Lipid synthesis from labeled acetate and glucose in the liver and adipose tissue after intravenous injection

Nobuko Iritani, Tomoe Hirakawa, Hitomi Fukuda,
Michiko Katsukawa, Akihiko Katsurada, Mika Kouno

Summary To investigate lipid synthesis from acetate and glucose, incorporations of labeled acetate and glucose into total lipids in livers and epididymal adipose tissues (adipose tissue) were followed after their intravenous injection in the tail vein of individual rat fed a fat-free or 10% corn oil diet. The incorporation of acetate into total lipids, mostly triacylglycerols (TAG), in the liver reached maximum 2 h after the injection, while the incorporation of glucose decreased more quickly. Incorporation of glucose into TAG was more greatly suppressed by dietary corn oil than that of acetate in the liver. Probably it could be because corn oil contained 53% linoleic acid, by which the glucose incorporation were suppressed in both glycolysis and lipogenesis, while the acetate incorporation was suppressed in lipogenesis only. In the adipose tissues, the incorporation of labeled acetate or glucose into total lipids was maximum 2~8 h after the injection, while the incorporation of glucose was very low, especially in rats fed corn oil diet. Moreover, the labeled acetate and glucose incorporations into total lipids in the liver were increased for 2 h, and then decreased in 2~8 h, but opposite to this in adipose tissue. Lipids synthesized from acetate and glucose in the liver appeared to be mostly transported to adipose tissue. It is suggested that as the labeled glucose rapidly decreased in the liver, TAG should be less derived from dietary carbohydrate than from dietary fat. Thus, TAG (generally called fat) synthesis from glucose in liver appeared to be activated in case of over eating, whereas usually derived from dietary fat even in normal eating.

INTRODUCTION

Although the conversion of dietary glucose into fat has been reported by many groups, the quantitative role of liver has not been established. Most reports indicated that the liver's role is minor in rodents (*1-9*). However, some reports has shown that, under conditions that promote rapid lipogenic rates, the liver may synthesize as much as 50% of the body's fatty acids in mice and rats (*7-9*).

In our previous studies, 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 (*10*). 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 (*11*). 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 extrahepatic 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, the time courses of incorporations of labeled glucose and acetate into total lipids, after intravenous injection of labeled acetate and glucose to rats were compared by following their radioactivities in liver and adipose tissue.

MATERIALS AND METHODS

Chemicals. [1-^{14}C] Acetic acid, sodium salt (37-111 MBq/mmol), and [^{14}C(U)] D-glucose (9.07 GBq/mmol) were purchased from Moravek Biochemicals Inc. (Brea, CA, USA). Reagents were obtained mostly from Wako Pure Chemical Industries (Osaka, Japan) and Sigma (St. Louis, MO, USA).

Animals. Male Wistar rats (Japan SLC Co., Hamamatsu, Japan), 4-wks old, were purchased and fed on a commercially available non-purified diet (No. MF, Oriental shiryou Co., Osaka, Japan) in our animal room. After 1 wk, the rats were food-deprived overnight and then fed ad libitum a fat-free diet or a 10% (weight) corn oil diet for 4 d. The composition of the fat-free diet was (g/100 g) sucrose, 70.8; casein, 20; cellulose, 5.0; salt mixture (*12*), 4.0; choline chloride, 0.1; and vitamin mixture (*12*), 0.1. Corn oil replaced sucrose for the corn oil diet. The major fatty acid compositions of the corn oil used were (%) *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℃) under an automatic lighting schedule (8:00 h to 20:00 h). Animals were allowed to consume the diet and water ad libitum.

Rats were killed 1, 2, 4, 8, and 16 h after the injection of labeled acetate or glucose, 4 d after the refeeding. Rats were given 74 kBq [1-^{14}C] acetate or 185 kBq [^{14}C (U)] D-glucose in 0.2 ml of water per 100g body weight by intravenous injection to the tail vein at 9:00 h, except at 17:00 h, to kill rats 16 h after the injection. We previously found that lipogenic enzyme induction (*13-17*) and TAG formation (*18*) had reached plateau in livers 3~4 d after refeeding experimental diets.

Aliquots of liver or adipose tissue were quickly removed, frozen with liquid nitrogen, and stored at -80℃ to extract total lipids as described below. All animal procedures were approved by the Animal Experimental Committee of the University of Tezukayama Gakuin University and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (*19*).

