Nitrogen Balance and Hepatic Gluconeogenesis in Rats Fed on Diets Containing Various Proportions of Carbohydrate and Fat

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Summary This study was conducted to investigate the effects of various proportions of dietary carbohydrate and fat in diet on protein and carbohydrate metabolism. The growth rate, nitrogen balance, urinary and serum urea levels, and the activities of key ureogenic and gluconeogenic enzymes in the livers were examined in the weanling and growing rats. The rats were raised on a 20% casein diet containing various proportions of carbohydrate and fat, viz. 30%, 50%, 50%, 30%, 60%, 60%, 20%, 20%, and 10% as calorie percent, respectively, for 10 days. For both weanling and growing rats, the growth rate was unaffected by the alteration in the proportions of carbohydrate and fat in the diets. However, in the rats fed on the 30% carbohydrate-50% fat diet, the urinary excretion of nitrogen and urea were reduced in both groups and these findings were reflected in the reduced serum urea level. Arginase activity decreased. In contrast, glucose-6-phosphatase activity was enhanced in the animals of the 30% carbohydrate-50% fat diet group as compared to the other groups. These results suggest that a low carbohydrate-high fat diet causes the reduction of urea formation and the enhancement of glucose formation at a fixed level of protein in the diets in weanling as well as in growing rats.

Key Words nitrogen balance, gluconeogenesis, arginase, glucose-6-phosphatase, dietary carbohydrate and fat

Considerable progress has been made in the understanding of alterations in dietary composition of nutrients often evoking metabolic changes in animals, induced by the alteration of a number of metabolic parameters following changes in

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the systemic levels of hormones (1, 2). Nakano et al. (3, 4) have reported that feeding high fat diet causes a decrease in the urinary output of nitrogen, accompanied by a reduction of hepatic amino acid-catabolizing enzymes, *e.g.*, threonine dehydratase. On the other hand, it has been shown that the feeding of carbohydrate-free and fat-rich or protein-rich diet causes the stimulation of carbohydrate synthesis in liver and kidney (5–7). The feeding of such a diet causes the decrease in the utilization of plasma glucose and liver glycogen, inducing insulin insensitivity (9–10). These disturbances in the regulation of carbohydrate metabolism might ultimately influence protein metabolism as observed by changes in nitrogen balance.

However, the above studies were done under extreme nutritional conditions such as carbohydrate-free or fat-free diet. The present study was, therefore, designed to observe the metabolic effects of the diets containing various proportions of dietary carbohydrate and fat on protein and carbohydrate metabolism in weanling and growing rats. An attempt was made to clarify the effect of these diets on the nitrogen balance and hepatic gluconeogenesis.

**METHODS**

*Animals and diets.* Male Wistar-albino rats, weanling and growing (about 140 g), (Japan Biological Material Center, Tokyo) were housed individually and fed on commercial stock diet (Japan Clea. Inc., Tokyo) for three days to acclimate to their surroundings. The animals were then randomly divided into 4 groups and were fed on one of the diets consisting of 30, 50, 60, or 70% of carbohydrate with 50, 30, 20 or 10% of fat, respectively (calorie ratio). Five rats were used in all experimental subgroups. The diets and water were provided *ad libitum* for all animals. The composition of the diets is shown in Table 1. Each diet contained an identical amount of protein and isocalorie in order to obtain a fixed level of urinary nitrogen excretion (11). The animal room was constantly kept at a temperature of 23°C and 55% relative humidity.

The food intake and body weight of each of the animals were determined daily during the experimental period. The urine and feces of rats for the final 2 days were collected by using individual metabolic cages. The urine samples were reserved by addition of toluene to flasks during the collection:

*Assay of nitrogen and urea content.* The urine samples were adjusted volumetrically with distilled water to 100 ml and aliquots were stored at −20°C. Individual feces was dried at 50°C and powdered with mortar and pestle. Aliquots of the diluted urine and powdered feces were taken for the duplicate analysis of total nitrogen content by the micro-Kjeldahl method.

Urinary and serum urea was determined by the method of Coulombe and Favreau (12).

