SOME LIPOGENIC ENZYME ACTIVITIES IN RAT LIVERS IN WHICH AN EXCESSIVE FAT ACCUMULATION OCCURRED DUE TO FEEDING LOW-LEVEL AMINO ACID MIXTURE DIETS

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(Received February 26, 1979)

Summary In liver homogenates of rats fed a low-level diet of Wheat-, Rice- or Miyazaki-pattern amino acid mixture, some enzymes such as glucose-6-phosphate dehydrogenase, ATP citrate lyase, fatty acid-synthesizing enzymes, malic enzyme and L-α-glycerophosphate dehydrogenase, whose activities are indicators of lipogenesis have been determined from the viewpoint of the mechanisms producing fatty liver. In the early experimental period, malic enzyme activity increased more markedly in rats fed low amino acid mixture diets than in the control group, and L-α-glycerophosphate dehydrogenase activity in the liver increased slightly. Conversely, ATP citrate lyase and fatty acid-synthesizing enzyme activities remained almost at control levels, or glucose-6-phosphate dehydrogenase activity tended to decrease. These results suggest that some other associated factors, such as depression of the lipid transfer system in the liver rather than accelerated lipogenesis itself, may be the main cause of the fatty livers produced under these nutritional conditions.

Keywords low amino acid mixture diets, lipogenic enzyme, fatty liver

In a preceding paper, rats given diets consisting of a low level of Wheat-, Rice- or Miyazaki-pattern amino acid mixture were biochemically and histochemically

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1 Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; CoA, Coenzyme A; acetyl CoA, acetyl Coenzyme A; G-6-P, glucose-6-phosphate; malonyl CoA, malonyl Coenzyme A; NAD+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide reduced form; NADP+, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; NEFA, non-esterified fatty acid; PEP, phosphoenol pyruvate; DHAP, dihydroxyacetone phosphate.
observed to have amount extensive fat accumulation in their livers (1). According to the intensity of fat accumulation in the liver, the order was Wheat-pattern diet group > Rice-pattern diet group > Miyazaki-pattern diet group ≥ control diet group. The lipids accumulated in the liver were mainly triacylglycerols consisting of long-chain fatty acids. On the other hand, in rats with fatty livers, serum triacylglycerol and NEFA decreased considerably compared with the controls. Free fatty acids, which may be a major factor in the pathogenesis of fatty liver, produced on these diets would not be excessively supplied to the liver.

Yoshida et al. (2) stated that in the process of producing fatty liver by feeding an amino acid-imbalanced diet, 14C-acetate was markedly incorporated into the fatty acids in the liver and from these data lipogenesis seemed to be accelerated. Furthermore, Stead et al. (3) reported that the lipid in fatty livers produced by protein depletion accumulated primarily as a consequence of elevated hepatic triacylglycerol synthesis. Whereas, in our preceding study, it was found that the generating capacity of NADPH by the action of glucose-6-phosphate dehydrogenase remained unchanged at control levels or somewhat decreased (4).

Each category of the several sources which Lieber (5) has described was investigated under nutritional conditions under which rats were given low amino acid mixture diets which produced fatty livers. In the present study, therefore, some enzyme activities in the liver homogenates as indicators of lipogenesis were determined from the viewpoint of the mechanisms producing fatty liver by feeding low-level amino acid mixture diets. Glucose-6-phosphate dehydrogenase was also re-investigated to observe changes in its activities.

MATERIALS AND METHODS

Materials. Adenosine diphosphate (ADP), nicotinamide adenine dinucleotide (NAD\(^+\)), reduced nicotinamide adenine dinucleotide (NADH) sodium salt, nicotinamide adenine dinucleotide phosphate (NADP\(^+\)), reduced nicotinamide adenine dinucleotide phosphate (NADPH), coenzyme A, malonyl coenzyme A, acetyl coenzyme A (acetyl Co A) and phosphoenol pyruvate (PEP) trisodium salt were purchased from the Sigma Chemical Co. Dihydroxyacetone phosphate dimethylketal crystalline dicyclohexyl ammonium salt and malate dehydrogenase [EC 1.1.1.37] were provided by the Boehringer Mannheim GmbH.

