DIETARY CHOLESTEROL INFLUENCES FASTING SERUM FREE AMINO ACIDS IN RATS FED DIETS CONTAINING DIFFERENT SUGARS

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Summary  Fasting serum aminograms were studied in rats fed a commercial stock diet or purified diets supplemented with or without 1% cholesterol and 0.25% sodium cholate. Sucrose, glucose or fructose served as a carbohydrate source for each purified diet. Accompanied by marked rises in serum cholesterol, the serum amino acid profile of rats fed glucose or fructose diets was modified significantly by dietary cholesterol. On feeding a glucose diet, dietary cholesterol caused decreases in Trp, Thr and Tyr and increase in Pro and Met. However, the concentration of total essential amino acids remained unchanged. Feeding a fructose diet resulted in a significant reduction of the amino acid level in comparison with that observed with glucose. This decrease was routinely compensated by the inclusion of cholesterol in the diet with the concentration of a number of amino acids being increased. Only Trp was decreased by this dietary manipulation. The serum aminogram of rats fed either a commercial stock diet or a sucrose diet was inconsiderably modified by dietary cholesterol. These data denote that dietary cholesterol influences the metabolic process of amino acids and that the response to cholesterol is modified by the carbohydrate source of the diet.

In addition to the hypercholesterolemic action, dietary cholesterol reduces activities of metabolically important enzymes involved in glycolysis, lipogenesis and cholesterogenesis (1–4). Differences in the sources of dietary carbohydrates perhaps influence these responses as might be inferred from their different effects on lipid metabolism (5, 6). Dietary cholesterol also appears to cause nonspecific reduction in hepatic protein synthesis in rats (7, 8), though the study of Clarkes et al. (9) indicates

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that dietary cholesterol probably has no depressive effect on total hepatic protein synthesis.

Recently, the interrelationship between dietary protein and the cholesterol metabolism has been watched with keen interest. Vegetable proteins, in comparison with animal proteins, have been found to be particularly effective in causing a lowering of plasma cholesterol levels regardless of the absence (10–13) or the presence (14, 15) of cholesterol in diets. The major factor contributing the difference in the cholesterol-lowering effect is considered to reside in the differences in their amino acid profiles (12, 15). However, the mechanism whereby differences in amino acid compositions between proteins can lead to a reduction in plasma cholesterol remains to be elucidated.

The amino acid profile of fasting plasma has been considered to be the most suitable reference pattern to assess the nutritive value of dietary proteins. Plasma amino acid concentrations thus can be used as response criteria for estimating the amino acid requirements (cf. 16). Hence, if specific amino acids effect the metabolic process of cholesterol and if cholesterol in turn influences the metabolism of amino acids, the measurement of the fasting plasma aminogram possibly gives a clue to the metabolic interaction between these two components.

The work reported herein was undertaken to gain basic information involved in the effect of dietary cholesterol on the serum aminogram of rats that had been fed different types of dietary sugars, sucrose, glucose and fructose. Rats fed a commercial stock diet were also tested for their amino acid profile. These studies seem to provide concurrently a useful approach to the selection of more preferable diet compositions for producing lower levels of plasma cholesterol.

EXPERIMENTAL

Animals. Male Wistar rats (Kyudo Co., Kumamoto) were used throughout the experiment. The animals were individually caged and given experimental diets and water for 14 days and sacrificed by decapitation after overnight fasting (16 hr). One group of rats were fed cholesterol-free diets and the other received diets containing 1% cholesterol and 0.25% sodium cholate in combination.

Trial 1: Rats were fed a powdered commercial stock diet (Oriental Yeast Co., NMF).

Trials 2 and 3: Rats were fed purified diets consisting of different types of sugars. The composition, in percent, was: casein, 20 (vitamin-free, ICN Pharmaceuticals, Inc., Cleveland); corn oil, 5; mineral mixture, 4; vitamin mixture (water soluble), 1; choline chloride, 0.15; cellulose powder, 2 and sugars to 100. The mineral and vitamin mixtures were according to HARPER (17) (Oriental Yeast Co.). Fat soluble vitamins were added as follows: vitamin A palmitate, 400 IU; vitamin D₃, 200 IU and dl-α-tocopherol acetate, 10 mg per 100 g of the diet. Cholesterol and cholate were added at the expense of sugar. The source of sugar was sucrose in trial 2 and glucose and fructose in trial 3.
Lipid analysis. Serum and liver lipids were extracted and analyzed for cholesterol and triglyceride as described elsewhere (18).

Amino acid analysis. Samples of sera were deproteinized with equal volumes of 6% sulfosalicylic acid solution (19). The denatured protein was sedimented by centrifugation and the supernatant was diluted with pH 2.72 lithium citrate buffer for amino acid analysis by ion-exchange chromatography using a JEOL JLC-6AH amino acid analyzer equipped with a JLC-DK dual channel integrator. The IRC-2 (Na+) and IRC-2 (Li+) columns were used for Lines 1 and 2, respectively. A standard amino acid mixture (Pierce Chemical Co., Rockford, Physiological ANB) was included in each automatic run of 15 samples.