*Preparation of tissue.* All preparation procedures were carried out at 0–4°C. The animals were sacrificed by decapitation between 1:00 and 1:30 pm, and the livers were quickly removed, rinsed with cold saline, blotted and weighed. For the
Table 1. Composition of the experimental diets.

| Ingredient                      | Dietary groups                          |
|---------------------------------|-----------------------------------------|
|                                 | 30% carb. | 50% carb. | 60% carb. | 70% carb. |
|                                 | -50% fat  | -30% fat  | -20% fat  | -10% fat  |
|                                 | (g/100 g diet) |          |          |           |
| z-Corn starch\(^1\)             | 30        | 50        | 60        | 70        |
| Vitamin-free casein\(^2\)       | 20        | 20        | 20        | 20        |
| Corn oil                        | 22        | 13        | 9         | 4.5       |
| Cellulose powder\(^3\)          | 23        | 12        | 6         | 0.5       |
| Salt mixture                    |           |           |           | 4         |
| Vitamin mixture\(^4\)           |           |           |           | 0.5       |
| Choline chloride                |           |           |           | 0.5       |
| Calorie (cal/100 g)             | 398       | 397       | 401       | 401       |
| Carbohydrate cal%               | 30        | 50        | 60        | 70        |
| Fat cal%                        | 50        | 30        | 20        | 10        |
| Protein cal%                    | 20        | 20        | 20        | 20        |

\(^1\) Nakarai Pharmaceutical Co., Osaka. \(^2\) Nutritional Biochemicals, Cleveland, Ohio. \(^3\) Tokyo Roshi Co., Tokyo. \(^4\) "Panvitan" purchased from Takeda Pharmaceutical Co., Osaka.

Enzyme assay. The activity of arginase was determined by the colorimetric method of Greenberg (13), as modified by Schimke (14). Briefly, preincubation were undertaken for 5 min at 37°C in the medium containing 0.1 ml of tissue homogenate (about 2 mg protein) and 1 mM MnSO\(_4\) and then the medium had 250 mM L-arginine added and was incubated for 10 min (1 ml of total volume). The reaction was stopped by the addition of 2.5 ml of 15% perchloric acid, followed by centrifuging for 10 min at 2,500 rpm. An aliquot of the supernatant was assayed for produced urea by the same procedure as mentioned above for the urine and serum. Enzyme activity of the arginase was expressed as µmol urea produced/10 min/mg protein of the homogenate.
The activity of glucose-6-phosphatase was determined according to the method of Segal and Washko (15). The assay mixture contained 10 mM potassium glucose-6-phosphate, 75 mM sodium cacodylate buffer, pH 6.0 and 0.5 ml of the supernatant (about 3 mg protein) in a final volume of 2 ml. The reaction was terminated by the addition of 0.4 ml of 30% trichloric acid, the precipitated protein was removed by centrifugation at 3,000 rpm for 5 min and the glucose in the supernatant was determined by the method of glucose oxidation using a glucostat (Fujisawa Pharmaceutical Co., Tokyo). Enzyme activity of the glucose-6-phosphatase was expressed as \( \mu \text{mol glucose produced/10 min/mg protein of the supernatant.} \)

Protein was measured by the method of Lowry et al. (16) using crystalline bovine serum albumin as a standard.

The data were statistically analyzed by using the Student’s \( t \)-test.

RESULTS

In both the weanling and growing rats, food consumption and weight gain were identical among the 4 groups (Table 2). The calorie efficiency ratio for the animals was thus unaffected by alteration of the proportion of carbohydrate and fat in the diets containing the same amount of protein.

The urinary excretion of nitrogen was significantly reduced in the 30% carbohydrate-50% fat diet group compared to those of the other groups in animals of both ages (Table 3). For both the weanling and growing rats, the urea content of urine was the lowest in the rats fed on the 30% carbohydrate-50% fat diet among the 4 groups. The rats of this group also had a low serum urea level compared to the other groups (Table 4). Although there was statistically no difference in levels of retained nitrogen among the 4 groups, the ratio of retained nitrogen to apparent absorbed nitrogen was significantly increased in the animals fed on the 30% carbohydrate-50% fat diet (Table 3). As the fecal nitrogen excretion was decreased by the increases in dietary fiber content, there was slight difference in real absorbed nitrogen levels of the 4 groups. However, it is unlikely that the small difference in the amount of fecal nitrogen contents affects the reduction of urinary nitrogen level in the low carbohydrate-high fat diet group, because of the increased ratio of retained nitrogen to apparent absorbed nitrogen.

