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Nutritional Regulation of Lipogenic Enzyme Gene Expression in Rat Epididymal Adipose Tissue[1]

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The time courses of gene expression, and the nutritional regulation of gene expression of lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase, ATP citrate-lyase, malic enzyme, and glucose-6-phosphate dehydrogenase) in epididymal adipose tissue after refeeding food-deprived rats have been investigated and compared with those in liver (previously reported). The mRNA concentrations of lipogenic enzymes reached maximum levels at 24 h after the refeeding in adipose tissue and at 8-16 h in liver, while the enzyme induction reached maximum at 48-72 h in both tissues. Moreover, the mRNAs were more strongly induced in adipose tissue than in liver, whereas the enzyme induction (except malic enzyme) was lower. In adipose tissue of rats fed a carbohydrate diet without protein, the mRNA concentrations of acetyl-CoA carboxylase, ATP-citrate lyase, malic enzyme, and fatty acid synthase reached comparable levels to those of the carbohydrate/protein diet group. The protein feeding increased the enzyme induction in adipose tissue. As regards reduction of gene expression, lipogenic enzyme mRNA concentrations were not so markedly reduced by starvation or polyunsaturated fatty acids in adipose tissue as in liver. The differences in regulation of lipogenic enzyme gene expression and induction between adipose tissue and liver can be ascribed to tissue specificity.

Key words: adipose tissue, lipogenic enzyme gene expression, nutritional regulation.

Studies on regulation of lipogenic enzyme gene expression have been conducted at the gene, cell, and animal levels. A number of studies of nutritional and hormonal regulation of the gene expression in rats, mice, and birds have been reported and reviewed (*1-7*). We have investigated the nutritional and hormonal regulation of gene expression of lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase, ATP citrate-lyase, malic enzyme, and glucose-6-phosphate dehydrogenase) in rat liver, following the cloning of cDNA species of the lipogenic enzymes (*8-12*). The gene expression and induction of liver lipogenic enzymes were coordinately altered with changes of the hormonal and nutritional conditions, whereas the magnitude of response to the changes was different among the enzymes. On the other hand, adipose tissue is another major tissue in which the lipogenic enzyme genes are expressed. Although Northern-blot analyses of RNA revealed that each mRNA of lipogenic enzymes of rat adipose tissue was the same as that of liver (*8-12*), the gene expression in adipose tissue may be considerably different from that in liver. Therefore, we have investigated the regulation of gene expression of lipogenic enzymes in rat epididymal adipose tissue during nutritional and hormonal manipulations, in comparison with that in liver.

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Abbreviation: adipose tissue, epididymal adipose tissue.

MATERIALS AND METHODS

Materials—[α-^{32}P]dCTP (111 TBq/mmol) was purchased from ICN Pharmaceuticals (Costa Mesa, CA). Nylon filter (Hybond N) was purchased from Amersham (Buckinghamshire, UK). Insulin assay kit was obtained from Eiken Chemical (Tokyo). Glucose assay kit was from Wako Pure Chemical Industries (Osaka). The other reagents were obtained mostly from Sigma (St. Louis, MO) and Wako.

Animals—Male Wistar rats (Japan SLC, Shizuoka), 5 weeks old (for Experiments 1, 2, and 3) or 6 weeks old (for Experiment 4) were used. The rats were fed laboratory synthetic diets, as described below. The rats were kept under an automatic lighting schedule from 0800 to 2000 h at 24°C. The animals were allowed water *ad libitum* and were given diet or treatment as described in "*Experimental Design.*"

After the experimental period, they were killed by decapitation while under diethyl ether anesthesia. An aliquot of each liver or epididymal adipose tissue was quickly removed and homogenized with 3 volumes of 0.25 mol/liter sucrose. The 10,000×*g* supernatant of the homogenate was centrifuged at 105,000×*g* for 45 min (Model L5, Type 40 rotor, Beckman Instruments, Palo Alto, CA). The 105,000×*g* supernatant was used for measurement of lipogenic enzyme activities. Another aliquot of the liver or adipose tissue was immediately frozen in liquid nitrogen and stored at −80°C to measure the mRNA concentration by dot blot or Northern blot

hybridization analysis.