\(^2\) Katayama, Y. (1975): Lipids metabolism of the rats fed the diets consisting of Wheat- or Rice-pattern amino acid mixtures. Abstract of Proceedings of the 53rd General Meeting of the Physiological Society of Japan. *J. Physiol. Soc. of Japan*, 37, 200.

\(^3\) Sigma Chemical Co., Ltd. (3500 De Kalb Str., St. Louis, Mo., 63178, U.S.A.): NAD (Grade III) (Lot. No. 60C-7600), NADH (Lot. No. 100C-6520), NADP (Lot. No. 21C-7070), NADPH (Lot. No. 73C-7030) (type I), acetyl Co A (Lot. No. 83C-2800), PEP (Lot. No. 62C-5080, 39B-5440), Co A (Grade II-A) (Lot. No. 25B-5241-9, 28B-8241-9), malonyl Co A (Lot. No. 83C-20019), ADP (Grade I) (Lot. No. 111C-7230), G-6-P (Lot No. 120C-6030).

\(^4\) C.F. Boehringer and Soehne GmbH (Mannheim, W. Germany): DHAP (Lot. No. 740332), Malate dehydrogenase [EC 1.1.1.37] (Lot. No. 7473531).
Adenosine triphosphate (ATP) disodium salt and 2-mercaptoethanol were purchased from the Kohjin Co., Ltd.\textsuperscript{5} and the Nakarai Chemical Co.,\textsuperscript{6} respectively. All the other chemicals used in this experiment were guaranteed reagents produced by the Wako Pure Chemical Co.\textsuperscript{7} and doubly-distilled water was used in the enzyme reactions of this study.

\textit{Animals and diets.} Four-week-old male rats of the Sprague-Dawley J. C. L. strain (Nikon Clea, Inc.\textsuperscript{8}) were individually housed in wire cages in a room which was automatically light-controlled to provide a 12-hr darkness and light cycle, at 22 ± 1°C, at 60\% of relative humidity. The rats, which were given standard rat pellets (CE-2, produced by Nikon Clea, Inc.\textsuperscript{8}) for 7 to 13 days before the experiment, were divided into four groups of five to ten rats with equal body weights (140–150 g). They were maintained on a 20\% casein diet as a control and on the three kinds of diets (5\% level of amino acid mixture) consisting of the Wheat-, Rice- or Miyazaki-pattern amino acid mixture for 1, 2, 3, and 4 weeks. The three groups given these experimental diets were: 1) Wheat-pattern diet group, 2) Rice-pattern diet group and 3) Miyazaki-pattern diet group, respectively\textsuperscript{(1)}.\textsuperscript{2} The details of these diets were described in our previous reports\textsuperscript{(1, 4)}.\textsuperscript{2} Food and tap water were given \textit{ad libitum}. The daily food intake and body weight for each animal were usually measured between 9:00 and 10:00 A.M. Rats were starved for 13 to 15 hr prior to sacrifice. Under light anesthesia with sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate (Ravonal\textsuperscript{9}), the livers of all experimental rats were rapidly excised.

\textit{Procedures for enzyme preparations.} The liver tissues removed were quickly washed with ice-cooled physiological saline solution and a washing medium was whipped up from the livers. After weighing, small pieces (2.0 g) from each liver tissue sample were homogenized in a Potter-Elvehjem glass homogenizer together with 8.0 ml of 0.25 M sucrose solution containing 0.02 M Tris-HCl buffer (pH 7.4), 0.1 mM MgCl\textsubscript{2} and 0.01 M 2-mercaptoethanol for the preparations of ATP citrate lyase, fatty acid-synthesizing enzymes and L-\alpha-glycerophosphate dehydrogenase or with the same volumes of 0.2 M KCl-buffer solution (pH 7.4) in the cases of glucose-6-phosphate dehydrogenase and malic enzyme preparations. These liver homogenates were centrifuged at 4,000 rpm for 20 min and the supernatants were ultracentrifuged at 105,000 × g for 30 min in a Marusan Superior 50S (refrigerated Ultracentrifuge produced by Sakuma Seisakusho, Ltd.\textsuperscript{10}). All procedures for enzyme preparations of livers were carried out in a cold room at 0 to 3°C.