In an attempt to reveal the reason for the lowered urinary excretion of nitrogen and urea in the 30% carbohydrate-50% fat diet, we observed the effect of these dietary regimens on hepatic urea formation and gluconeogenesis, using the same animals as used for the analysis of urinary nitrogen and urea. As shown in Fig. 1, the lowest arginase activity was found in the rats fed on the 30% carbohydrate-50% fat diet in both the weanling and growing animals. This decline of arginase activity was related to the decreased level of urinary nitrogen and urea. On the other hand, an increase of the glucose-6-phosphatase activity on feeding the 30% carbohydrate-50% fat diet was greater in the growing rats than in the weanling rats. It was found
Table 2. Effects of dietary carbohydrate and fat levels on growth, food consumption and calorie efficiency ratio in rats.

| Dietary groups   | Initial body wt. (g) | Final body wt. (g) | Weight gain (g/day) | Food consumption (g/day) | Calorie efficiency ratio²) (g wt. gain/100 cal) |
|------------------|----------------------|--------------------|---------------------|--------------------------|-----------------------------------------------|
| **Weanling**     |                      |                    |                     |                          |                                               |
| 30% carb.¹)–50% fat (a)⁴) | 43.7 ± 1.6³)         | 101.9 ± 5.9        | 6.5 ± 0.5           | 9.5 ± 0.4                  | 17.6 ± 1.5                                    |
| 50% carb.–30% fat (b)    | 44.6 ± 2.1           | 106.2 ± 3.4        | 6.2 ± 0.4           | 10.3 ± 0.4                 | 15.4 ± 1.3                                    |
| 60% carb.–20% fat (c)    | 44.1 ± 1.6           | 112.6 ± 2.2        | 6.7 ± 0.2           | 10.3 ± 0.4                 | 16.2 ± 0.8                                    |
| 70% carb.–10% fat (d)    | 44.0 ± 1.6           | 109.7 ± 4.3        | 6.6 ± 0.3           | 9.4 ± 0.4                  | 17.2 ± 0.5                                    |
| **Growing**       |                      |                    |                     |                          |                                               |
| 30% carb.–50% fat (e)    | 142.2 ± 2.2          | 231.2 ± 1.5        | 8.9 ± 0.8           | 17.9 ± 0.3                 | 12.3 ± 0.3                                    |
| 50% carb.–30% fat (f)    | 142.6 ± 2.5          | 232.3 ± 2.5        | 8.9 ± 0.4           | 17.9 ± 0.2                 | 12.6 ± 0.5                                    |
| 60% carb.–20% fat (g)    | 143.3 ± 1.6          | 230.8 ± 2.7        | 8.8 ± 0.3           | 17.1 ± 0.3                 | 12.7 ± 0.5                                    |
| 70% carb.–10% fat (h)    | 143.0 ± 1.7          | 233.0 ± 2.2        | 8.9 ± 0.2           | 18.1 ± 0.7                 | 10.9 ± 0.3                                    |

¹) carb., carbohydrate.  ²) Weight gain/calorie intake × 100. The calorie intake was calculated from the food consumption.
³) Means ± S.E.M. ⁴) The experimental groups were designated (a) to (h), respectively, in order to represent each matched pair with statistical significance. There were no statistical differences in each column of data.
Table 3. Effects of dietary carbohydrate and fat levels on nitrogen balance.