Experimental Design—Experiment 1: To measure the time courses of mRNA concentrations and enzyme activities of lipogenic enzymes, the rats were deprived of diet for 2 days and then refed a carbohydrate/protein diet for 8, 16, 24, 48, or 72 h. The carbohydrate/protein (fat-free) diet contained 67.9% sucrose, 18% casein, 9.5% cellulose, 0.1% choline chloride, 4% salt mixture, and vitamins (*13*).

Experiment 2: To measure the effects of dietary nutrients on mRNA concentrations and enzyme activities of lipogenic enzymes, the food-deprived rats were refed for 24 and 72 h, respectively, with a carbohydrate/protein diet (CP), an 85% carbohydrate diet without protein (C), an 85% protein diet without carbohydrate (P), or a 57% carbohydrate/18% protein/10% fat diet (CPF). Ten percent sucrose was substituted by hydrogenated fat or corn oil in the CPF diet. The animals were given equal energy-containing diet for body wt (10 g/100 g as rats fed the CP diet) among the dietary groups. Only the rats which consumed the equal-energy containing diets were used for the experiment. As the diet intake was limited to the amount consumed by the carbohydrate (without protein) diet group, the diet quantities consumed were 10-15% less than usual.

Experiment 3: Male Wistar rats, 6 weeks old, were made diabetic by intravenous injection of streptozotocin (6 mg/100 g) after starvation for 20 h. Blood glucose was assayed 3 days after streptozotocin treatment and rats with blood glucose concentrations of over 16.7 mmol/liter were used for experiments. The diabetic rats consumed a glucose diet *ad libitum*. The composition of the glucose diet was the same as that of CP diet, except that sucrose was replaced with glucose. For insulin-treatment, diabetic rats fed on the glucose diet were injected with 3.43 nmol of Actrapid insulin intraperitoneally and 5.15 nmol of Lente insulin subcutaneously. For continuous insulin treatment (to measure the enzyme activities), the diabetic rats were subcutaneously injected with 8.58 nmol of Lente insulin/day (3.43 nmol in the morning and 5.15 nmol in the evening). The animals were subcutaneously injected with Lente and Actrapid insulin (obtained from Novo Industry A/S, Denmark, and Shimizu Pharmaceutical, Shizuoka, respectively).

Dot Blot Hybridization Assay—The cDNA species were cloned as described in our previous reports (*8-12*). The genomic clone of rat rRNA was obtained from the Japanese Cancer Research Resources Bank. A 1 kb *Bam*HI/*Eco*RI fragment of this clone was isolated and used as a probe for 18S rRNA. Total RNA was isolated from the tissues by acid guanidium thiocyanate-phenol-chloroform extraction (*14*). The cDNAs were labeled by use of a multiprimer DNA labeling system kit (Amersham) with [α-^{32}P]dCTP. To measure the mRNA concentrations of lipogenic enzymes, the total RNA (5 μg) was denatured with formamide at 65°C for 15 min, spotted on a nylon filter, and then irradiated with UV for 5 min. The filter was prehybridized and then hybridized as described previously (*10*). Relative densities of the hybridization signals were determined by scanning the autoradiograms at 525 nm and normalized to the values of 18S rRNA. The validity of the determination was established previously (*15*).

Northern Blot Hybridization Analysis—Northern blot analysis of RNA was performed as described by Gonzales and Kasper (*16*). Total RNA was denatured and electrophoresed on a 0.8% agarose gel containing 2.2 mol/liter formaldehyde. The gel was blotted onto a nylon filter according to Thomas (*17*). Prehybridization, hybridization, and autoradiography were carried out by using the dot-blot hybridization method. Details were as described previously (*10*).

The mRNA concentrations were measured by the dot blot hybridization method, and a half of the results was confirmed by Northern blot analysis. The autoradiograms of the Northern blot analysis are shown in Fig. 1. The two mRNAs of different size for each of fatty acid synthase and malic enzyme both code for the respective active enzymes (*18, 19*). The two bands were coordinately altered by fasting/refeeding (Fig. 2). The relative densities of hybridization signals showed parallel changes in Northern blot and dot blot analyses, as shown in Fig. 2.