Lipid extraction, fractionation, and analysis. Total lipids of the liver and adipose tissue were extracted according to the method of Folch et al. (*20*) and 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). 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%. TAG concentration was measured with Triglyceride G-test Wako kit (Osaka, Japan) (*21*). 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 significant differences among the groups were compared by the Student t-test in Fig. 1. A two way ANOVA was followed by an inspection of all differences between pairs of means using the least significant difference test (*22*). Differences were considered significant at $P<0.05$.

RESULTS

Pilot experiment

To search experimental methods for amount of labeled acetate, addition of unlabeled acetate (substrate) and administration way of labeled acetate (oral or intravenous), pilot experiments were conducted. Data were shown in Fig. 1. The methods of pilot experiment were mostly the same (as written in materials and methods) to the main experiments, unless written in the legend of Fig. 1.

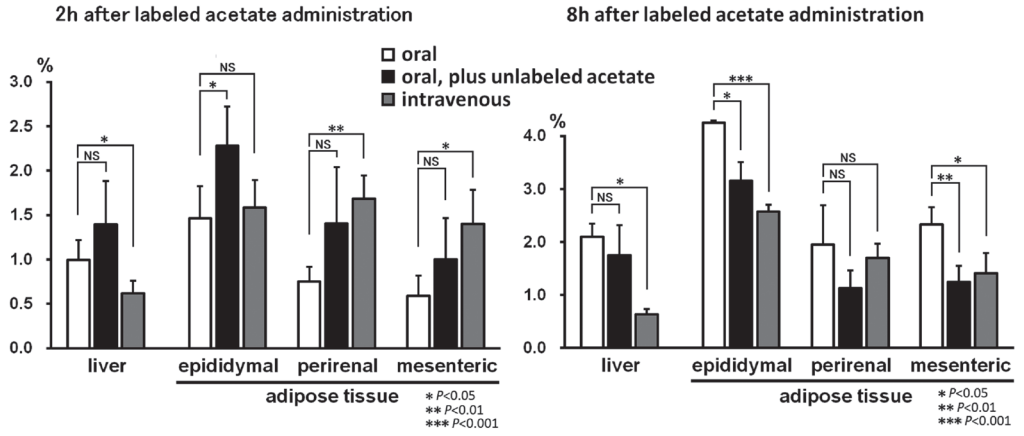

Figure 1 Relative incorporation percents of labeled acetate into total lipids/g tissue per total injection in liver and adipose tissue after oral or intravenous administration (pilot experiment). The methods of pilot experiment were mostly the same to the main experiments, unless written in this legend. Rats were food-deprived overnight and refed a fat free-diet for 4 days. Rats were orally given 185 kBq [1-^{14}C] acetic acid, sodium salt in 0.5 ml saline /100 g bw (white bar). Some rats were orally given the labeled acetate in 0.5 ml of 50 mM unlabeled acetate/100g bw (black bar). Some other rats were intravenously injected with the labeled acetate in 0.2 ml saline /100g bw (gray bar). Rats were killed 2 h, or 8 h after administration. Total and total lipids radioactivities in tissues were measured. 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 administration) were shown as an index of the relative values of the incorporation percent of acetate into total lipids. Mean ± SD (n=5-7). t-Test between oral administration with and without unlabeled acetate, and t-test between oral and intravenous administrations without unlabeled acetate were shown in figure 1. NS, not significantly different.

In liver, incorporation of labeled acetate into lipids (at maximum, 2 h after labeled acetate administration) was not affected with or without unlabeled acetate in oral administration, and the incorporation was more by oral administration than by intravenous injection without

unlabeled acetate. In adipose tissues, incorporation of labeled acetate into lipids (at maximum, 8 h after labeled acetate administration) was decreased with unlabeled acetate in oral administration, and the incorporation was more by oral than by intravenous injection without unlabeled acetate in epididymal but not significantly different in other adipose tissues.

It is very difficult to find the physiological concentration of acetate (substrate). Lipid synthesis from labeled acetate could be measured in physiologically normal condition by intravenous injection without labeled acetate. Consequently, labeled acetate was intravenously injected without unlabeled acetate, to neglect the influences of food digestion in stomach, intestinal absorption and dilution of specific activitiy of labeled acetate.