| Dietary groups          | Nitrogen intake | Excreted nitrogen | Retained\(^2\) nitrogen | Retained N/ Absorbed N\(^3\) × 100 (%) |
|------------------------|----------------|-------------------|--------------------------|--------------------------------------|
|                        |                | Feces (mg/2 days/rat) | Urine                   |                                       |
| Weanling               |                |                   |                          |                                       |
| 30% carb.\(^1\)–50% fat (a)\(^3\) | 820 ± 58\(^4\) | 45 ± 6 a)–c)\(^6\) | 140 ± 8 a)–b) c) d)\(^\ast\) | 635 ± 16 | 82.3 ± 0.5 a)–b) c) d)\(^\ast\)*** |
| 50% carb.–30% fat (b)  | 813 ± 45      | 26 ± 7 a)–d)\(^\ast\) | 181 ± 8                  | 606 ± 55 | 77.1 ± 0.6                           |
| 60% carb.–20% fat (c)  | 845 ± 23      | 20 ± 4             | 196 ± 7                  | 629 ± 27 | 77.8 ± 0.9                           |
| 70% carb.–10% fat (d)  | 840 ± 32      | 14 ± 2             | 204 ± 15                 | 622 ± 17 | 75.1 ± 1.0                           |
| Growing                |                |                   |                          |                                       |
| 30% carb.–5% fat (e)   | 1188 ± 67     | 80 ± 8 e)–h)\(^\ast\) | 238 ± 27 e)–f) g) h)\(^\ast\) | 870 ± 69 | 79.6 ± 2.9 e)–f) g) h)\(^\ast\)*** |
| 50% carb.–3% fat (f)   | 1248 ± 35     | 67 ± 1 f)–h)\(^\ast\) | 395 ± 18 f)–h)\(^\ast\) | 786 ± 20 | 67.6 ± 2.0                           |
| 60% carb.–20% fat (g)  | 1217 ± 30     | 49 ± 6             | 420 ± 42                 | 748 ± 52 | 64.0 ± 3.2                           |
| 70% carb.–10% fat (h)  | 1276 ± 62     | 31 ± 6             | 466 ± 8                  | 779 ± 40 | 63.6 ± 3.7                           |

\(^1\) carb., carbohydrate.  
\(^2\) Retained nitrogen = nitrogen intake – (feces-N + urine-N). The nitrogen intake was calculated from the amount of food consumption.  
\(^3\) Absorbed N was calculated by subtraction of feces-N from the nitrogen intake.  
\(^4\) Means ± S.E.M.  
\(^5\) The experimental groups were designated (a) to (h), respectively, in order to represent each matched pair with statistical significance.  
\(^6\) t-test analysis: *p < 0.05; **p < 0.02; ***p < 0.01.
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Table 4. Effects of dietary carbohydrate and fat levels on serum and urinary urea in rats.

| Dietary groups | Urea Serum (mg/100 ml) | Urea Urine (mg/total urea/2 days) |
|----------------|------------------------|-----------------------------------|
| Weanling       |                        |                                   |
| 30% carb. 1)-50% fat (a) 1) | 25 ± 1 3) a)-d) 4)*) | 177 ± 14 a)-b) c) d)* |
| 50% carb. -30% fat (b) | 27 ± 1 b)-d)*        | 232 ± 30                          |
| 60% carb. -20% fat (c) | 27 ± 2                 | 296 ± 28                          |
| 70% carb.-10% fat (d) | 30 ± 2                 | 388 ± 42                          |
| Growing        |                        |                                   |
| 30% carb.-50% fat (e) | 25 ± 1 e)-f) g)*, e)-h)** | 360 ± 20 e)-f) g) h)* |
| 50% carb.-30% fat (f) | 31 ± 1                 | 634 ± 64                          |
| 60% carb.-20% fat (g) | 29 ± 1                 | 702 ± 77                          |
| 70% carb.-10% fat (h) | 32 ± 1                 | 805 ± 102                         |

1) carb., carbohydrate. 2) The experimental groups were designated (a) to (h), respectively, in a similar way as indicated in Table 3. 3) Means±S.E. 4) t-test analysis: *p<0.05; **p<0.01.

that a relationship had existed between arginase activity and glucose-6-phosphatase activity in the liver of rats fed on the diets consisting of various proportions of carbohydrate and fat (Fig. 1). This data suggested that in rats fed on the 30% carbohydrate-50% fat diet, the decline of the urinary nitrogen excretion was associated with the enhanced gluconeogenesis.

