Labeled triolein turnover after oral administration in rats

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Summary

The dietary triacylglycerol turnover was investigated by using labeled triolein. After oral administration of labeled triolein to rats fed a fat-free or 10% corn oil diet for 3 days (d), the time courses for the triolein in the liver, plasma and epididymal adipose tissue (adipose tissue) were examined for 10 d. After the administration of labeled triolein to rats fed the fat-free diet, the incorporation (dpm/g) into total lipids of the liver and adipose tissue each reached the maximum in 8 hour and was about 7 times higher in the adipose tissue than in the liver. The half-lives of total lipid radioactivities after the maximum were 0.39 d and 2.58 d, respectively, in the rapid and slow phases of the decay curve in the liver, and 4.78 d in only one phase of the adipose tissue. Radioactivity after administration of labeled triolein was mostly found in the oleic acid in the tissues. The half-life of oleic acid was 3.92 d in the adipose tissues. Although dietary corn oil reduced the triolein incorporation into total lipids and oleic acid in comparison with the fat-free diet, it did not affect the half-lives. Thus the labeled oleic acid stayed abundantly intact for a long time in the adipose tissue, whereas it was only slightly incorporated into total lipids and quickly metabolized in the liver. It appeared that non-essential fatty acids may be mostly endogenous in the liver but may be exogenous and endogenous in adipose tissue.

Introduction

A number of investigators have shown the nutritional and hormonal regulation of lipogenic enzyme gene expressions and enzyme activities in the liver in animal studies, an example of which is described in one review (1). In comparison to fatty acid synthesis in the liver, the turnover of triacylglycerols (TAG) is not so clear, particularly in epididymal adipose tissue (adipose tissue). TAG turnover as well as fatty acid synthesis should be involved in TAG accumulation. In early studies, it has been 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 (2-5). It is likely that most of these newly made fatty acids are subsequently transported to the extrahepatic

tissues for storage and use. Borensztain and Getz (5) estimated that about 50% of the ^{14}C-labeled TAG fatty acids in adipose tissue had been synthesized by the liver after injection of labeled glucose and then transported to the adipose tissue. However, Baker *et al.* (6) concluded that, after intravenous injection of [U-^{14}C] glucose in rats, virtually all of the radioactivity found in the adipose tissue of the rats was actually synthesized by the adipose tissue itself. These studies reported only the results for a short time after the injection of labeled glucose.

We previously found that linolenic acid, an exogenous polyunsaturated fatty acids (PUFA) used as a marker, was quickly in 1 h (hour) incorporated into liver lipids, and lipogenic enzyme mRNA gene expression was quickly in 2 h suppressed by dietary PUFA (7). The linolenic acid continued to increase during the first 4 h, then decreased and almost disappeared by 48 h. Therefore, exogenous fatty acids and TAG apparently are quickly incorporated into the liver lipids and quickly disappear. Although plenty of them was quickly metabolized and expired as CO_2, the residue remained for a longer time in the adipose tissue (8, 9). Gordis (8) found that little exchange of TAG fatty acids occurred in rat adipose tissue in storage even after 2 months, and most TAG molecules in adipose depots are stored intact until mobilized. Carmaniu and Herrera (10) reported that the highest proportion of radioactivity from [1-^{14}C]-palmitate appeared in the esterified fatty acid in adipose tissue.

In the present experiment, in order to investigate the turnover of TAG in liver and particularly in adipose tissue, the time courses for lipid elimination after oral administration of radioactive triolein were examined. The half-lives of the radioactivities in total lipids and oleic acid were calculated from the time courses, and compared in the liver and adipose tissue. The effects of dietary fat on them were also investigated.

Materials and methods

Chemicals. [9, 10-^{3}H (N)]-triolein (148-740 GBq/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA, U.S.A.). Reagents were obtained mostly from Wako Pure Chemical Industries (Osaka, Japan) and Sigma (St. Louis, MO, U.S.A.).