Analyses—Acetyl-CoA carboxylase [EC 6.4.1.2] activity was assayed by the $H^{14}CO_3^-$-fixation method (*20*). To attain full activity, the enzyme was first preincubated with 10 mmol/liter citrate (*20*). Fatty acid synthase [EC 2.3.1.85] activity was assayed according to Hsu *et al.* (*21*). Malic enzyme [EC 1.1.1.40] was assayed according to Ochoa (*22*) and glucose-6-phosphate dehydrogenase [EC 1.1.1.49], according to Glock and McLean (*23*). ATP-citrate lyase [EC 4.1.3.8] activity was assayed as described by Takeda *et al.* (*24*). The enzyme activities in the supernatant of the tissue homogenates are shown as mU/mg protein, when 1 mU is the amount catalyzing the formation of 1 nmol of product/min at 37°C for acetyl-CoA carboxylase, fatty acid

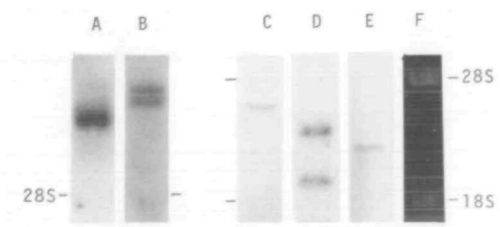

Fig. 1. **Northern blot analysis of RNA in adipose tissue.** A, B, C, D, and E show RNA bands of acetyl-CoA carboxylase, fatty acid synthase, ATP citrate-lyase, malic enzyme, and glucose-6-phosphate dehydrogenase, respectively. F shows 28S and 18S ribosomal RNA bands visualized by staining with ethidium bromide. Twenty micrograms of total RNA in adipose tissue of rats refed a carbohydrate/protein diet was applied to each lane.

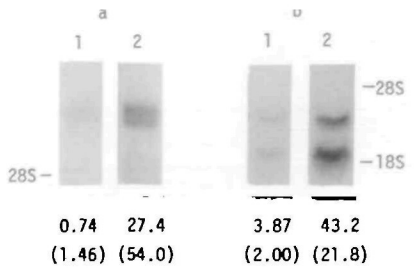

Fig. 2. **Effects of fasting/refeeding on Northern blot analyses of fatty acid synthase and malic enzyme mRNAs in adipose tissue.** Lanes a1 and a2 show mRNA bands of fatty acid synthase for fasted and fasted/refed rats, respectively, and lane b1 and b2, the bands of malic enzyme for the rats, respectively. Arbitrary units (sum of the two bands of each mRNA) of the hybridization signals (normalized to the value of 18S rRNA) measured by Northern blotting are shown below lanes and those measured by dot blot, in parentheses.

synthase, and ATP-citrate lyase, and the utilization of 1 nmol of NADP/min for malic enzyme and glucose-6-phosphate dehydrogenase. Protein was determined by the method of Lowry *et al.* (*25*). RNA content was measured by the Munro-Fleck method (*26*).

Statistical Analysis—One-way or two-way ANOVA was followed by inspection of all differences between pairs of means by using the least significant difference test (*27*). Differences were considered significant at $p<0.05$.

RESULTS

Changes in mRNA and Enzyme Induction after Feeding a Carbohydrate/Protein Diet (Experiment 1)—Time courses for mRNA concentrations and activities of lipogenic enzymes after refeeding a carbohydrate/protein diet (CP) to food-deprived rats are shown in Fig. 3. Although the time courses in liver were given in our previous reports (*5, 8*), those in liver and adipose tissue of the same animals were also compared here. The mRNA concentrations in adipose