\textit{Biochemical assay.} The contents of enzyme protein were determined by the method of LOWRY \textit{et al.} (6).
Assay of enzyme activities. In order to perform spectrophotometric determinations of these enzyme activities, two quartz microcuvettes with a light path of 1.0 cm were used in this study and the assay of enzyme reactions was carried out at 20 ± 1°C. A settled volume of each enzyme solution was usually used, and if necessary, the enzyme solution was diluted with each buffer to obtain a linear relationship between optical densities of co-factors at 340 nm and the time course. The reaction progress was estimated against a reference cuvette containing all the components without the co-factors or the enzymes, and the optical density changes in its progress were automatically measured in the scanning time course of 7.5 min at 340 nm with a Hitachi Recording Spectrophotometer (Model 323, Hitachi, Ltd., Tokyo, Japan).

For glucose-6-phosphate dehydrogenase and malic enzyme, the rate of NADPH formation is a measure of each enzyme's activity and measuring can be performed by taking the average of the increasing optical density changes per minute at 340 nm. On the other hand, for ATP citrate lyase and L-α-glycerophosphate dehydrogenase or fatty acid-synthesizing enzymes, the rate of NADH or NADPH oxidation is a measure of each enzyme's activity. From data taken by the following procedure, the activity of each enzyme was calculated according to the general formula indicated by BERGMeyer (7, 8) and represented as μmol of NADPH formed/min/mg of protein for D-glucose-6-phosphate dehydrogenase and malic enzyme or μmol of oxidized NADH/min/mg of protein for ATP citrate lyase and L-α-glycerophosphate dehydrogenase and μmol of oxidized NADPH/min/mg of protein for the fatty acid-synthesizing enzymes.

1) D-Glucose-6-phosphate NADP⁺: oxidoreductase[EC 1.1.1.49] (G-6-PDH) The activities of G-6-PDH were assayed spectrophotometrically by the method of LOHR and WALLER (9). The reaction medium consisted of 40 μmol of triethanolamine buffer (pH 7.5) containing 0.005 M ethylenediamine-tetraacetate, 6 μmol of MgCl₂, 260 μmol of NADP⁺, 0.5 μmol of G-6-P, enzyme solution (0.1 ml) and doubly-distilled water to a final volume of 1.0 ml. The reaction was started by the addition of the supernatant of the liver homogenate.

2) L-Malate NADP⁺: oxidoreductase [EC 1.1.1.40] (malic enzyme) Malic enzyme assay was performed according to the modified method described by OCHOA (10). The reaction medium consisted of 300 μmol of tris-(hydroxymethyl)aminomethane (pH 7.4), 2.5 μmol of MnCl₂, 0.135 μmol of NADP⁺, 1.5 μmol of sodium L-malate, enzyme solution (0.20 ml) and doubly-distilled water in a final volume of 3.0 ml. The reaction was initiated by the addition of NADP⁺ and its progress was automatically measured against a blank cell containing all the components except for NADP⁺.

3) ATP : citrate oxaloacetate-lyase (CoA-acetylating and ATP-dephosphorylating)[EC 4.1.3.8] (ATP citrate lyase). This enzyme assay was performed by the modified method of TAKEDA et al. (11-14) which was initially employed by SRERE (15-18). In order to follow the

11 Hitachi, Ltd. (2, Nishikubo-Sakuragawacho, Minato-ku, Tokyo 105, Japan).
reaction of ATP citrate lyase, its reaction was coupled with a malate dehydrogenase reaction in which oxaloacetate formed by the ATP citrate lyase was converted to malate by the addition of NADH in the presence of malate dehydrogenase. Thus, the activity of the ATP citrate lyase was measured from oxidation of NADH in the following reaction mixture. The reaction mixture contained 200 µmol of tris-(hydroxymethyl)aminomethane-HCl buffer (pH 8.4), 10 µmol of MgCl₂, 1.3 µmol of Coenzyme A, 10 µmol of 2-mercaptoethanol, 20 µmol of potassium citrate, 20 µg (24 units) of malate dehydrogenase, 0.2 µmol of NADH, 5 µmol of ATP, the enzyme solution (0.1 ml) and doubly-distilled water to make a final volume of 1.0 ml. The reaction was started by the addition of ATP and automatically followed by a decrease in optical density against a reference cell containing all the components without NADH.

4) Fatty acid-synthesizing enzymes [EC 2.3.1.38 etc.]