When kinetic analysis of the hepatic arginase activity was done, the $K_m$ value of the enzyme was the same among the 4 groups in animals of both ages (20 mm), although the $V_{max}$ value of the rats fed on the 30% carbohydrate-50% fat diet at either age was the highest among the groups. Furthermore, the $K_m$ value of the enzyme in the weanling rats was the same as that in the growing rats, but the $V_{max}$ value was higher in the growing rats than the weanling rats (Fig. 2). It is suggested that the changes in enzyme activity of the arginase associated with altering of the dietary composition of carbohydrate and fat could be due to differences in content of enzyme protein. In addition, it is possible that there are no differences in the enzyme proteins from animals of either age, but the high level of enzyme activities in the 4 groups of growing rats as compared with the weanling rats could be due to an increase in the content of enzyme protein.

DISCUSSION

The experiments reported here demonstrate that the growth of the animals of either age group was not affected by the dietary regimen of this study, but that the...
protein and carbohydrate metabolism were influenced. In respect of those influences, the weanling rats provided results similar to those obtained in the growing rats. The inhibitory effect of the low carbohydrate-high fat diet on the urinary excretion of nitrogen is undoubtedly reflected by the decreases in urinary and serum urea levels. These findings were also confirmed by the decreased arginase activity in the liver. Thus, the reduction of urinary nitrogen could be caused by the enhanced utilization of nitrogen due to some metabolic change in the animals.

It is well known that both dietary carbohydrate and fat have a "protein sparing action" which is often given as the reason for the inhibitory effect of diet on urinary nitrogen output, and it is greater with a carbohydrate diet than with a fat diet (11). However, Munro and Thompson (17) have reported that the inhibitory effect of carbohydrate on the urinary nitrogen output is equal to that caused by the fat-rich diet. Nakano et al. (3, 4) have also shown that the amount of urinary nitrogen excretion is less in rats fed on the high fat diet compared with the high carbohydrate diet.
diet. The inhibitory effect of the 30% carbohydrate–50% fat diet on urinary nitrogen excretion is in good agreement with the data provided by those investigations.

The question may be asked why there was significantly decreased urea formation in the animals fed on low carbohydrate-high fat diet. The answer is probably provided by the findings of enhancement of nitrogen utilization. Hepatic glucose-6-phosphatase activity was significantly enhanced in the rats fed on the 30% carbohydrate–50% fat diet. Although we did not observe incorporation of carbon skeletons of amino acids into glucose or glycogen and we cannot prove in this experiment whether glucose formation is provided by pyruvate rather than glycerol, it is considered that an enhancement of amino acid utilization in the rats would have occurred, resulting in inhibition of the degradation rate of some amino acids as donors of the carbon skeletons. Hayase et al. recently proposed that liver levels of free amino acids may regulate the rate of urea formation (21). Nitrogen balance is the sum of gains and losses of nitrogen from various compartments of the body. Therefore, nitrogen retention does not necessarily mean protein anabolism only, such as synthesis of protein. It has been reported that the protein-sparing action might be attributable to protein synthesis in the muscle (18). However, the reduction

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of urinary nitrogen excretion would be involved in more complicated biochemical changes.

According to the observation by Eisenstein et al. (7), the enhancement of glucose formation in the rats fed on high fat-carbohydrate free diet could be provided by another mechanism without a rise of glucagon secretion, which stimulate gluconeogenesis in rats fed high protein-carbohydrate free diet (19). It has been reported that the fasting glucose level in animals fed on high fat-carbohydrate free diet is increased due to the delayed glucose utilization (7, 9), consequently causing a high level of fasting plasma insulin and diminishing insulin sensitivity (1, 8, 9). Enhanced insulin and decreased glucagon levels would cause inhibition of activity of hepatic amino acid-degrading enzymes, e.g., threonine dehydratase and arginase (2, 20).

In view of these facts described above, our findings concerning the decreased urea formation and the elevated hepatic glucose formation by the feeding of the low carbohydrate-high fat diet support the following possibility. The enhanced glucose-6-phosphatase activity is possibly unaccompanied by an increase of glucagon secretion, because of high-fat feeding. Accordingly, the inhibition of urea formation may be caused by increasing insulin secretion resulted from delaying plasma glucose utilization in animals of the high-fat feeding. As urea cycle metabolism decreases, the enlarged nitrogen pool would facilitate the use of amino acids from the pool for glucose formation. Further work is needed to elucidate this hypothesis.