RESULTS

Plasma and liver lipid concentrations

Table 1 summarizes growth, food intake, liver weight and the concentration of serum and liver lipids. In each trial, growth and food intake were not influenced, but liver weight increased by dietary cholesterol. On feeding cholesterol-free diets, the weight gain was considerably lower and liver weight was significantly greater in rats fed fructose than in the animals fed glucose.

Feeding cholesterol resulted in a significant increase in serum cholesterol, the magnitude of the increase being influenced by the type of diets fed. Though there may be some limitation for a direct comparison of serum and liver lipid levels due to differences in body weights of rats, the concentration of serum cholesterol of rats fed a commercial diet was markedly lower than that of rats fed purified diets irrespective of the presence or the absence of cholesterol in the diet. The rise in serum cholesterol due to feeding an atherogenic diet was marked in rats fed monosaccharide than that in rats fed sucrose. The response of liver cholesterol also resembled that of a serum counterpart.

Serum triglyceride levels tended to decrease by feeding cholesterol. In contrast to the case of serum, rats fed a commercial diet supplemented with cholesterol had the highest level of liver triglyceride and the increase due to feeding cholesterol was rather moderate with monosaccharides. Thus, the depressing effect of dietary cholesterol on the release of triglyceride from the liver to blood stream was particularly marked in rats fed a commercial diet. Alternately, older rats appeared to have a propensity to accumulate more triglyceride in the liver.

Serum aminograms

Figures 1 to 3 illustrate effects of cholesterol feeding on fasting serum aminograms. The concentration of serum total essential amino acids is shown in Table 2. In rats fed a commercial diet, dietary cholesterol exerted only an inconsiderable effect on the amino acid composition (Fig. 1). Only a slight but significant increase in Pro and decrease in Trp were observed.
Table 1. Growth, food intake, liver weight and serum and liver lipids.a

| Dietary regimens | Dietary cholesterol | Body weight (g) | Food intake (g/day) | Liver weight (g/100 g body weight) | Serum lipids (mg/100 ml) | Liver lipids (mg/g) |
|------------------|---------------------|-----------------|---------------------|-----------------------------------|--------------------------|-------------------|
|                  | Initial            | Gain            |                     |                                   |                          |                   |
| Commercial diet  | −                   | 253 ± 5         | 43 ± 3              | 21 ± 1                           | 3.14 ± 0.36              | 64.1 ± 5.3        |
| (6)              |                     | 253 ± 6         | 44 ± 2              | 22 ± 1                           | 3.97 ± 0.51              | 95.3 ± 2.24       |
|                   | +                   | 253 ± 6         | 44 ± 2              | 22 ± 1                           | 3.97 ± 0.51              | 57.2 ± 4.0        |
| Sucrose diet     | −                   | 188 ± 3         | 69 ± 6              | 20 ± 1                           | 5.30 ± 0.24              | 96.3 ± 9.8        |
| (7)              |                     | 189 ± 3         | 70 ± 3              | 21 ± 1                           | 6.44 ± 0.23d             | 141 ± 5.6         |
|                   | +                   | 189 ± 3         | 70 ± 3              | 21 ± 1                           | 6.44 ± 0.23d             | 68.5 ± 7.8e       |
| Glucose diet     | −                   | 158 ± 4         | 76 ± 5              | 18 ± 1                           | 3.44 ± 0.06              | 103 ± 6           |
| (6)              |                     | 159 ± 3         | 77 ± 6              | 17 ± 1                           | 4.73 ± 0.18e             | 303 ± 43e         |
|                   | +                   | 159 ± 3         | 77 ± 6              | 17 ± 1                           | 4.73 ± 0.18e             | 121 ± 12          |
| Fructose diet    | −                   | 157 ± 3         | 55 ± 8              | 16 ± 1                           | 3.91 ± 0.13              | 114 ± 3           |
| (6)              |                     | 158 ± 3         | 57 ± 4              | 16 ± 1                           | 4.88 ± 0.16e             | 276 ± 23e         |
|                   | +                   | 158 ± 3         | 57 ± 4              | 16 ± 1                           | 4.88 ± 0.16e             | 94.7 ± 11.0d      |

a Values are the means ± SEM. b Numbers of rats. c, d, and e Significantly different from the corresponding cholesterol free groups at p < 0.05, p < 0.01 and p < 0.001, respectively. The difference in liver weight between rats fed cholesterol free glucose and fructose is significant at p < 0.01.
Fig. 1. Effect of dietary cholesterol on fasting serum aminogram of rats fed a commercial stock diet. Values are the means of 6 rats. ●, Cholesterol free; ◆, cholesterol supplemented. Differences in the concentrations of Trp and Pro are significant at $p<0.05$. Only trace amounts of Asp were detected.