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

After intravenous injection of labeled acetate to rats fed the fat-free diet, acetate incorporation into total lipids in the liver reached a maximum in 2 h, then quickly decreased to 28% of the maximum after 8h and to 19% after 16 h. In rats fed the corn oil diet, incorporation in the liver was low after 2 h, 39% of that in rats fed the fat-free diet. Incorporation of labeled glucose into total lipids in the liver was more quickly increased and then decreased than that of acetate.

In adipose tissue, however, labeled acetate incorporation into total lipids in rats fed the fat-free diet was still low after 1 h, became greatly elevated to a maximum in 2h to 8h, and then decreased to 61% of the maximum level after 16 h. Acetate incorporations in both dietary groups were in parallel. In rats fed the corn oil diet, acetate incorporation into total lipids in adipose tissue was markedly lower than that in rats fed the fat-free diet. Incorporation of labeled glucose into total lipids in adipose tissue of rats fed the fat-free diet was comparable to that of acetate. However, glucose incorporation into total lipids was markedly suppressed by dietary corn oil, more so than that of incorporation of acetate.

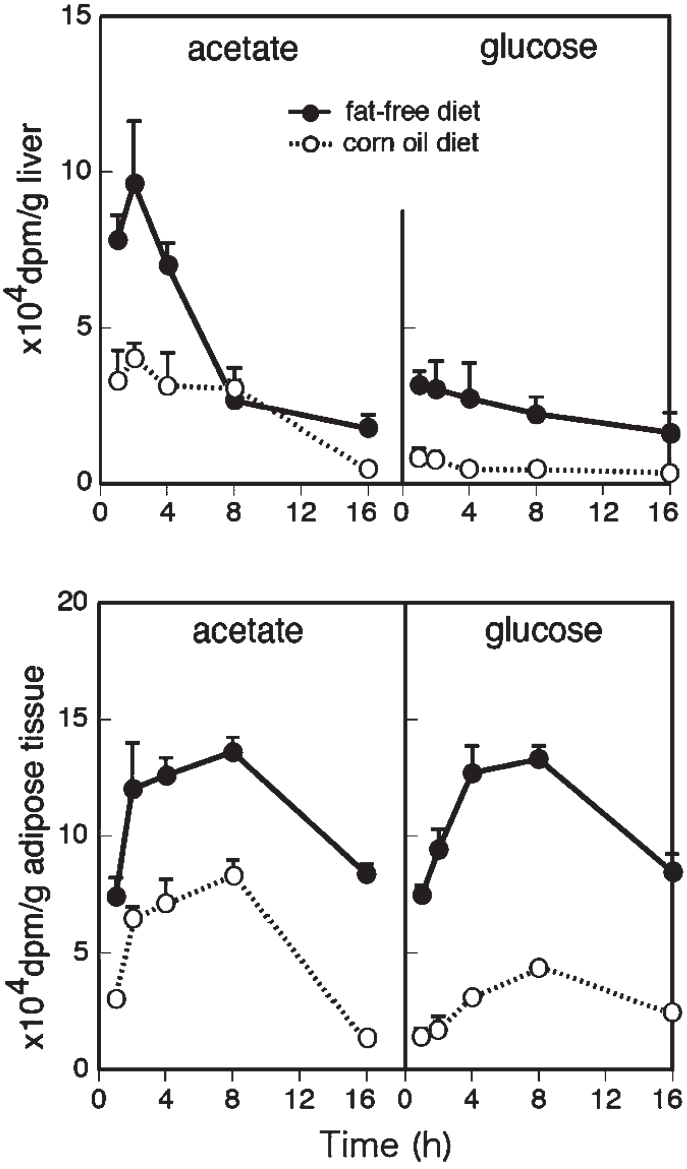

Figure 2. Time courses for total lipids radioactivities in the liver and epididymal adipose tissues for 16 h after intravenous injection of labeled acetate or glucose to rats. Rats were food-deprived overnight and refed a fat-free or 10% corn oil diet for 4 d. Rats were then intravenously injected with 74kBq [1-^{14}C] acetic acid, sodium salt/100g bw, or 185kBq [^{14}C (U)] D-glucose/100g bw. Rats were killed 1, 2, 4, 8, and 16 h after injection. Body weights, when killed, were (g) 84.3±2.43 and 84.1±2.89, respectively, in rats fed the fat-free diet and the corn oil diet. Total lipids radioactivities in tissues were measured. The time courses of radioactivities in tissues are shown by dpm/g. Mean±SD (n=4). A two-way ANOVA for total lipid radioactivities after injection of labeled acetate in each tissue: D (diet), T (time), DxT, $p<0.001$ in the liver; D, T, $p<0.001$ in adipose tissue. A two-way ANOVA for total lipid radioactivities after injection of labeled glucose in each tissue: D, $p<0.001$ in the liver; D, T, $p<0.001$ in adipose tissue.

Relative incorporation percents of labeled acetate and glucose into total lipids/g tissue per total injection

The relative incorporation percents of labeled acetate and glucose into total lipids at maximum, after 2 h in the liver and 8 h in adipose tissue, following intravenous injection are shown in Table 1（data from Fig. 2）. Acetate and glucose incorporation percents into total lipids/g liver per total injection（used as an index of incorporations of acetate and glucose into total lipids）were 1.26% and 0.46%, respectively, of the total injection 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. Thus, incorporation ratios of glucose into total lipids/g liver per total injection were 46%, greatly less than those of acetate in both dietary groups. Incorporation ratios of glucose into total lipids were 26%, more greatly suppressed by the corn oil diet than those of acetate.