Animals. Male Wistar rats (Japan SLC Co., Hamamatsu, Japan), 4.5-week old (75-80 g body weight), fed on a commercially available non-purified diet (No. MF, Oriental Shiryo Co., Osaka, Japan) were food-deprived overnight and then fed a fat-free diet or a 10 % (by weight) corn oil diet. The composition of the fat-free diet was (g/kg) sucrose, 720.7; casein, 200; cellulose, 50; salt mixture (11), 27.3; choline chloride, 1; vitamin mixture (11), 1. Corn oil replaced sucrose for the corn oil diet. The major fatty acid compositions of corn oil used were (% in total fatty acids) 16:0,

9.47; 18:0, 1.93; 18:1, 33.8 and 18:2, 52.9. Rats were individually housed in wire-bottomed cages in a temperature-controlled room (24°C) under an automatic lighting schedule (08.00 h to 20.00 h). The animals were allowed to consume diet and water ad libitum.

The experimental design was as follows. The rats were orally given 1.85 MBq [9, 10-^{3}H (N)]-triolein in 5 ml of 20% (v/v) ethanol per kg body weight by a stomach tube at 09.00 h 3 d after feeding the fat-free or 10% corn oil diet, and then killed 1, 2, 4, 8, 16 h, and 1, 2, 4, 7, 10 d after the labeled triolein administration. Care and treatment of experimental animals were in accordance with the Guide for the Care and Use of Laboratory Animals (12).

Aliquots of liver, plasma and adipose tissue of the rats were immediately frozen in liquid nitrogen, and stored at -80°C to extract total lipids as described below. The total lipids were used for thin layer chromatography (TLC), gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC).

Lipid extraction, fractionation and analysis. Total lipids of liver, plasma and adipose tissue were extracted according to the method of Folch *et al.* (13) and separated by TLC on silica gel H (Merck, Darmstadt, Germany). The fractions of TAG were separated by TLC with a solvent of petroleum ether/ether/acetic acid (80:20:1, by vol.). The silica gel zone corresponding to TAG was identified by comparison with 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, v/v). The recovery of TAG from the zones was over 90%. Each TAG amount was corrected by each recovery. For GLC analysis, after saponification of the lipids with 1.79 M-KOH ethanol at 60°C for 1 h, the aqueous phase was washed with petroleum ether and acidified. The fractions were extracted with petroleum ether. 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, Kyoto, Japan), poly (ethylene glycol) coated in a tube 0.25 mm x 25 m was programmed to increase from 60°C to 230°C at 6°C/min and finally to maintain at 230°C for 20 min. The carrier gas was nitrogen.

HPLC analysis. Major endogenous fatty acids were separated and collected by HPLC. The quantities of fatty acids were measured using gas chromatography, the radioactivity was measured using a scintillation counter (LSC-5100, Aloka, Tokyo, Japan) and the specific activities (dpm/mg fatty acid) were calculated. HPLC analysis was performed with a Waters Associates (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, v/v).

Statistical analysis. Two-way ANOVA was followed by an inspection of all difference between pairs of means using the least significant difference test (14). Differences were considered significant at $P<0.05$.

Results and discussion

Time courses for total lipid radioactivities in liver, plasma and adipose tissues after the oral administration of labeled triolein to rats. After the oral administration of labeled triolein to rats fed the fat-free diet, the incorporation into total lipids of the liver and adipose tissue each reached the maximum in 8 h (Fig. 1). The incorporation per tissue (dpm/g) at the maximum was 7 times higher in the adipose tissue than in the liver (Fig. 1 upper panel). The labeled triolein was incorporated only slightly into the total lipids in the liver and quickly decreased to 18 % of the maximum in 1d and almost disappeared in 2d, whereas the triolein was greatly incorporated in the adipose tissue and 25% of the maximum remained even after 10 d (Fig. 2). The exogenous total lipids stayed for much longer and more abundantly in the adipose tissue than in the liver. In the rats fed the corn oil diet, the incorporations at each maximum were about 50% and 80%, respectively, of those in the liver and adipose tissue of the rats fed the fat-free diet (Fig. 1). The incorporation of labeled triolein into plasma lipids quickly decreased without increasing. The triolein incorporations (% incorporation of dpm/g) into total radioactivities and total lipids for 16 h are shown in Fig. 1. The triolein incorporation into total lipids at each maximum was about 74, 86 and 92 %, respectively, of the total radioactivities in the liver, plasma and adipose tissue of rats fed the fat-free diet (Fig. 1). In the adipose tissue, almost all radioactivities were incorporated in to lipids.

Kalopissis *et al.* (15) reported that, in fat-fed rats, the initial cellular uptake of [1-^{14}C] oleate in vitro was decreased by 25%, its esterification to TAG and phospholipids by 50% and its incorporation into VLDL TAG by 70%. Thus, it is suggested that the cellular uptake of the labeled oleate, the esterification and the incorporation into VLDL may be decreased in the rats fed corn oil in the present experiment.