The activities of the enzymes were determined by the method of Martin et al. (19-21). The reaction medium contained 50 µmol of potassium phosphate buffer (pH 7.0), 3 µmol of 2-mercaptoethanol, 0.2 µmol of NADPH, 0.05 µmol of malonyl-coenzyme A, 0.05 µmol of acetyl-coenzyme A, enzyme and doubly-distilled water in a final volume of 1.0 ml. The reaction was initiated by the addition of 200 µl of diluted enzyme solution.

5) Glycerol-1-phosphate dehydrogenase [EC 1.1.1.8] (l-α-glycerophosphate DH)

The activities of l-α-glycerophosphate DH were measured in the reaction system described by Bücher et al. (22-24). The reaction medium in a final volume of 1.0 ml contained 47.5 µmol of triethanolamine buffer (pH 7.6), 0.35 µmol of dihydroxyacetone phosphate, 0.2 µmol of NADH, 10 µl of enzyme solution and doubly-distilled water. The enzyme reaction was started by the addition of the enzyme solution and its progress by changes in optical density were determined against a blank cell containing all the components without NADH.

Statistics

In the tables, each value represents the mean ± half-range of the confidence interval (confidence limit) at p < 0.05. Statistical analysis was performed by the paired t test for the differences between means.

RESULTS

Body weights

Growth results in control rats and in the three experimental groups corresponded with our previous data (1, 4).² Thus, the control rats fed 20% casein diet had an almost linear increase in body weight gain at a rate of about 6 g per day. The body weight of rats in the Miyazaki-pattern diet group did not change from the initial level during the first 3 days and then slightly increased at a rate of about 4 g per day, showing a gain of 86 g for 27 days. On the other hand, in the Rice-pattern diet group, body weight decreased slightly during the first 5 to 7 days. Thereafter, it increased at a rate of 2 g per day with a final body weight gain of about 40 g. For the
Wheat-pattern diet group, however, body weight decreased to about 5 to 10 g below the initial level. This lower level continued for 17 days and finally recovered to the initial level after 27 days. There were statistically significant differences between the control group and the experimental diet groups at the 95% level.

**Food intake**

In the Rice-pattern diet group, cumulative food intake per rat did not differ from that of the control group. In the Wheat-pattern diet group, food intake per rat tended to decrease slightly, while in the Miyazaki-pattern diet group it tended to increase slightly over that of the control group. There was, however, no significance in the difference in food intake among the 4 groups. Consequently, no obvious difference in rats' food intake among these diet groups and the control group was observed.

**Liver wet weights (Table 1)**

In the Wheat- and Rice-pattern diet groups, wet weights of the livers significantly decreased compared with the control group during all experimental periods \((p < 0.05)\). On the other hand, those of the Miyazaki-pattern diet group had a tendency to decrease slightly but these decreases were not significant as compared

| Weeks | 1       | 2       | 3       | 4       |
|-------|---------|---------|---------|---------|
| Wheat-pattern diet group | 5.3 ± 0.3* | 4.6 ± 1.0* | 4.8 ± 1.4* | 4.6 ± 0.5***        |
| Rice-pattern diet group  | 5.6 ± 0.2* | 4.7 ± 0.9* | 4.9 ± 0.7* | 5.2 ± 0.4*         |
| Miyazaki-pattern diet group | 6.0 ± 1.1 | 6.6 ± 1.8 | 5.7 ± 1.5 | 6.0 ± 1.0*        |
| Control diet group        | 7.4 ± 2.2 | 7.2 ± 2.5 | 7.5 ± 2.8 | 8.8 ± 1.5         |

Each value represents the mean ± half range of the confidence interval (confidence limit) at 95% level.

* Indicates statistically significant difference from control at \(p < 0.05\).

**Table 2.** d-Glucose-6-phosphate NADP⁺: oxidoreductase (G-6-PDH) activities.

Control group, 54 ± 12 µmol/min/mg protein

| Weeks | 1         | 2         | 3         | 4         |
|-------|-----------|-----------|-----------|-----------|
| Wheat-pattern diet group | 25 ± 7*   | 30 ± 10*  | 28 ± 11*  | 31 ± 9*   |
| Rice-pattern diet group  | 37 ± 11   | 42 ± 21   | 29 ± 12*  | 31 ± 12*  |
| Miyazaki-pattern diet group | 29 ± 5* | 35 ± 15   | 34 ± 20   | 46 ± 22   |

Each value represents the mean ± half range of the confidence interval (confidence limit) at 95% level.

