Labeled Acetate Incorporation into Lipids and Lipid Elimination after Oral Administration in Rat Liver and Adipose Tissue

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Summary To investigate the incorporation of acetate into fatty acids and their turnover, the time courses for the incorporation of labeled acetate into lipids in the liver and epididymal adipose tissue (adipose tissue) after the oral administration to rats were examined for 10 d. The labeled acetate was abundantly incorporated into lipids, mainly into triacylglycerols (TAG) in the liver, reached a maximum at 2 h after the administration and then quickly decreased. In the adipose tissue, the incorporation of the acetate 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 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 both dietary groups. The half-lives of esterified C16:0 and C18:1 in the decreasing phase were 5.4 and 8.9 h, respectively, in the liver, and 4.3 and 5.6 d, in the 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 adipose tissues and to stay there longer. Moreover, it is remarkable that 30% of the acetate radioactivities administered were found after 2 h in the whole liver: 75% of the products from the acetate at the maximum were lipids and 61% of the lipids, TAG. The major products from acetate in the liver were lipids.

Key Words acetate incorporation, time course, half-life, triacylglycerols, total lipids

The nutritional and hormonal regulation of lipogenic enzyme gene expressions and enzyme activities in the liver has been studied by many investigators using animals, as described in one review (1). We previously reported that the incorporation of $^3$H$_2$O into the fatty acid fraction was already high 1 h after the intraperitoneal injection into rats (2). When linolenic acid was considered to be an index of exogenous polyunsaturated fatty acid (PUFA), linolenic acid was quickly (within 1 h) incorporated into the liver lipids, and quickly (within 2 h) suppressed lipogenic enzyme mRNA gene expressions (3). 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 triacylglycerol (TAG)-fatty acids were quickly synthesized from substrates, and exogenous TAG-fatty acids were quickly incorporated into liver lipids and disappeared. On the other hand, in the adipose tissues, the turnover of TAG 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 (4–7). 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 (5). Borensztain and Getz (6) 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. (5) concluded that, after intravenous injection of [U-$^{14}$C]glucose in mice, virtually all of the radioactivity found in the adipose tissue of the mice was actually synthesized by the adipose tissue itself.

We have found that a small part of labeled exogenous triolein orally given to rats stayed intact for a long time in adipose tissue, whereas the labeled triolein was only slightly incorporated into lipids and quickly metabolized in the liver (8). Thus it appears 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 adipose tissue.

The aim of our studies including Ref. 8) is to trace exogenous and endogenous TAG turnover after oral intake of TAG 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 (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 (9), 4.0; choline chloride, 0.1; vitamin mixture (9), 0.1. Corn oil replaced sucrose for the corn oil diet. The major fatty acid compositions of the 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°C) under an automatic lighting schedule (08:00 to 20:00 h). The animals were allowed to consume diet and water ad libitum.

The rats were orally given 3.7 MBq [1-14C]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 heparinized blood at 4°C for 20 min at 1,200~g. The liver, plasma and adipose tissue were immediately frozen in liquid nitrogen, and stored at -80°C 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 (10).

Lipid extraction, fractionation and analysis. Total lipids of liver, plasma and adipose tissue were extracted according to the method of Folch et al. (11). The fractions of TAG 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 TAG 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 TAG from the zones was over 90%. Each TAG amount was corrected by the recovery. After saponification of the lipids with 1.79 mol/L 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.

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°C to 230°C at 6°C/min and finally to maintain at 230°C 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. (12). 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 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 acetonitril/water (85 : 15 by volume).

Statistical analysis. Two-way ANOVA was followed by an inspection of all difference between pairs of means using the least significant difference test (13). Differences were considered significant at p<0.05.

RESULTS

Time courses for incorporation of labeled acetate into total lipids in liver and 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

![Fig. 1. Time courses for total lipid radioactivity in liver, plasma and epididymal adipose tissue for 10 d after oral administration of labeled acetate to rats. Rats were food-deprived overnight and re-fed a fat-free or 10% corn oil diet for 3 d. Then rats were orally given 3.7 MBq [1-14C]acetate acid, sodium salt. Mean±SD (n=4). Two-way ANOVA for diet (D) and time (T); D, T, D×T, p<0.001 in the liver, plasma and adipose tissue.](image-url)
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 2 h after the administration. The time courses for incorporation into plasma lipids were similar to those in the liver.