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, similar to the liver.

Table 1 Relative incorporation percents of labeled acetate and glucose into total lipids/g tissue per total injection in liver and epididymal adipose tissue after intravenous injection.

Dietary groups	acetate	glucose
	%	
Liver		
fat-free diet	1.26±0.20	0.46±0.14
corn oil diet	0.58±0.08	0.12±0.09
Adipose tissue		
fat-free diet	1.91±0.12	1.99±0.26
corn oil diet	1.16±0.11	0.64±0.09

Data are from Fig. 2. Percents of the radioactivities incorporated into total lipids/g tissue per total injection at maximum (2 h in the liver and 8 h in 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, S, D, $p<0.001$ in the liver; and D, $p<0.001$, SxD, $p<0.05$ in adipose tissue.

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 2h after intravenous injection are shown in Table 2. 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 incorporations into TAG were 31% and 7%, respectively.

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

Dietary groups	acetate		glucose	
		%		
	TL	TAG	TL	TAG
fat-free diet	73.7±12.2	53.9 ±15.6	44.1±8.17	31.2±8.13
corn oil diet	45.9±6.41	18.5±5.12	15.4±4.87	6.83±1.27

Incorporation percents of labeled acetate and glucose into total lipids/total radioactivities in the liver 2 h after intravenous injection were calculated from the results of Fig. 2. Mean ± SD (n=4). A two-way ANOVA for substrate (S) and diet (D) in radioactivities of total lipids in liver: S, D, SxD, $p<0.001$ in dietary group.

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 3. 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 of 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).

Table 3. Incorporation percents of labeled glucose into fatty acids and glycerol/total lipids in the liver and epididymal adipose tissue.

Dietary groups	Liver		Adipose tissue	
	fatty acids	glycerol	fatty acids	glycerol
		%		
fat-free diet	74.7±7.26	12.7±4.26	64.8±6.65	23.7±2.80
corn oil diet	60.9±8.05	26.1±5.25	56.4±6.57	33.9±6.97

Results were obtained from the same rats in Fig.2. 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 (Ti) and diet (D) in glucose radioactivities into glycerol in total lipids: Ti, D, $P<0.05$.

DISCUSSION

A major discrepancy has existed in the literatures with respect to the role of liver in fatty acid synthesis from glucose carbon and from other carbon sources (*1-9*). Most reports indicated that the liver's role is minor in rodents. However, Hems et al. (*7*) have calculated that at least 60% of all fatty acid synthesized de novo from all carbon sources is made by the liver. Borensztain et al. (*8*) estimated that about 50% of ^{14}C-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. (*9*) 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. In adipose tissue, however, incorporations were opposite to those in the liver, until 8h 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 extrahepatic tissues for storage and use.