* Indicates statistically significant difference from the control at \(p < 0.05\).
with the control level except at the 4th week. These results corresponded with our previous data (1, 4).

Each enzyme activity in rat liver showed a similar value in all control groups during the experimental period (1 to 4 weeks), therefore, the mean value of each enzyme activity in the control group was calculated from values of all control rats.

Glucose-6-phosphate dehydrogenase. The activities of this enzyme are shown in Table 2, together with the control level. The Wheat-pattern diet group showed many more reductions in glucose-6-phosphate dehydrogenase compared to the control level at every experimental interval (p < 0.05). On the other hand, the Rice-pattern diet group also showed considerable decreases in enzyme activity compared to control levels. At the 3rd and 4th week feeding intervals, these reductions were significant, but not at 1st and 2nd week feeding intervals. No considerable reduction in activity for the Miyazaki-pattern diet group was observed at any of the weekly intervals except for the first feeding interval. There were no significant differences among the other three diet groups. In the results from our previous study the activities in the liver homogenates remained at control level or tended to decrease slightly in every experimental group (4). Whereas in the present study all experimental diet groups showed obvious reductions to some extent.

Malic enzyme. The activity of this enzyme in every experimental diet group was remarkably higher than control level (25 ± 6 μmol/min/mg of protein) during all the experimental periods and the difference in the enzyme's activities was significant at p < 0.05 (Table 3). In the latter period of the experiment the activities tended to decrease gradually. There were no significant differences in enzyme activity among the three experimental diet groups throughout all the periods tested. However, the intensity of this enzyme's activity was in the order of Miyazaki-pattern diet group > Rice-pattern diet group > Wheat-pattern diet group > control group.

ATP citrate lyase. Table 4 shows the activities of ATP citrate lyase in liver homogenates together with the control level. The activities of this enzyme in the Wheat-pattern diet group remained at the control level throughout the 1 to 4 week experimental periods. Those in the Rice- and Miyazaki-pattern diet groups, however, tended to increase slightly during the initial period of the experiment and

| Weeks          | 1            | 2            | 3            | 4            |
|----------------|--------------|--------------|--------------|--------------|
| Wheat-pattern diet group | 93 ± 19*     | 88 ± 22*     | 75 ± 26*     | 87 ± 27*     |
| Rice-pattern diet group    | 96 ± 25*     | 117 ± 23*    | 74 ± 26*     | 83 ± 29*     |
| Miyazaki-pattern diet group | 124 ± 36*    | 134 ± 51*    | 86 ± 21*     | 110 ± 24*    |

Each value represents the mean ± half range of the confidence interval (confidence limit) at 95% level.

* Indicates statistical significance from the control level at p < 0.05.
Table 4. ATP: citrate oxaloacetate-lyase (ATP citrate lyase) activities.
Control group, 10.1 ± 1.9 μmol/min/mg protein

| Weeks       | 1          | 2          | 3          | 4          |
|-------------|------------|------------|------------|------------|
| Wheat-pattern diet group | 10.0±1.6   | 10.9±2.0   | 11.2±3.8   | 9.2±2.6    |
| Rice-pattern diet group    | 13.7±3.5*  | 10.6±4.0   | 11.5±4.9   | 9.4±1.0**  |
| Miyazaki-pattern diet group| 13.2±4.3   | 11.1±4.5   | 11.6±5.3   | 7.8±2.7**  |

Each value represents the mean ± half range of the confidence interval (confidence limit) at 95% level.
* Indicates significance compared with the control group at p<0.05.
** Indicates statistical significance from each of the values at 1 week experimental interval at p<0.05.

Table 5. Fatty acid-synthesizing enzyme activities.
Control group, 1.9±0.5 μmol/min/mg protein

| Weeks       | 1          | 2          | 3          | 4          |
|-------------|------------|------------|------------|------------|
| Wheat-pattern diet group | 1.6±0.5    | 1.3±0.7    | 2.0±1.0    | 1.4±0.3    |
| Rice-pattern diet group    | 2.1±0.5    | 1.7±0.7    | 1.8±0.5    | 1.5±0.4    |
| Miyazaki-pattern diet group| 2.5±1.1    | 1.7±0.6    | 2.3±1.0    | 1.7±0.6    |

Each value represents the mean ± half range of the confidence interval (confidence limit) at 95% level.