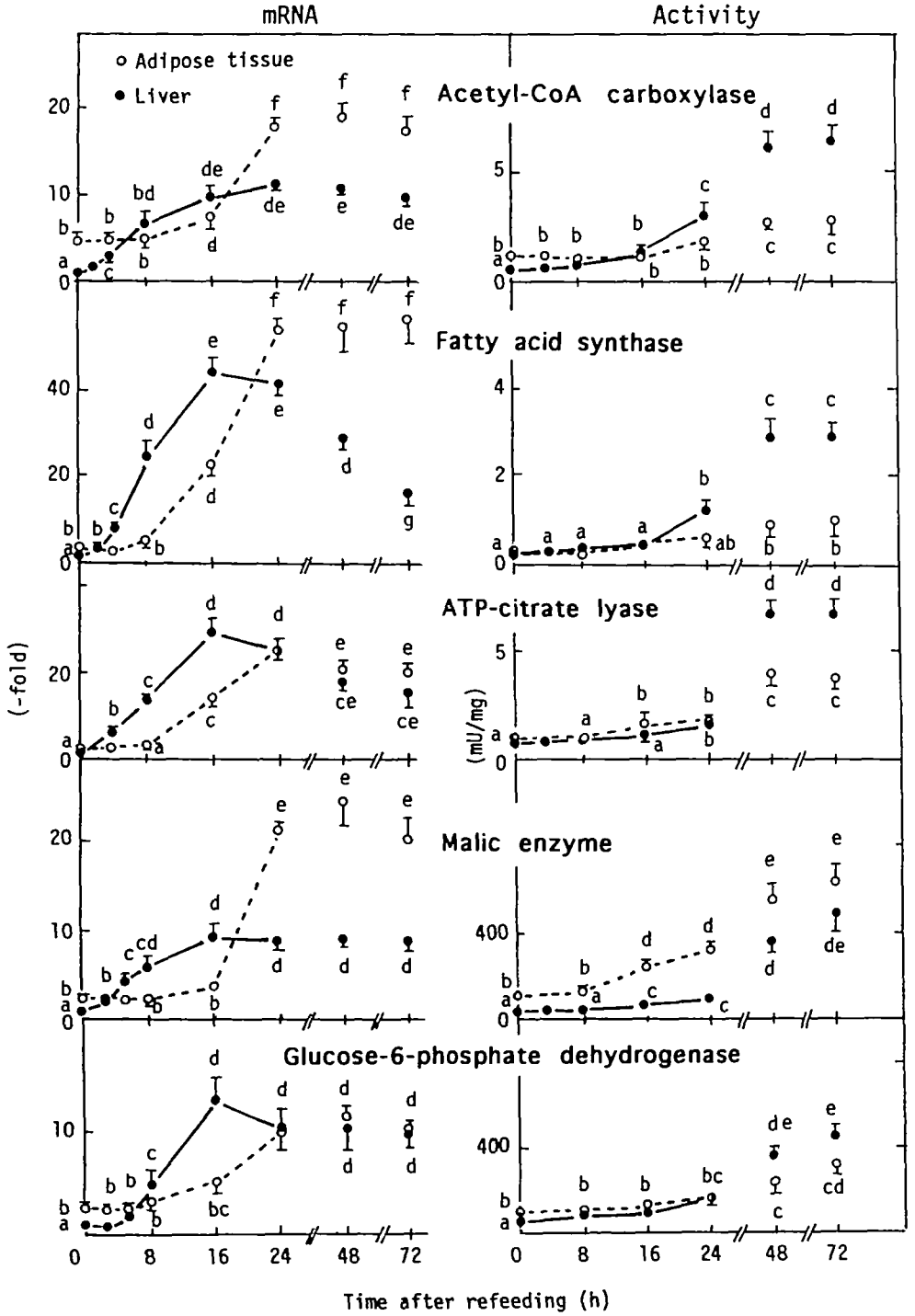

Fig. 3. **Changes in mRNA concentrations and activities of lipogenic enzymes in adipose tissue and liver after feeding the carbohydrate/protein diet to food-deprived rats.** After refeeding the carbohydrate/protein diet to food-deprived rats, time courses of mRNA concentrations and enzyme activities of lipogenic enzymes in adipose tissues and livers were followed. The animals were killed at the times indicated after refeeding. Mean ± SD ($n=5$-6). The mean values with different superscripts are significantly different ($p<0.05$) among times and tissues (two-way ANOVA).

tissue were significantly increased 16 h after refeeding, but reached maximum levels at 24 h, while those in liver reached maximum at 16 h. The lag time for mRNA induction after refeeding was longer in adipose tissue than in liver. The maximum mRNA concentrations of acetyl-CoA carboxylase, fatty acid synthase, and malic enzyme were significantly higher in adipose tissue than in liver, and those of other enzymes were similar between adipose tissue and liver. The enzyme activities reached maximum levels 48-72 h after refeeding in both adipose tissue and liver. However, the activities of lipogenic enzymes (excepting malic enzyme) were markedly lower in adipose tissue than in liver. Only malic enzyme activities were higher in adipose tissue. Changes in the enzyme activities of lipogenic enzymes were accompanied by proportional changes in the quantities of immunochemically reactive proteins during the dietary and insulin manipulations.

