In our previous study, after oral administration of labeled acetate to rats fed a fat-free diet, acetate incorporation into total lipids in the liver increased quickly and abundantly, reaching a maximum after 2 h, and then quickly decreased and almost disappeared after 2 d \((1)\). In adipose tissue, however, incorporation increased after 2 h, reaching a maximum after 8 h, then gradually decreased, and remained at 28% of the maximum even after 10 d. The major products from acetate in the liver were lipids, 67% of which were triacylglycerols (TAG) in fed states.

After oral administration of labeled triolein to rats, exogenous oleic acid stayed intact and abundant for a long time in adipose tissue, whereas it was only slightly incorporated into lipids and was quickly metabolized in the liver \((2)\). TAG and other lipids appeared to be quickly synthesized in the liver and also disappear quickly. The most of these newly made fatty acids in the liver appeared to be subsequently transported to extra hepatic tissues for storage and use. To measure the newly made fatty acids in the liver, time after the administration of labeled glucose or acetate is the most important factor. In the present study, to compare incorporations of acetate and glucose in tissue total lipids and TAG, the time courses for labeled acetate and glucose incorporations into total lipids in the liver were parallel to those in plasma, but opposite to those in adipose tissue. TAG synthesized from acetate and glucose in the liver appeared to be mostly transported to adipose tissue. Thus, it is suggested that as the labeled glucose rapidly decreased in the liver, plasma and adipose tissue, TAG should be less derived from dietary carbohydrate than from dietary fat.

**Key Words** acetate, glucose, lipids, triacylglycerols
Acetate and Glucose Incorporations into Lipids

in livers 2–3 d and 3 d, respectively, after refeeding experimental diets. Rats were given 7.4 kBq \([1-^{14}C]\) acetic acid, sodium salt/kg bw, or 18.5 kBq \([^{14}C(U)]\) d-glucose/kg bw. Rats were killed 1, 2, 4, 8, and 16 h after injection. Total, total lipids and TAG radioactivities in tissues were measured. The time courses of the radioactivities in tissues are shown by dpm/g or mL. Mean±SD (n=4). Statistical analyses are shown in Fig. 2.

The rats were anesthetized with isoflurane using anesthetic vaporizer (MK Vapo, Muromachi Kikai Co., Ltd., Tokyo, Japan). The blood was taken using a heparinized syringe from the inferior vena cava of rats under the anesthesia and the plasma was obtained by centrifuging heparinized blood at 4˚C for 10 min at 1,200 \(x\)g.

The liver, plasma and adipose tissue were quickly removed, frozen with liquid nitrogen, and stored at \(-80^\circ\)C to extract total lipids as described below. Four rats were treated once by three skilled collaborators within 10 min. The rats injected with labeled acetate and glucose were killed on different days. 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 the liver, plasma, and adipose tissue were extracted according to the method of Folch et al. (11) and TAG were separated by thin-layer chromatography on silica gel H (Merck, Darmstadt, Germany) 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. Silica gel zones were scraped and lipids were extracted with chloroform/methanol (1:1, by volume). The recovery of TAG from these zones was over 90%. The TAG concentrations were measured with Triglyceride G-test Wako kit (Wako Pure Chemical Industries, Ltd.) (12). Each TAG amount was corrected by the recovery.

After saponification of total lipids, the sterol fraction was removed with petroleum ether. The water soluble fraction was acidified and then the fatty acid fraction was extracted with petroleum ether. Glycerol remained in the acidified water soluble fraction. Incorporations of labeled acetate or glucose into fatty acids or glycerol were measured by their radioactivities.

**Statistical analysis.** The main effects were analyzed by two- or three-way ANOVA using the Tukey post hoc test (13). Mean comparisons were conducted by using a Bonferroni t test when the main effect was significant. If an interaction between main effects was found, the mean comparison was done conditionally. All analyses were performed with SPSS software (Version 21.0, IBM). Data were expressed as mean±SD and significance was set at p<0.05.