Labeled triolein incorporation into TAG in liver, plasma and adipose tissues after oral administration to rats. After oral administration of labeled triolein to rats, the time courses of triolein incorporation into total lipids and TAG for 16 h are shown in Fig. 3. The

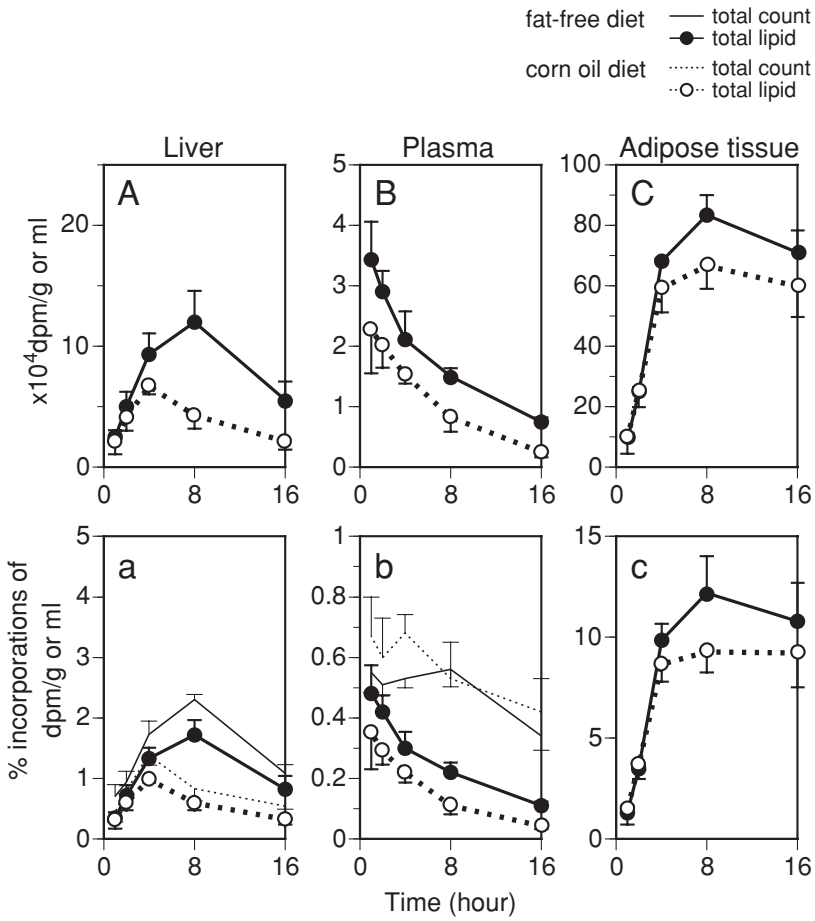

Fig. 1. Time courses for total lipid radioactivities in liver, plasma and epididymal adipose tissues for 1-16h after oral administration of labeled triolein to rats. Rats were food-deprived for overnight and refed a fat-free or 10% corn oil diet for 3 d. Then the rats were orally given 1.85 MBq [9, 10- ^{3}H (N)]-triolein. The rats were killed 1, 2, 4, 8 and 16 h after the administration. Total lipid radioactivities in liver (A, a), plasma (B, b) and adipose tissues (C, c) were measured. Total lipid radioactivities are shown by dpm/g or ml in the upper panel and by % incorporation in the lower panel. Mean ± SD (n=4). Two-way ANOVA for D (diet) and T (time) between rats fed the fat-free diet and corn oil diet: D, T, P<0.001, D x T, P< 0.005 in liver, D, T, P<0.001 in plasma and D, P< 0.02, T, P<0.001 in adipose tissue.