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 Fig. 2. As glucose was very quickly and abundantly metabolized, the quantity of fatty acid synthesis should be changed by the measured time. A major discrepancy existed in the literatures could be ascribed to the measured time after the injection.

In the liver, incorporation of labeled glucose into total lipids and TAG per total radioactivity of intravenous administration did not increase much, then more quickly and more abundantly decreased, over the incorporation of labeled acetate.

Moreover, glucose incorporation into total lipids and TAG was extremely suppressed by dietary corn oil in the liver, and was so more than acetate incorporation. Glucose should be quickly used in many physiological pathways and decrease quickly. It is suggested that glucokinase and pyruvate kinase were also suppressed by polyunsaturated fatty acids (PUFA) in glycolysis before acetyl-CoA formation in the liver, as we previously reported (*23*). Thus, it is suggested that fatty acid synthesis from glucose should be suppressed by PUFA before (in glycolysis) and after (in lipogenesis) acetyl-CoA (Fig. 3). It appeared that fatty acid synthesis from acetyl-CoA from glucose (derived from dietary carbohydrates) should be more greatly suppressed by PUFA than synthesis from acetyl-CoA derived from fatty acid oxidation of

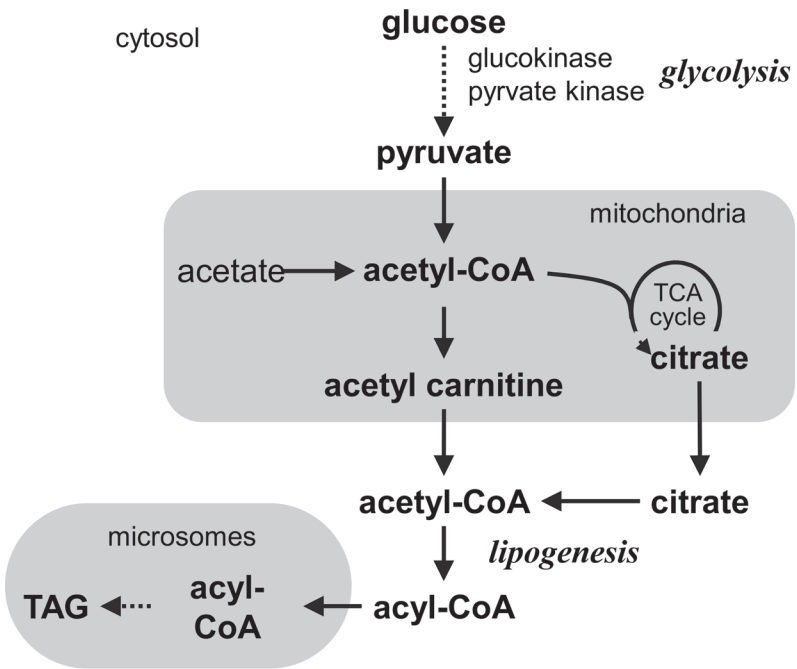

Figure 3

dietary fat. In adipose tissue, incorporation of labeled acetate and glucose into total lipids increased more slowly than in the liver, and both acetate and glucose incorporations into total lipids were higher in rats fed the fat-free diet than in those fed the corn oil diet, similarly to that in the liver. Thus, dietary fat appeared to be incorporated into fat more abundantly than dietary carbohydrate.

In conclusion, it is suggested that as the labeled glucose rapidly decreased in the liver and adipose tissue, TAG should be less derived from dietary carbohydrate than from dietary fat. Thus, TAG（generally called *fat*）synthesis from glucose in liver appeared to be activated in case of over eating, whereas usually derived from dietary fat even in normal eating.

REFERENCES

1) Jansen GR, Hutchison CF, Zanetti ME. 1966. Studies on lipogenesis in vivo. Effect of dietary fat or starvation on conversion of［^{14}C］glucose into fat and turnover of newly synthesized fat. *Biochem J* 99: 323-332.

2) Jansen GR, Zanetti ME, Hutchison CF. 1968. Studies on lipogenesis in vivo. Lipogenesis during extended periods of re-feeding after starvation. *Biochem J* 106: 345-353.

3) Bjorntorp J, Krotkiewski PM, Larsson B, Somlo-Sziics Z. 1970. Effects of feeding states on lipid radioactivity in liver, muscle and adipose tissue after injection of labeled glucose in the rat. *Acta Physiol Scand* 80: 29-38.