**RESULTS**

**Time courses for incorporations of labeled acetate and glucose into total lipids and TAG in the liver, plasma and adipose tissue after intravenous injection**

Time courses of incorporations of labeled acetate and glucose into total lipids and TAG in liver, plasma and adipose tissue after injection are shown in Fig. 1, and statistical analyses for total lipids and TAG are shown in Fig. 2. Total radioactivities in tissues are also shown in Fig. 3. Statistical analyses for incorporation of labeled acetate and glucose into total lipids and TAG in the liver, plasma and adipose tissues after the intravenous injection. A three-way ANOVA for substrate (S, acetate and glucose), diet (D, fat-free diet and corn oil diet) and time (T, time after injection of labeled substrate) in Fig. 1 are shown in Fig. 2. Mean±SD (n=4). NS: not significantly different. Means without a common letter differ, p<0.05.

| Fat-free diet | Corn oil diet |
|---------------|--------------|
| Body (g)      | 84.3±2.43    | 84.1±2.89    |
| Liver (g/100 g bw) | 6.01±0.97  | 5.39±1.01    |
| Adipose tissue (g/100 g bw) | 0.31±0.05  | 0.32±0.12    |
| Plasma TAG (mg/dL) | 136±10.4   | 146±17.6     |
| Liver TAG (mg/g) | 20.3±2.27  | 18.8±3.32    |

Body, liver and adipose tissue weights of rats, when killed, are shown. Mean±SD (n=20 for each dietary group). TAG concentrations of plasma and liver are also shown (n=8 for each dietary group). These data are not significantly different between the dietary groups. Food intake (per day/100 g bw) was (g) 11.8±0.70 and 11.8±0.79, (kcal) 43.4±2.40 and 49.3±3.30, respectively, in rats fed the fat-free diet and corn oil diet.
1. Time courses of incorporations of labeled acetate and glucose into total lipids/g tissue per total injection in liver and adipose tissue after intravenous injection.

|                     | Acetate (%)            | Glucose (%)            | p value |
|---------------------|------------------------|------------------------|---------|
|                     | Fat-free diet          | Corn oil diet          | Fat-free diet | Corn oil diet |         |
| Liver               | 1.26±0.20              | 0.58±0.08              |          | 0.46±0.14     | 0.12±0.09 | S p<0.000 |
| Adipose tissue      | 1.91±0.12^a            | 1.16±0.11^b            |          | 1.99±0.26^a   | 0.64±0.09^c | D p<0.000 S\times D NS |

Data are from Fig. 1. Percents of the radioactivities incorporated into total lipids/g tissue per total injection at maximum (2 h in the liver and 8 h in the adipose tissue after labeled acetate or glucose injection) were used as an index of the relative values of the incorporation percent of acetate or glucose into total lipids. Mean±SD (n=4). A two-way ANOVA for substrate (S) and diet (D) in radioactivities of total lipids. Means without a common letter differ, p<0.05. NS, not significantly different.

Table 3. Incorporation percents of labeled acetate and glucose into total lipids and TAG/total radioactivities in the liver 2 h after intravenous injection.

|                     | Acetate (%)            | Glucose (%)            | p value |
|---------------------|------------------------|------------------------|---------|
|                     | Fat-free diet          | Corn oil diet          | Fat-free diet | Corn oil diet |         |
| Total lipids        | 73.7±12.2^a            | 45.9±6.41^b            |          | 44.1±8.17^b   | 15.4±4.87^c | S p<0.000 D p<0.000 S\times D p<0.000 |
| TAG                 | 53.9±15.6^a            | 18.5±5.12^c            |          | 31.2±8.13^b   | 6.83±1.27^d | S p<0.000 D p<0.000 S\times D p<0.000 |

Incorporation percents of labeled acetate and glucose into total lipids or TAG/total radioactivities in the liver 2 h after intravenous injection were calculated from the results of Fig. 1. Mean±SD (n=4). A two-way ANOVA for substrate (S) and diet (D) in radioactivities of total lipids or TAG in liver. Means without a common letter differ, p<0.05.
Incorporation percents of labeled glucose into fatty acids and glycerol/total lipids in the liver and adipose tissue.