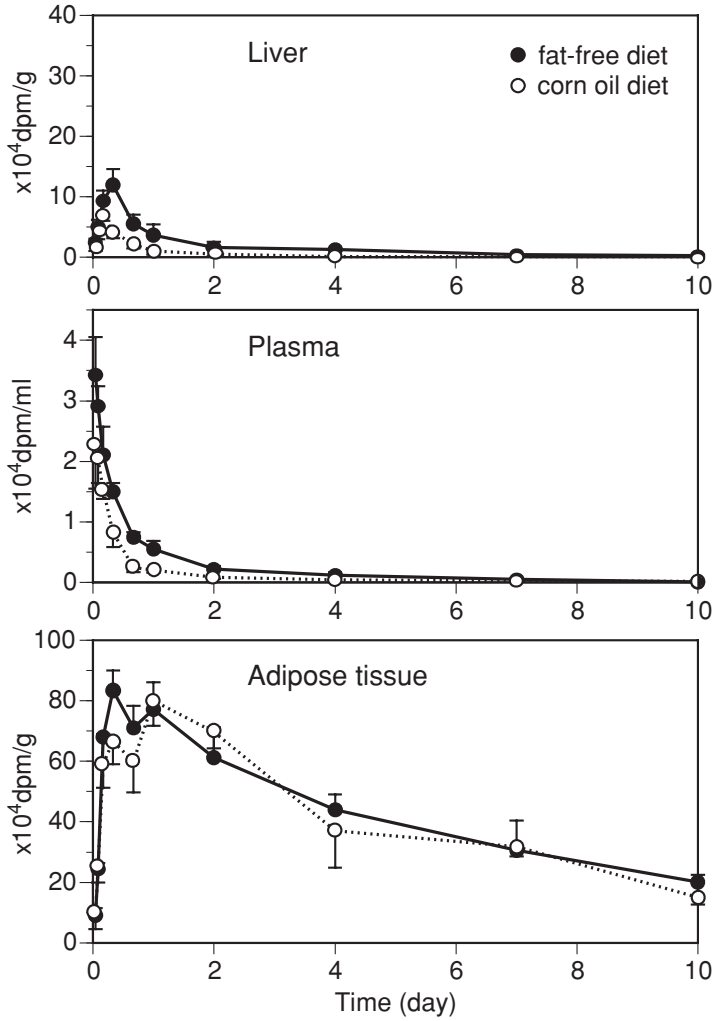

Fig. 2. Time courses for total lipid radioactivities in liver, plasma and epididymal adipose tissues for 1h-10d after oral administration of labeled triolein to rats. Rats were food-deprived for overnight and refed a fat-free or 10% corn oil diet for 3 d. Then the rats were orally given 1.85 MBq [9, 10- ^{3}H (N)]-triolein. The rats were killed 1, 2, 4, 7 and 10 d after the administration. Total lipid radioactivities in tissues were measured. Total lipid radioactivities are shown by dpm/g or ml in the figures. Mean ± SD (n=4). Two-way ANOVA for D (diet) and T (time) between rats fed the fat-free diet and corn oil diet: D, T, D x T, P< 0.001 in liver, D, T, P<0.001, D x T, P< 0.02 in plasma and T, P<0.001 in adipose tissue.

incorporation into TAG in the liver was about parallel to that into total lipids: 77% (average) of that into total lipids 2-16 h after administration in the rats fed the fat-free diet, and 66% (average) of that in the rats fed the corn oil diet (Fig. 3). The incorporation into TAG in the adipose tissue was also parallel to that into total lipids: 91% (average) of that into total lipids in the rats fed the fat-free diet, and 78% (average) in those fed the corn oil diet. The incorporation of exogenous triolein into total lipids and TAG was significantly lower in the livers and adipose tissues of rats fed the corn oil diet than in those fed the fat-free diet. In rats fed the corn oil diet, it was found that dietary PUFA decreased the triolein incorporation into total lipids due to the reduction of the incorporation into TAG in the tissues. The incorporation of labeled triolein into plasma TAG quickly decreased without increasing similarly to the total lipids.

TAG were separated by TLC from total lipids, and the TAG radioactivities were corrected by the recovery. The triolein incorporation of total lipids could be more accurately measured without the TLC process than that of TAG. As the incorporation into TAG was about parallel to that of total lipids, the incorporations were shown by total lipid radioactivity. Changes in total lipids are considered to be correlated to those in TAG.