Table 6. Glycerol-1-phosphate dehydrogenase (L-a-glycerophosphate DH).
Control group, 13±3 μmol/min/mg protein

| Weeks       | 1          | 2          | 3          | 4          |
|-------------|------------|------------|------------|------------|
| Wheat-pattern diet group | 17±5       | 20±9       | 15±7       | 16±8       |
| Rice-pattern diet group    | 16±6       | 17±4       | 16±6       | 15±4       |
| Miyazaki-pattern diet group| 20±9       | 20±10      | 12±7       | 16±6       |

Each value represents the mean ± half range of the confidence interval (confidence limit) at 95% level.

thereafter to decrease gradually, being significantly lower at the 4 week interval than each of the values at the 1 week interval. For the Rice-pattern diet group, the activity at the 1 week interval was statistically different from the control level at p < 0.05. From these data, it is likely that ATP citrate lyase activities in the liver homogenates remained almost at control level, although some of them tended to increase slightly.

**Fatty acid-synthesizing enzymes.** The activities of the fatty acid-synthesizing enzymes in the liver homogenates are shown in Table 5, together with the control level. The activities of this enzyme in each diet group were nearly the same as the control level during all the experimental periods. For the Rice-pattern diet group, however, there was a tendency for enzyme activities to decrease gradually after the 2 week interval.
L-α-Glycerophosphate dehydrogenase. This activity is shown in Table 6. The activities of this enzyme in all the experimental groups tended to increase slightly compared with the control level throughout the 1 to 2 week experimental periods although they recovered to the control level halfway through the experimental period. But no significant increases in this activity at the initial feeding period were observed, nor were there any significant differences among the experimental groups.

DISCUSSION

Some enzyme activities related to lipogenesis in the liver homogenates of rats with fatty livers due to feeding low-level amino acid mixture diets were determined in the present study. The L-α-glycerophosphate dehydrogenase catalyzes the reaction from dihydroxyacetone phosphate, an intermediate of glycolysis, to L-α-glycerophosphate, which is a major source of glycerol in the biosynthesis of triacylglycerols and phospholipids in the liver. It is generally believed that in liver tissues, ATP glycerol-phosphotransferase acts as the main promoter of glycerol formation in lipogenesis only when the supply of fatty acids from adipose tissues into the liver increases (25). From our previous results, the transfer of fatty acids from adipose tissues to the liver as serum NEFA did not increase but gradually decreased, showing a considerable reduction throughout the course of these diets. Therefore, L-α-glycerophosphate dehydrogenase activity, rather than ATP glycerol-phosphotransferase, was estimated in the present study. In the results of this study, the activities of L-α-glycerophosphate dehydrogenase were slightly higher than the control level at the initial stage of the experimental period and thereafter recovered to control level after the halfway point of the experimental period.

In a previous report (4), we stated that pyruvate kinase activity in the liver of the rats given amino acid mixture diets at low levels increased slightly at the beginning of the experimental period. Because of increased pyruvate kinase activity at the beginning of the experimental period in our previous report, glycolysis may have been activated slightly. LEVEILLE et al. (26) have indicated that the activity of the key rate-limiting enzyme of the glycolytic and lipogenic reactions in liver tissues from rats given large amounts of a high-carbohydrate diet was accelerated and consequentially lipogenesis was increased in liver tissues. Acetyl coenzyme A produced by pyruvate dehydrogenase in a mitochondrion diffuses into the microsome through the mitochondrial membrane at a very slow rate (27). On the other hand, there is evidence that in liver tissues the citrate as a precursor is incorporated into fatty acid as much as the acetate is (28–30). As SPENCER and BHADURI (28–31) pointed out, citrate produced in the mitochondrion moved very easily into the microsomes through the mitochondrial membrane and then was converted by an ATP citrate lyase to oxaloacetate and acetyl coenzyme A, which was incorporated into fatty acids. On the other hand, in a comparison of the activities of some lipogenic enzymes in meal-feeding and nibbling, TAKEDA (32) has indicated that ATP citrate lyase, acetyl coenzyme A carboxylase, and NADP+:
malate dehydrogenase did play major roles in key rate-limiting enzymes of lipogenesis. To confirm their possible contribution within lipogenesis, we determined ATP citrate lyase activity in the liver which produced extensive fatty accumulation by low amino acid mixture diet feedings. ATP citrate lyase activity in the control diet group in the present study was 10.1 μmol/min/mg of protein, corresponding with the normal value (10.3 μmol/min/mg of protein) of the specific activity of the crude enzyme in Takeda's results (11–14). As described in the results, however, ATP citrate lyase activities remained unchanged at the control level in many of the experimental rats, except that the activity was statistically higher only at the 1st week feeding point in the Rice-pattern diet group.