|                | Liver (%)                  |                  | Adipose tissue (%)                  | p value |
|----------------|----------------------------|------------------|------------------------------------|---------|
|                | Fat-free diet              | Corn oil diet    | Fat-free diet                       | Corn oil diet      |
| Fatty acids    | 74.7±7.26                 | 60.9±8.05        | 64.8±6.65                          | 56.4±6.57         |
| Glycerol       | 12.7±4.26                 | 26.1±5.25        | 23.7±2.80                          | 33.9±6.97         |

Results were obtained from the same rats as in Fig.1. The incorporation percents of radioactivities into fatty acids and glycerol in total lipids after 2 h are shown. Mean±SD (n=4). A two-way ANOVA for tissue (Ts) and diet (D) in glucose radioactivities into fatty acids and glycerol in total lipids.

In adipose tissue, acetate and glucose incorporation percents into total lipids/g tissue for the total injection were comparable in rats fed the fat-free diet. In rats fed the corn oil diet, acetate and glucose incorporations into total lipids/g liver were 0.58% and 0.12%, respectively, of the total injection of radioactivities, and were lower than those in rats fed the fat-free diet (diet p<0.000). Thus, incorporation ratios of glucose into total lipids/g liver per total injection were greatly less than those of acetate in both dietary groups (substrate p<0.000). Incorporation ratios of glucose into total lipids were more greatly suppressed by the corn oil diet than those of acetate (p<0.001).

In adipose tissue, acetate and glucose incorporation percents into total lipids/g tissue for the total injection were comparable in rats fed the fat-free diet. In rats fed the corn oil diet, however, acetate incorporation was 61% of that in rats fed the fat-free diet, and glucose incorporation was only 32% in adipose tissue (diet p<0.000), similar to the liver.

Incorporation percents of labeled acetate and glucose into total lipids and TAG per total radioactivities in the liver

Incorporation percents of labeled acetate and glucose into total lipids and TAG per total radioactivities in the liver are shown in Table 3. Values were calculated from data at 2 h in Fig. 1. There were effects of substrate (p<0.000) and diet (p<0.000) and substrate x diet interaction (p<0.000) for the incorporation into total lipids and TAG. Acetate incorporations into total lipids were about 74% and 46% of total radioactivities, respectively, in the livers of rats fed the fat-free diet and corn oil diet, and the incorporations into TAG were about 54% and 19%. Glucose incorporations into total lipids were about 44% and 15% of total radioactivities, respectively, in the liver of rats fed the fat-free diet and corn oil diet, and the incorporations into TAG were 31% and 7%, respectively. The incorporations of glucose into total lipids and TAG were more strongly suppressed by dietary corn oil than those of acetate.

After injection of labeled acetate, radioactivity was incorporated into total lipids, mostly into TAG, in parallel to the total radioactivities until 16 h in the liver (Fig. 1A). After injection of labeled glucose, however, the total radioactivity was already low after 1 h, so was not incorporated much into total lipids and TAG (Fig. 1D).

Incorporations of labeled acetate and glucose into total lipids in plasma were similarly decreased with incorporations in the liver (Fig. 1B, E). However, incorporations in adipose tissues were opposite to those in the liver and plasma, 2–8 h after injection of labeled acetate and glucose (Fig. 1C, F).

Incorporation percents of labeled glucose into fatty acids and glycerol/total lipids

Incorporation percents of labeled glucose into fatty acids and glycerol per total lipids are shown in Table 4. There was an effect of tissue (p<0.036) and diet (p<0.005) for mean incorporation percent of labeled glucose in fatty acids and an effect of tissue (p<0.000) and diet (p<0.000) for glycerol, but there was no significant tissue x diet interaction. In the liver, labeled glucose was incorporated into fatty acids and glycerol, 75% and 13% of total lipids, respectively, in rats fed the fat-free diet, and 61% and 26%, respectively, in rats fed the corn oil diet. In adipose tissue, labeled glucose was incorporated into fatty acids and glycerol, 65% and 24% of total lipids, respectively, in rats fed the fat-free diet, and 56% and 34%, respectively, in rats fed the corn oil diet. Glucose incorporation into glycerol (relative to fatty acids) was more abundant in adipose tissue than in liver in both dietary groups. Glucose incorporation into glycerol was more abundant in both the liver and adipose tissue in rats fed the corn oil diet than in those fed the fat-free diet.