Half-lives. Using data shown in Fig. 1 and 2, semi-logarithmic plots of total lipid radioactivities against time during the decreasing phases in the rat liver, plasma and adipose tissue are shown in Fig. 4. Half-lives were calculated by computer analysis. The decay curves were about parallel between the rats fed the fat-free diet and those fed the corn oil diet. Triolein was rapidly metabolized at an early stage (the rapid phase) after administration and the residue was slowly metabolized (the slow phase). The half-lives in the livers were 0.39 d and 0.33 d, respectively, in the rapid phases of the rats fed the fat-free diet and corn oil diet, and 2.58 d and 2.54 d, respectively, in the slow phases of both dietary groups. The decay curves in plasma were also about parallel between the rats fed the fat-free diet and those fed the corn oil diet. The half-lives of the rapid phases in the plasma were 0.33 d and 0.26 d, respectively, in the rats fed the fat-free diet and corn oil diet, and 2.30 d and 2.63 d, respectively, in the slow phases of both dietary groups. The half-lives in the slow phases were similar in the liver and plasma, whereas those in the rapid phases were rather shorter in the plasma.

In the adipose tissue, the decay curves were similar in the two dietary groups. The half-lives were similar in only one phase, 4.78 d and 4.10 d, respectively, in the rats fed the fat-free diet and those fed the corn oil diet. The half-lives were similar in the liver and plasma, but much longer in the adipose tissue than in the liver and plasma.

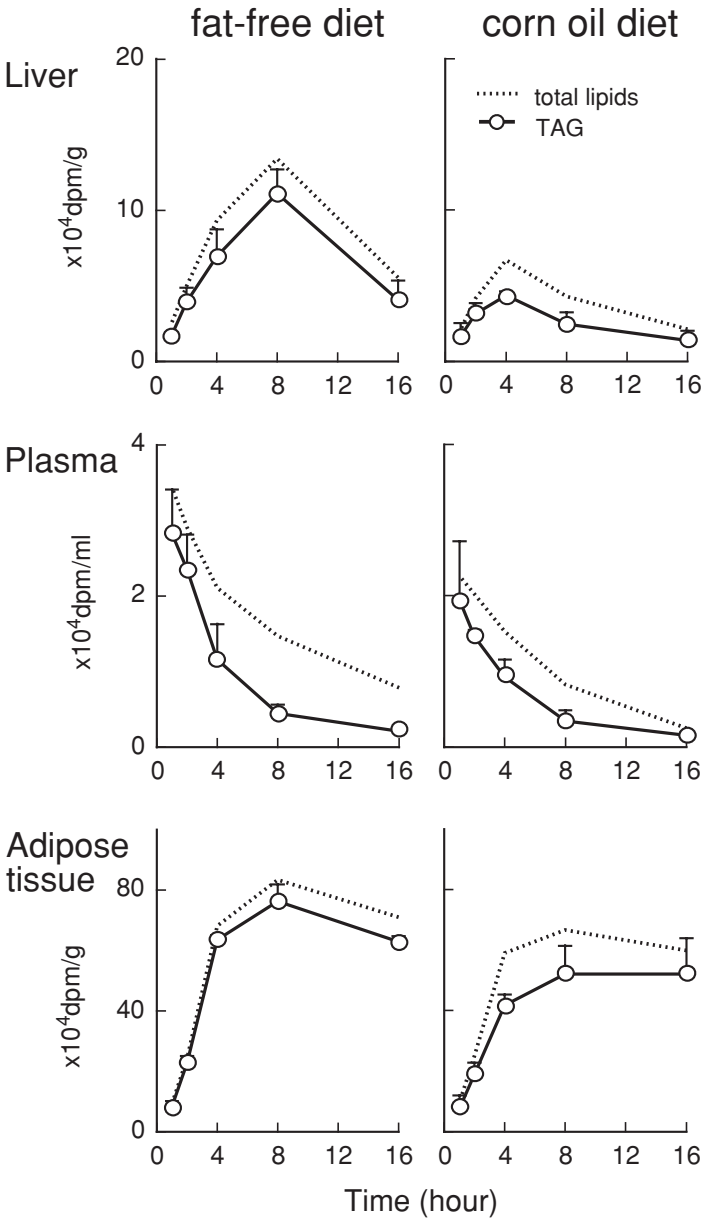

Fig. 3. Labeled triolein incorporation into TAG in liver, plasma and adipose tissues after oral administration to rats. Rats were orally given the labeled triolein as described in the legend of Fig. 1. The labeled triolein incorporation into TAG was shown in comparison with the incorporation into total lipids (taken from Fig. 1). Mean ± SD (n=4). Each figure on the left shows the results for rats fed the fat-free diet, and the figure on right, those for rats fed the corn oil diet. Two-way ANOVA for D (diet) and T (time) between rats fed the fat-free diet and corn oil diet: D, T, D x T, P<0.001 in liver, D, P< 0.05, T, P<0.001 in plasma and D, T, P<0.001, D x T, P<0.05 in adipose tissue.