In the adipose tissue, however, the labeled acetate incorporation into total lipids after oral administration to rats fed the corn oil 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 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 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 adipose tissue after oral administration**

After oral administration of labeled acetate to rats, the time courses of acetate incorporation into total lipids and TAG in the liver 2 h after oral administration to rats are shown in Table 1(A). The percent incorporations of the acetate in whole liver per total administration to whole body are shown in Table 1(A). As shown in the table, about 30% of total acetate administration was incorporated into the liver. The incorporations of total acetate administration into total lipids and TAG in the liver were 21 and 14%, respectively. Moreover, the relative incorporations at the maximum, 2 h after the labeled acetate administration are shown in Table 1(B). It was remarkable that, as shown in the table, 75% of the products from the acetate at the maximum were lipids in the liver, and 61% of the lipids was TAG. 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 TAG.

**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 adipose tissue of rats fed the fat-free or corn oil diet are shown in Fig. 3. The time courses in the 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...
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 for 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, F×T, p<0.001 in liver, plasma and adipose tissues; in rats fed corn oil diet (lower): F, T, F×T, p<0.001 in liver, F, T, p<0.001, F×T, p<0.01 in plasma, F, T, p<0.001 in 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 C16:0, C16:1, C18:1, D, T, p<0.001, D×T, p<0.01 in liver, D, T, p<0.001, D×T, p<0.05 in plasma and adipose tissue; for C18:0, T, p<0.001 in liver, D, T, p<0.001 in plasma, D, p<0.001 in adipose tissue.

Half-lives of endogenous fatty acids

Using data shown in Fig. 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 Fig. 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 adipose tissue are shown, as the radioactivities decreased slowly. The half-lives of C16:0 and C18:1 were 5.4 and 8.9 h, respectively, in the liver of the rats fed the fat-free diet, 4.5 and 6.7 h, respectively, in the plasma, and 4.3 and 5.6 d, respectively, in the adipose tissue. The half-lives were similar in the liver and plasma, but much longer in the adipose tissue. The half-lives of C18:1 were slightly longer than those of C16:0. In the rats fed the corn oil diet, the half-lives of 16:0 and 18:1 were 4.5 and 8.9 h, respectively, in the liver; 4.7 and 5.2 h, respectively, in the plasma; and 4.4 and 4.8 d, respectively, in the adipose tissue. Thus the half-lives were similar in the 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. We previously found that the induction of lipogenic enzymes (acyetyl-CoA carboxylase, fatty acid synthase and others) in liver (1) and in adipose tissue (14) 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% corn oil diet for 3 d (15). Therefore, feeding the diet for 3 d was considered to be enough for fatty acid synthesis to reach a steady state in the liver and adipose tissue.

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 (14). Thus, fatty acid synthesis ability should be much lower in the adipose tissue than in the liver. Esterified fatty acids are synthesized abundantly in the liver, and the time courses for labeled acetate incorpora-
After oral administration of labeled acetate to rats fed the fat-free diet, the acetate incorporation into total lipids (mainly TAG) was markedly lower in the liver, plasma and 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 both dietary groups. It appeared that dietary corn oil suppressed the acetate incorporation into fatty acids but did not affect the fatty acid elimination.

In our previous study (8), 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 adipose tissue. In the present experiment, TAG and other lipids were abundantly synthesized in the liver and appeared to be transported to adipose tissue. Thus, it is suggested that esterified fatty acids (non-essential) might be scarcely exogenous and mostly endogenous in the liver, whereas more exogenous fatty acids may be contained in adipose tissue. The previous results support the present results. Gordis (17) also found that most TAG molecules in adipose depots are stored intact until mobilized. Moreover, Carmaniu and Herrera (18) reported that the highest proportion of radioactivity from \([1-14C]palmitate\) appeared in the esterified fatty acid in adipose tissue.

In summary, the labeled acetate was abundantly incorporated into lipids (mainly into TAG) in the liver, reached a maximum at 2h after the administration and then quickly decreased. The acetate incorporation into the lipids was markedly lower in the liver, plasma and 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 both dietary groups. The half-lives were very short in the liver, but were much longer in the adipose tissue. In the adipose tissue, the incorporation of the acetate reached a maximum after 8 h and slowly decreased. The time courses for incorporation into plasma lipids were parallel to those in the liver. Thus the esterified fatty acids synthesized from acetate in the liver appeared to be abundantly transported to adipose tissues and to stay there longer. As previously suggested (8), the esterified fatty acids (non-essential) should be mostly endogenous in the liver, whereas some exogenous fatty acids should be contained in adipose tissue.

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