Effects of Dietary Nutrients on mRNA and Enzyme Induction (Experiment 2)—The mRNA concentrations and activities of lipogenic enzymes in adipose tissue were measured at maximum levels, at 24 and 72 h, respectively, after refeeding either a carbohydrate/protein (CP), carbohydrate (C), protein (P), or carbohydrate/protein/fat (CPF) diet. The results are shown in Fig. 4. In rats fed the carbohydrate diet without protein (C), the mRNA concentrations of acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase, and malic enzyme were elevated to the levels seen in the animals on the carbohydrate/protein diet (CP). Even the enzyme induction of acetyl-CoA carboxylase, ATP-citrate lyase, and malic enzyme reached comparable levels to those in the carbohydrate/protein diet group. The enzyme induction of fatty acid synthase and glucose-6-phosphate dehydrogenase was not increased so greatly by carbohydrate only (C diet) as by carbohydrate plus protein (CP diet). The mRNA and enzyme inductions seen with the protein diet without carbohydrate (P) were about 50% of those in the carbohydrate/protein diet group.

In rats fed a 10% corn oil diet (CPF), the mRNA inductions in the adipose tissue were reduced to 50-60% of those of the carbohydrate/protein diet group, but they were reduced to 20-40% in the liver (*8-12*). In the liver, the mRNA and enzyme inductions of lipogenic enzymes were significantly reduced by dietary polyunsaturated fat relative to hydrogenated fat. In the adipose tissue, however, the mRNA and enzyme inductions of fatty acid synthase and malic enzyme were significantly reduced by corn oil relative to hydrogenated fat, while those of other lipogenic enzymes were not significantly reduced.

Effects of Insulin on mRNA and Enzyme Induction (Experiment 3)—The mRNA concentrations and enzyme activities of lipogenic enzymes in liver and adipose tissue of diabetic rats were low (at 0 time in Fig. 5) relative to normal (at 72 h in Fig. 3), but increased to normal after the insulin treatment (Fig. 5). Relative densities of the hybridization signals were compared for mRNA concentrations of diabetic and normal rats.

The time courses of mRNA concentrations and enzyme activities after insulin treatment of the diabetic rats are