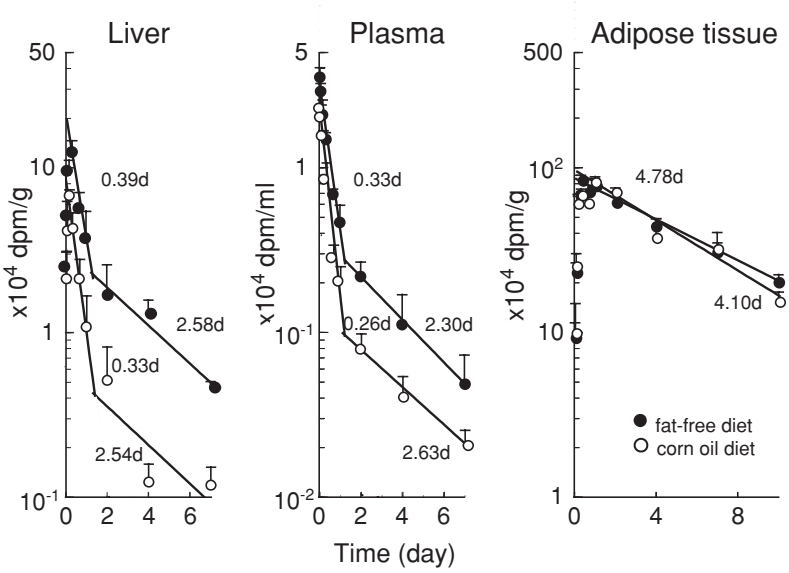

Fig. 4. Semi-logarithmic plots of total lipid radioactivities in liver, plasma and adipose tissues after oral administration of labeled triolein to rats. The total lipid radioactivities (dpm/g or ml) in the liver, plasma and adipose tissues were plotted against time on semi-logarithmic graphs. The half-lives are written on the graphs.

Moreover, the half-lives for the specific activities of oleic acid in adipose tissue were calculated from Fig. 5. The half-lives for the specific activities of oleic acid in adipose tissue were close to those of the total lipids, and were 3.92 d and 3.43 d, respectively, in the rats fed the fat-free diet and the corn oil diet. The turnover rate of oleic acid was similar in both dietary groups.

Thus, the decay curves in liver, plasma and adipose tissue were about parallel between the rats fed the fat-free diet and those fed the corn oil diet. Although dietary corn oil reduced the triolein incorporation into lipids, it did not affect on the half-lives. It is suggested that dietary PUFA did not affect on the decay of TAG but suppressed the labeled triolein incorporation into the liver TAG (Fig. 3). The cellular uptake of the labeled oleate, the esterification and the incorporation into VLDL may be decreased in the rats fed corn oil in the present experiment (15).

Time courses for specific activities of fatty acids in liver, plasma and adipose tissues after oral administration of rats. After oral administration of triolein to rats, the labeled triolein incorporation into each fatty acid (major fatty acids except essential fatty acids) in the liver, plasma and adipose tissue of rats is shown as specific activities (dpm/mg fatty acid) in Fig. 5. After the oral administration of triolein, the time course for the specific activities of oleic acid was