4) Baker N, Huebotter RT. 1973. LiDotzenic activation after nibbling and gorging in mice. *J Lipid Res* 14: 87-97.

5) Kannan R, Palmquist DL, Baker N. 1976. Contribution of intermuscular fat to lipogenesis from dietary glucose carbon in mice. *Biochim Biophys Acta* 431: 225-232.

6) Palmquist DL, Learn DB, Baker N. 1977. Re-evaluation of lipogenic activation in nibbling and gorging rats. *J Nutr* 107: 502-509.

7) Hems DA, Rath EA, Verrinder TR. 1975. Fatty acid synthesis in liver and adipose tissue of normal and genetically obese（ob/ob）mice during the 24-hour cycle. *Biochem J* 150: 167-173.

8) Borensztain J, Getz GS. 1972. The contribution of lipogenesis in situ to the accumulation of fat by rat adipose tissue. *Biochim Biophys Acta* 280: 86-93.

9) Baker N, Learn DB, Bruckdorfer KR. 1978. Re-evaluation of lipogenesis from dietary glucose carbon in liver and carcass of mice. *J Lipid Res* 19: 879-893.

10) Kimura T, Fukuda H, Iritani N. 2005. Labeled acetate incorporation into lipids and lipid elimination after oral administration in rat liver and adipose tissue. *J Nutr Sci Vitaminol* 51: 104-109.

11) Iritani N, Kimura T, Fukuda H, Sugimoto T. 2004. Differences in labeled triolein turnover after oral administration between liver and adipose tissue of rats. *Brit J Nutr* 93:53-58.

12) Reeves PG, Nielsen FH, Fahey GC Jr. 1993. Purified diets for laboratory rodents: Final report of the American Institute of Nutrition and Hoc Writing Committee on the reformation of the AIN-76A rodent Diet. *J Nutr* 123: 1939-1951.

13) Iritani N. 1992. Nutritional and hormonal regulation of lipogenic-enzyme gene expression in rat liver. A review, *Eur J Biochem* 205: 433-442.
14) Katsurada A, Iritani N, Fukuda H, Matsumura Y, Nishimoto N, Noguchi, T, Tanaka T. 1990. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of acetyl-CoA carboxylase in rat liver. *Eur J Biochem* 190: 435-441.
15) Katsurada A, Iritani N, Fukuda H, Matsumura Y, Nishimoto N, Noguchi T, Tanaka T. 1990. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of fatty acid synthase in rat liver. *Eur J Biochem* 190: 427-433.
16) Katsurada A, Iritani N, Fukuda H, Matsumura, Y Noguchi T, Tanaka T. 1989. Effects of nutrients and insulin on transcriptional and post-transcriptional regulation of glucose-6-phosphate dehydrogenase synthesis in rat liver. *Biochim Biophys Acta* 1006: 104-110.
17) Fukuda H, Katsurada A, Iritani N. 1992. Effects of nutrients and hormones on gene expression of ATP citrate-lyase in rat liver. *Eur J Biochem* 209: 217-222.
18) Iritani N, Nagasima K, Fukuda H, Katurada A, Tanaka T. 1986. Effects of Dietary proteins on lipogenic enzymes in rat liver. *J Nutr* 116: 190-197.
19) National Research Council.1985. Guide for the care and use of laboratory animals. Publication no. 85-23 (rev.), National Institute of Health, Bethesda, MD.
20) Folch J, Lees M, Sloane-Stanley GH. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497-509.
21) Spayd RW, Bruschi B, Burdick BA, Dappen GM, Eikenberry JN, Esders TW, Figueras J, Goodhue CT, LaRossa DD, Nelson RW, Rand RN, Wu TW. 1978. Multilayer film elements for clinical analysis: applications to representative chemical determinations. *Clin Chem* 24: 1343-1350.
22) Snedecor GW, Cochran WG. 1967. *Statistical Methods*. p285-338. Iowa State University Press, Ames, IA.
23) Iritani N, Fukuda H, Tada K, Itoh A, Noguchi T. 1995. Diet differentially regulates glucokinase and L-type pyruvate kinase gene expression in rat liver. *J. Nutr* 125: 2945-2952.