver. Thus, the major product from acetate was lipids, and 61% of the lipids were triacylglycerols in the liver. In the epididymal adipose tissue, the acetate incorporations into total lipids and triacylglycerols at the 8 h were 89% and 86%, respectively, of the total radioactivities, and almost all the lipids were triacylglycerols.

After the labeled acetate administration, the acetate incorporation into total lipids（Fig. 1）and fatty acids（Fig. 3）at 16 h was lower than those before and after 8 h. This observation may be ascribed to diurnal rhythm of lipogenesis. Because we previously found diurnal variations of

lipogenesis in the rat liver, after the intraperitoneal injection of tritiated water and the lipogenesis was low at 0900 h (22). The rats were orally given the labeled acetate at 1700 h and sacrificed at 0900 h next day in the present experiment.

The acetate incorporation into lipids (mainly triacylglycerols) was markedly lower in the liver, plasma and epididymal adipose tissue of rats fed the corn oil diet than in those fed the fat-free diet. However, the half-lives of esterified fatty acids were similar in the both dietary groups. It appeared that dietary corn oil suppressed the acetate incorporation into fatty acids but did not affect on the fatty acid elimination.

In our previous study (11), the exogenous radioactive triolein was slightly incorporated into lipids and quickly decreased in the liver, whereas a small part of the triolein remained intact for a long time in the epididymal adipose tissue. In the present experiment, triacylglycerols and other lipids were abundantly synthesized in the liver and appeared to be transported to epididymal adipose tissue. Thus, it is suggested that esterified fatty acids (non-essential) might be scarecely exogenous and mostly endogenous in the liver, whereas some more exogenous fatty acids may be contained in epididymal adipose tissue. The previous results support the present results. Gordis (23) also found that most triacylglycerols molecules in adipose depots are stored intact until mobilized. Moreover, Carmaniu and Herrera (24) reported that the highest proportion of radioactivity from [1-^{14}C]-palmitate appeared in the esterified fatty acid in epididymal adipose tissue.

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