Acetyl coenzyme A produced by ATP citrate lyase from the citrate built up long-chain fatty acids by acetyl coenzyme A carboxylase and fatty acid synthetase through the process of formation of malonyl coenzyme A. In the present study, the enzyme preparation of fatty acid-synthesizing enzymes in the liver homogenate may also have contained some parts by which a reaction of fatty acids elongation is mediated in the presence of NADPH together with the action of fatty acid synthetase in the incubation medium. We referred to this enzyme complex as fatty acid-synthesizing enzymes in this study. As shown in the results, the activities of this enzyme in each diet group were nearly the same as the control level during all experimental periods.

As is well known, the participation of reduced nicotinamide adenine dinucleotide phosphate is necessary for the biosynthesis of fatty acids (31, 33). It is generally recognized that NADPH is generated in reactions catalyzed by hexose monophosphate pathway dehydrogenases, malic enzyme and iso-citrate dehydrogenase (34, 35). In our preceding reports, the activity of glucose-6-phosphate dehydrogenase under these feeding conditions did not differ from the control level nor did it show a tendency to be lower than the control level (4). If lipogenesis in the liver is accelerated under these nutritional conditions, we assume that glucose-6-phosphate dehydrogenase in the liver may rise above the control (36–38). We therefore carefully re-investigated the activity of glucose-6-phosphate dehydrogenase as compared to the control group. However, the activity of this enzyme fell below the control level rather than increasing. Pandey, Fitch and Chakoff (39, 40) have reported that NADP⁺: malate dehydrogenase (decarboxylating) activity increased along with an accelerated activity of the hexose monophosphate pathway dehydrogenases as an adaptation to lipogenesis that occurred due to a high carbohydrate diet.

Therefore, NADP⁺: malate dehydrogenase activity was also investigated in the present study. Malic enzyme activities in all experimental diet groups increased notably from the control level during all experimental diet feeding intervals. The intensity of the activity of this enzyme, however, tended to be in the order of Miyazaki-pattern diet group > Rice-pattern diet group > Wheat-pattern diet group >> control. This order was, however, contrary to the intensity of fat retention in liver tissues (1). The increased activity of the malic enzyme alone could
not account for all of the kinetics of extensive fat accumulation in the liver under these nutritional conditions. Some enzyme which had increased in activity in the initial experimental period tended toward a progressive decrease after the halfway point of the experimental period. These phenomena may have resulted from depletion of the enzyme proteins caused by supplementation of low-level amino acid mixture or by disturbances in the amino acid pattern in the diets. This conception is supported by our previous evidence that protein contents of the supernatants from liver tissues gradually decreased as the experimental periods progressed (4).

When our present data was summarized together with our preceding results, early in the experimental period malic enzyme activity increased more markedly in rats given the low amino acid mixture diets than in control rats, and pyruvate kinase and L-α-glycerophosphate dehydrogenase in liver homogenates increased slightly. Conversely, the activities of ATP citrate lyase and fatty acid-synthesizing enzymes remained almost at the control level, or rather, glucose-6-phosphate dehydrogenase activity tended to decrease compared with control rats. Under these nutritional conditions, the acceleration of lipogenesis in liver tissues is undeniable. However, these results suggested that some other associated factors, such as depression of lipid transfer mechanisms in the liver rather than accelerated lipogenesis itself, may be the main cause of the fatty liver produced under these nutritional conditions.

We would like to thank the Ajinomoto Co., Ltd. for providing all of the crystalline amino acids.

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