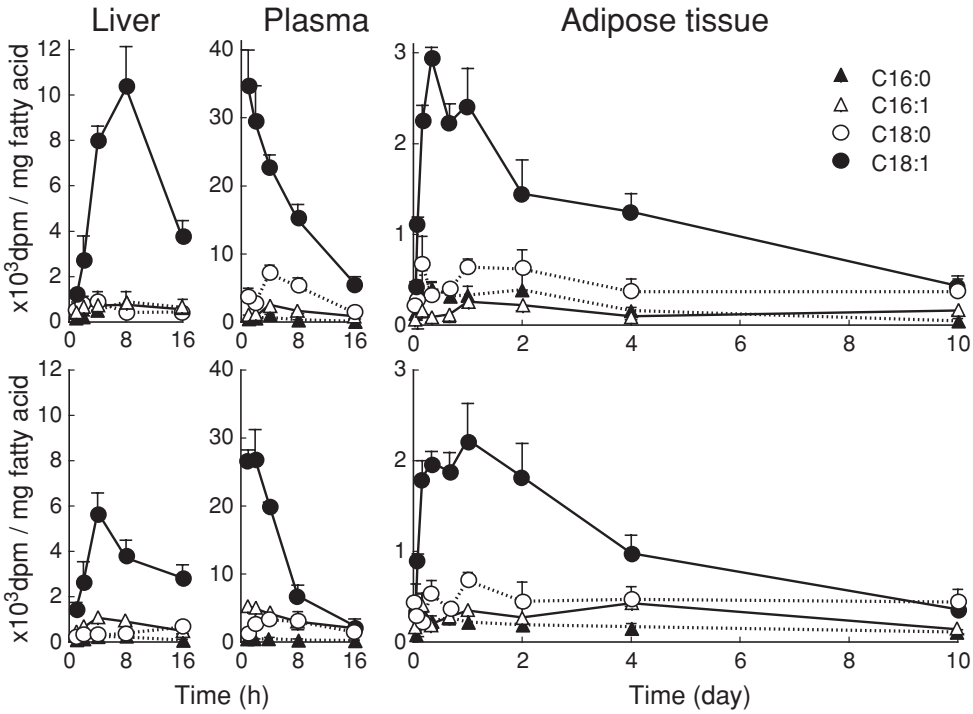

Fig. 5. Time courses for specific activities of each fatty acid in liver, plasma and adipose tissues after oral administration of triolein to rats. Rats were orally given the labeled triolein as described in the legend of Fig. 2. Mean ± SD (n=4). Two-way ANOVA for oleic acid from other fatty acids: F (other fatty acids), T (time), F x T, P<0.001 in liver, plasma and adipose tissues of rats fed the fat-free (upper) or corn oil (lower) diet. Two-way ANOVA for oleic acid between rats fed the fat-free and corn oil diet: T, D, T x D, P<0.05 in liver, plasma and adipose tissues.

similar to that of total lipids in the tissues. Radioactivity after administration of labeled triolein was mostly found in the oleic acid fraction in the liver, plasma and adipose tissue. The specific activities of oleic acid were markedly higher than those of the other fatty acids, which were very slight in both the fat-free and corn oil diet groups. The oleic acid was scarcely metabolized to other fatty acids. The specific activities of oleic acid were significantly lower in rats fed the corn oil diet than in those fed the fat-free diet.

Gordis (8) concluded that most TAG molecules in adipose depots are stored intact until mobilized. Moreover, Carmaniu and Herrera (10) reported that the highest proportion of radioactivity from [1-^{14}C]-palmitate appeared in the esterified fatty acid in adipose tissue. These reports support our present results. Thus, labeled triolein may be mostly stored as intact triolein. The incorporation of labeled triolein into plasma lipids, in contrast to the increase in

adipose tissue, quickly decreased similarly to that in the liver. Triolein appeared to be transported from liver to adipose tissue.

Moreover, in the 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 adipose tissue (mg/g tissue) were about 4% of those in the liver (16). Thus, fatty acid synthesis ability should be much lower in the adipose tissue than in the liver. In the liver, however, the labeled acetate was quickly and abundantly incorporated into fatty acids of TAG (as is well known), whereas the exogenous labeled triolein incorporation was slight and quickly decreased. The half-lives of radioactivity in the plasma after oral administration of labeled triolein were similar to those of liver. Therefore, it appeared that total lipids and TAG synthesized in the liver should be abundantly transported to the adipose tissues via plasma.

In the present experiment, the rats were fed the fat-free or corn oil diet for 3 d after fasting overnight. Because we previously found that the induction of lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase and others) in liver (1) and in adipose tissue (16) reached a steady state 3 d after the refeeding. Lipogenic enzyme activities and TAG concentrations in the liver were lowered to minimum levels in rats given the 10% (by weight) corn oil diet for 3 d (17). Dietary perilla oil (5%) began to change the fatty acid compositions of liver 1 h after feeding, the exogenous fatty acids increased for the first 4 h, then decreased and almost disappeared by 48 h (7). Therefore, feeding the diet for 3 d was considered to be enough for fatty acid compositions to reach a steady state in the liver. Lambert et al. (18) showed that different types of fat given in the diet for 21 d have different effects on the hepatic uptake and metabolism of lipids carried in chylomicron remnants of the corresponding fatty acid composition. Adaptive changes, which occur in the rat liver on long-term feeding of different types of fat, may have effects on the uptake and metabolism of chylomicron remnants.

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