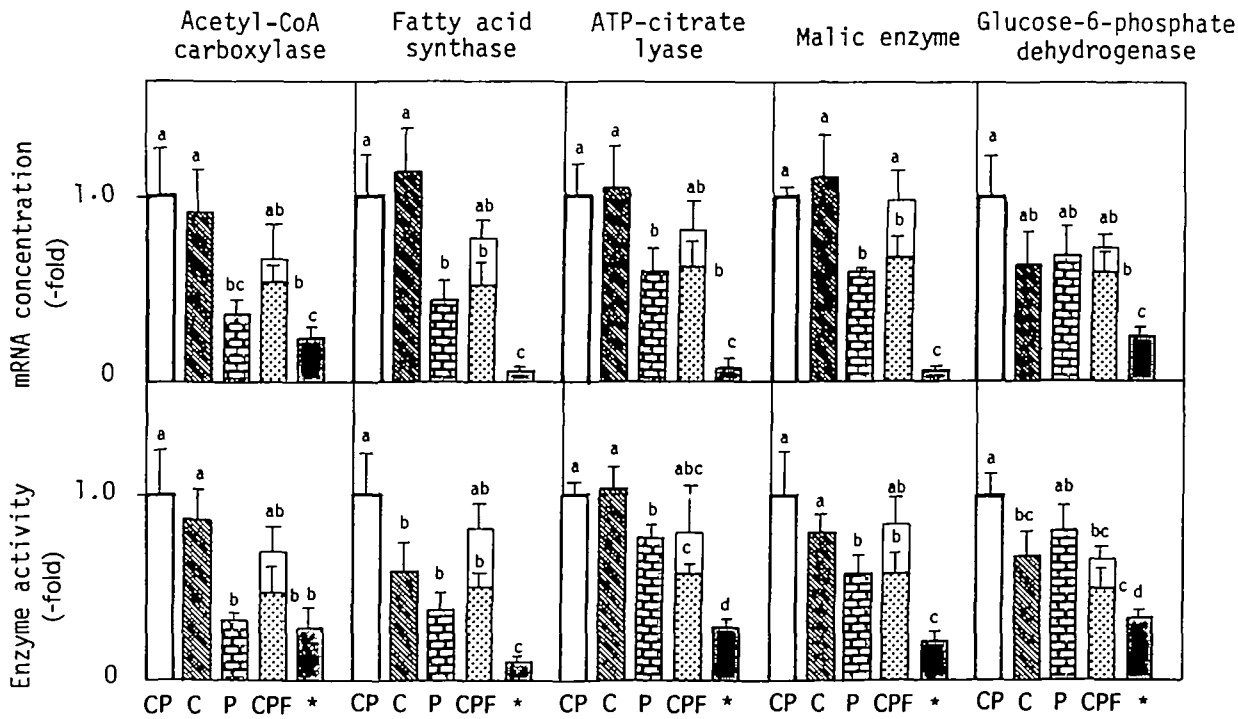

Fig. 4. **Effects of dietary nutrients on mRNA concentrations and activities of lipogenic enzymes in rat adipose tissue.** Food-deprived rats (* each right end column) consumed diets containing the same amount of calories of either a carbohydrate/protein (CP), a carbohydrate (C), a protein (P), or a carbohydrate/protein/corn oil or hydrogenated fat (CPF) diet and then were killed after 3 days. The upper and lower lines show the results for the hydrogenated fat and corn oil groups, respectively, in CPF columns. The results are normalized to the values for the CP group. The enzyme activities of acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase, malic enzyme, and glucose-6-phosphate dehydrogenase in the CP group were 1.33±0.25, 0.80±0.18, 4.17±0.76, 516±78.1, and 189±37.0 mU/mg protein, respectively. Mean±SD (n=6-9). The mean values with different superscripts are significantly different (at $p<0.05$) among diets.

shown in Fig. 5. After insulin treatment, the mRNA concentrations and enzyme activities in adipose tissue reached maximum at 16-24 and 48-72 h, respectively (similarly in liver). The lag time for mRNA inductions, seen after refeeding the carbohydrate/protein diet to food-deprived (normal) rats (Fig. 3), was not seen after the insulin treatment of diabetic rats. Although the lipogenic enzyme mRNA inductions after insulin treatment were generally higher in adipose tissue than in liver, the enzyme inductions (except malic enzyme) were markedly low in adipose tissue. Therefore, the enzyme inductions were markedly low in comparison with the mRNA inductions in adipose tissue.

On the other hand, in the diabetic state, the mRNA concentrations of lipogenic enzymes were generally higher in adipose tissue than in liver. In particular, glucose-6-phosphate dehydrogenase mRNA concentration was about 4-fold higher in the adipose tissue. Therefore, the insulin-dependent increase (fold) in the mRNA concentration of glucose-6-phosphate dehydrogenase was not so great in adipose tissue as in liver.