On the other hand, although labeled acetate was abundantly incorporated into fatty acids, incorporation of acetate into glycerol was negligible in the liver and adipose tissue (data not shown).

**DISCUSSION**

A major discrepancy has existed in the literature with respect to the role of the liver in fatty acid synthesis from glucose carbon and from other carbon sources (14–22). Most reports indicate that the liver’s role is minor in rodents. However, Hems et al. (20) calculated that at least 60% of all fatty acid synthesized de novo from all carbon sources was made by the liver. Borensztain and Getz (21) estimated that about 50% of 14C-labeled TAG...
fatty acids in adipose tissue had been synthesized by the liver after injection of labeled glucose and then transported to adipose tissue. Baker et al. (22) have shown according to their extensive research that, under conditions that promote rapid lipogenic rates, the liver synthesized as much as 50% of the body’s fatty acids from all 2-carbon units in mice.

In the present investigation, we have found that, after intravenous injections of labeled acetate or glucose to rats fed the fat-free diet, acetate incorporation into total lipids in the liver increased quickly and abundantly, reaching a maximum after 2 h, and then quickly decreased to 28% of the maximum after 8 h. In adipose tissue, however, incorporation reached a maximum in 2–8 h, then gradually decreased, and remained at 61% of the maximum even after 16 h. Incorporations of labeled acetate and glucose into total lipids in plasma quickly decreased similarly to those in the liver. In adipose tissue, however, incorporations were opposite to those in the liver and plasma, until 8 h after injection of labeled acetate and glucose. TAG synthesized from acetate and glucose in the liver appeared to be transported to adipose tissues and stay there longer. Thus it is likely that most of these newly made fatty acids in the liver are subsequently transported to extra hepatic tissues for storage and use.

For reference, the incorporation ratios of labeled glucose into total lipids of tissues (per g tissue or mL plasma) 2 h after intravenous injection to rats fed the 10% corn oil diet are shown next. The incorporation ratios relative to liver were 1.00 (liver), 1.25 (adipose tissue), 0.43 (lung), 0.42 (kidney), 0.14 (brain), 0.29 (intestine), 0.11 (red blood cells by mL), and 0.67 (plasma by mL). Mean values of 2–4 rats. The liver appeared to be the major lipid maker.

After intravenous injections of labeled acetate and glucose to rats, the incorporations of labeled acetate and glucose into total lipids were greatly changed by time after the injection, as shown in Figs. 1 and 2. As glucose was very quickly and abundantly metabolized, the quantity of fatty acid synthesis should have been changed by the measured time. A major discrepancy existing in the literature on lipid (mostly TAG) synthesis could be ascribed to the measured time after the injection.

In the liver, glucose incorporation into total lipids was more suppressed by dietary corn oil than the acetate incorporation. Enzyme activities of glucokinase and pyruvate kinase in glycolysis were also suppressed by dietary corn oil than the acetate incorporation. Glucose incorporation into TAG synthesized from acetate and glucose should be suppressed by PUFAs before (in glycolysis) and also after (in lipogenesis) acetyl-CoA.

It appeared that fatty acid synthesis from acetyl-CoA derived from dietary glucose should be more greatly suppressed by PUFAs than synthesis from acetyl-CoA derived from fatty acid oxidation of dietary fat. Moreover, glucose should be quickly used in many physiological pathways and decrease quickly. Thus, it is suggested that, as the injected glucose was rapidly decreased in the liver, plasma and adipose tissue, body fat should be less derived from dietary carbohydrate than from dietary fat.

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