On the basis of present results, it could be concluded that the diet containing less than 30% carbohydrate with high fat caused the reduction of urinary nitrogen excretion accompanying the enhancement of gluconeogenesis.

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REFERENCES

1) Zaragoza, N., and Felber, J. P. (1970): Studies on the metabolic effects induced in the rat by a high fat diet. Horm. Metab. Res., 2, 323–329.

2) Izzo, J. L., and Glasser, S. R. (1961): Comparative effects of glucagon, hydrocortisone and epinephrine on the protein metabolism of the fasting rat. Endocrinology, 68, 189–198.

3) Nakano, K., and Ashida, K. (1970): Effect of dietary carbohydrate and fat on amino acid-degrading enzymes in relation to their protein sparing action. J. Nutr., 100, 208–216.

4) Nakano, K., Kurimoto, S., and Ashida, K. (1971): Effect of previous high fat diet on body protein metabolism in rats. J. Nutr., 101, 895–900.

5) Evans, R. M., and Scholz, R. W. (1973): Development of renal gluconeogenesis in chicks fed high fat and high protein “carbohydrate-free” diets. J. Nutr., 103, 242–250.

6) Suzuki, H., and Fuwa, H. (1970): Influence of dietary composition on the capacity of glucose formation in liver of rats. Agric. Biol. Chem., 34, 80–87.

J. Nutr. Sci. Vitaminol.
7) Eisenstein, A. B., Strack, I., and Steiner, A. (1974): Increased hepatic gluconeogenesis without a rise of glucagon secretion in rats fed a high fat diet. Diabetes, 23, 869–875.

8) Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A. (1963): The glucose-fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet, 1, 785–789.

9) Schalch, D. S., and Kipnis, D. M. (1965): Abnormality in carbohydrate tolerance associated with elevated plasma nonesterified fatty acids. J. Clin. Invest., 44, 2010–2020.

10) Switzer, B. R., Zana, T., Niehaus, N. J., and Edozien, J. C. (1974): Effect of diet on fasting plasma immunoreactive insulin. Fed. Proc., 33, 669–769.

11) Munro, H. N. (1951): Carbohydrate and fat as factors in protein utilization and metabolism. Physiol. Rev., 31, 449–488.

12) Coulombe, J. J., and Favreau, L. (1963): A new simple semimicro method for colorimetric determination of urea. Clin. Chem., 9, 102–108.

13) Greenberg, D. M. (1955): Arginase, in Methods in Enzymology, ed. by Colowick, S. P., and Kaplan, N. O., Academic Press, New York, Vol. II, pp. 368–374.

14) Schimke, R. T. (1962): Adaptive characteristics of urea cycle enzymes in the rat. J. Biol. Chem., 237, 459–468.

15) Segal, H. L., and Washko, M. E. (1959): Studies of liver glucose-6-phosphatase. J. Biol. Chem., 234, 1937–1941.

16) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem., 193, 265–275.

17) Munro, H. N., and Thompson, W. S. T. (1955): The relationship of carbohydrate metabolism to protein metabolism. J. Nutr., 56, 139–150.

18) Munro, H. N., Black, J. G., and Thompson, W. S. T. (1959): The mode of action of dietary carbohydrate on protein metabolism. Brit. J. Nutr., 13, 475–485.

19) Eisenstein, A. B., Strak, I., and Steiner, A. (1974): Glucagon stimulation of hepatic gluconeogenesis in rats fed a high protein, carbohydrate-free diet. Metabolism, 23, 15–23.

20) Mondon, C. E., and Mortimore, G. E. (1967): Effect of insulin on amino acid release and urea formation in perfused rat liver. Am. J. Physiol., 212, 173–178.

21) Hayase, K., Yokogoshi, H., and Yoshida, A. (1980): Effect of dietary proteins and amino acid deficiencies on urinary excretion of nitrogen and the urea synthesizing system in rats. J. Nutr., 110, 1327–1337.