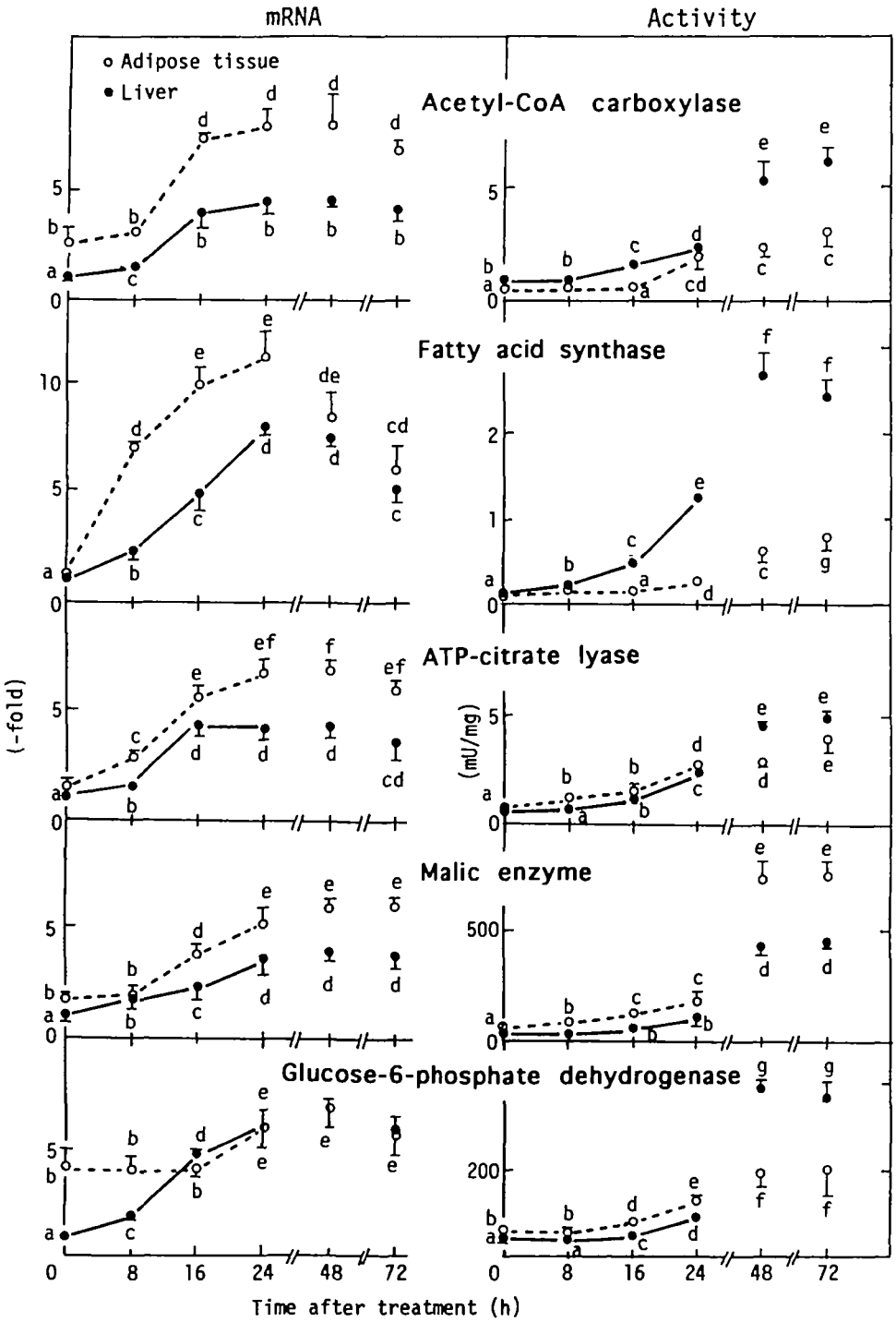

Fig. 5. **Changes in mRNA concentrations and activities of lipogenic enzymes of adipose tissue and liver after insulin treatment of diabetic rats.** The time courses of mRNA concentrations and activities of lipogenic enzymes in adipose tissues and in liver were followed. Mean±SD (n=6-9). The mean values with different superscripts are significantly different ($p<0.05$) among times and tissues (two-way ANOVA).

TABLE I. **Changes in contents of total RNA and lipogenic enzyme mRNAs in whole liver and epididymal adipose tissue.**

Conditions	Total RNA (mg/whole tissue)	mRNA contents (fold)		
		Acetyl-CoA carboxylase	Fatty acid synthase	Malic enzyme
Liver				
Fasted	50.9±7.60[a]	1.00±0.25[a]	1.00±0.11[a]	1.00±0.21[a]
Refed	71.4±8.32[b]	16.3±1.38[b]	58.2±6.51[b]	12.9±1.70[c]
Diabetic	55.6±3.20[a]	1.72±0.17[c]	1.30±0.15[c]	1.45±0.23[c]
Diabetic +ins*	92.5±7.42[c]	8.39±1.10[d]	20.3±2.39[d]	8.90±1.63[d]
Adipose tissue				
Fasted	1.43±0.33[a]	0.13±0.03[a]	0.04±0.006[a]	0.06±0.01[a]
Refed	2.00±0.28[b]	0.71±0.12[b]	2.12±0.22[b]	0.85±0.18[b]
Diabetic	1.06±0.16[c]	0.08±0.02[c]	0.03±0.004[c]	0.04±0.01[c]
Diabetic +ins	1.35±0.27[ac]	0.33±0.03[d]	0.41±0.04[d]	0.18±0.03[d]

* + ins: The diabetic rats were treated with insulin as described in "MATERIALS AND METHODS" and then killed 24 h after the first treatment. The mRNA contents were normalized to the value for liver of fasted rats. Mean±SD (n=5-6). The mean values of total RNA and all the mRNA contents of whole liver are significantly higher ($p<0.001$) than the corresponding values of whole adipose tissue. The mean values of total RNA and mRNA contents with different superscripts are significantly different ($p<0.05$) among the conditions in each tissue.

Changes in mRNA Contents of Lipogenic Enzymes in the Whole Liver and Adipose Tissue—The total RNA contents in liver and adipose tissue of fasted, fasted/refed, diabetic, and diabetic/insulin-treated rats (at 0 and 24 h in Figs. 3 and 5) were measured. The mRNA contents of lipogenic enzymes in the whole tissues were calculated from the mRNA concentrations per total RNA (shown in Figs. 3 and 5) and the total RNA contents. Some of them are shown in Table I. The mRNA contents in the liver were very much higher than those in the adipose tissue under any conditions. The mRNA contents in the whole liver were significantly higher in diabetic rats than in normal/fasted rats, whereas those in the whole adipose tissue showed an opposite relation. The mRNA contents of the tissues after insulin treatment of diabetic rats did not reach those after refeeding the carbohydrate/protein diet to the fasted rats.

DISCUSSION

In the present study, we have examined the differences in regulation of gene expression of lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase, ATP citrate-lyase, malic enzyme, and glucose-6-phosphate dehydrogenase) between adipose tissue and liver. Regulation of lipogenic enzyme mRNA concentrations (relative to RNA) and enzyme induction (relative to cytosol protein) in the adipose tissue during nutritional and insulin manipulations is discussed below, in comparison with that in liver. After refeeding a carbohydrate/protein diet to food-deprived rats, the mRNA concentrations of lipogenic enzymes reached maximum levels at 16 h in liver, but reached these levels more slowly (at 24 h) in adipose tissue. The late induction of mRNAs in adipose tissue can be ascribed to a lag time for the induction. As the lag time for the mRNA inductions was longer in adipose tissue than in liver, the response of gene expression to carbohydrate appeared to be slow. Moreover, although the magnitudes of mRNA concentrations were similar or significantly higher in adipose tissue in comparison with liver, the enzyme inductions (excepting malic enzyme) were significantly lower in adipose tissue. It is suggested that the lipogenic enzyme mRNAs were not so efficiently translated to proteins in adipose tissue as in liver. The protein/RNA ratios in liver and adipose tissue of rats fed the 67% carbohydrate/18% protein diet (in Experiment 2) were 25.5±2.14 and 9.04±1.53 (n=3), respectively, and those in rats fed the 85% protein diet were 32.4±2.00 and 16.2±2.41, respectively. The protein synthesis was higher in the liver than in the adipose tissue, and was increased by the high protein diet in both tissues, particularly in adipose tissue. This supports the idea that the efficiency of the mRNA translation to enzyme proteins is lower in adipose tissue than in liver.

In adipose tissue of rats fed the carbohydrate diet without protein (C diet), the mRNAs of acetyl-CoA carboxylase, ATP-citrate lyase, malic enzyme, and fatty acid synthase in adipose tissue were induced and the enzymes (except fatty acid synthase) were also induced. In the liver, however, the mRNAs of the enzymes except fatty acid synthase were induced, but only the enzymes of acetyl-CoA carboxylase and ATP citrate-lyase were induced (*8-12*). Generally, the gene expression of lipogenic enzymes appeared to be more dependent on carbohydrate feeding in the adipose tissue than in the liver. Similarly, in cultured adipose tissue of rat, glucose alone, *via* an increase in intracellular glucose-6-phosphate level, was capable of increasing fatty acid synthase mRNA levels (*28*), although in the presence of insulin and glucose the mRNA levels of fatty acid synthase were increased in hepatocytes (*29, 30*).

As regards the reduction of gene expression, however, lipogenic enzyme mRNA concentrations were not so markedly reduced by starvation, by polyunsaturated fatty acids or by diabetes in adipose tissue as in liver. As the fatty acids of adipose tissue mostly exist as esters of triacylglycerols, whose major fatty acids are saturated or monounsaturated, the proportions of polyunsaturated fatty acids are low. Consequently, the polyunsaturated fatty acid suppression of lipogenic enzyme gene expression should be weak in adipose tissue. Thus, the differences of lipogenic enzyme gene expression between adipose tissue and liver can be ascribed to tissue specificity. The tissue specificity may be ascribed to the low proportions of polyunsaturated fatty acids and to the low efficiency of translation to the enzyme proteins (due to the low concentration of protein) in the adipose tissue.

On the other hand, total RNA content was very much higher in whole liver than in whole adipose tissue. Therefore, changes in the lipogenic enzyme gene expression should be much greater in liver than in adipose tissue during any nutritional manipulation.

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