Table 2. Concentrations of serum essential amino acids.

| Dietary regimens       | Serum essential amino acids ($\mu$moles/100 ml) | Cholesterol free | Cholesterol added |
|------------------------|-----------------------------------------------|------------------|-------------------|
|                        |                                               |                  |                   |
| Commercial diet        |                                               | 92.6 ± 3.9 (44.6$^b$) | 97.5 ± 7.7 (43.2) |
| Sucrose diet           |                                               | 139 ± 6.8 (38.5)  | 135 ± 4.5 (39.7)  |
| Glucose diet           |                                               | 181 ± 22.6 (49.1) | 168 ± 39.6 (50.4) |
| Fructose diet          |                                               | 126 ± 5.4 (54.7)  | 158 ± 11.3 (44.0) |

$^a$ Values are the means ± SEM (numbers of rats are shown in Table 1). $^b$ Percentage of essential to total amino acids. Differences in the concentration of essential amino acids between rats fed cholesterol-free glucose and fructose, and between rats fed fructose supplemented with and without cholesterol are significant at $p<0.05$, respectively.
When sucrose was used as a source of dietary sugar, serum amino acids were not demonstrably influenced by cholesterol, except for a moderate decrease in Thr, though the pattern not completely resembled that observed in the preceding experiment (Fig. 2).

The aminogram of rats fed a glucose diet was conspicuously modified by cholesterol (Fig. 3A). The changes were characterized by significant decreases in Trp, Thr and Tyr and increases in Pro and Met. The differences were particularly marked in Thr, Tyr and Met. However, there was no demonstrable differences in the concentration of total essential amino acids, though the percentage to total amino acids was quite lower than that observed in other trials (Table 2).

The concentration of amino acids in rats fed a cholesterol-free fructose diet was strikingly lower than that observed with rats fed the corresponding glucose diet (Fig. 3B). The magnitude of the decrease in non-essential amino acids was much greater than that of essential counterparts (Table 2). Addition of cholesterol to this diet resulted in significant increases in a number of amino acids (Lys, Asp, Thr, Ser, Asn, Glu, Pro, Gly, Ala, Val, Leu, Tyr, and Phe). Only Trp decreased significantly. As the results of these alterations, the concentration and pattern of serum amino acids became roughly comparable with that of rats fed glucose or sucrose.
Fig. 3. Effect of dietary cholesterol on fasting serum aminogram of rats fed a glucose diet (A) or a fructose diet (B). Values are the means of 6 rats. ●, Cholesterol free; ○, cholesterol supplemented. In a glucose diet (A), differences in the concentrations of Trp, Thr and Tyr are significant at $p<0.05$, and Pro and Met at $p<0.001$. In a fructose diet (B), differences in the concentrations of Asp, Pro, Gly, Ala and Leu are significant at $p<0.05$, and Trp, Arg, Thr, Ser, Asn, Glu, Val, Ile, Tyr and Phe at $p<0.001$.

Dietary cholesterol did not influence the concentration of ornithine, taurine, $\alpha$-aminobutyric acid, urea and ammonia when rats were fed a stock diet or a sucrose diet. Cholesterol feeding caused a significant decrease in the concentration of urea in rats on glucose (134 $\pm$ 22 vs. 52.4 $\pm$ 10.1 $\mu$moles/100 ml, $p<0.01$) and increase in that of rats on fructose (56.5 $\pm$ 16.2 vs. 196 $\pm$ 7 $\mu$moles/100 ml, $p<0.01$). The ornithine level also increased in the latter (3.56 $\pm$ 0.41 vs. 17.5 $\pm$ 8.3 $\mu$moles/100 ml, $p<0.01$).

DISCUSSION

The rise in serum cholesterol due to feeding cholesterol appeared to be influenced by the type of dietary sugars. Because of a relatively small difference in initial body weight between trials 2 and 3, a comparison may be permitted. Thus, the magnitude of hypercholesterolemia demonstrated in rats fed glucose or fructose appeared much more marked than that observed with sucrose feeding. These responses in general agreed with those reported (20).
Modification by cholesterol on the metabolism of amino acids was remarkable when rats were fed monosaccharide diets. This was particularly marked with a fructose diet. Alternately, it was likely that in general the extent of changes in serum aminograms seemed greater in rats with severe hypercholesterolemia. However, cholesterol-induced changes in the profile of rats on glucose or on fructose were not necessarily parallel with each other, rather numbers of amino acids responded inversely. This result agreed with the observation that the serum aminogram of rats fed a sucrose diet remained routinely unchanged by dietary cholesterol.

The concentration or composition of plasma amino acids are found to be modified under various nutritional and physiological status of the animals. Insulin causes a decrease in the concentration of amino acids in the serum of intact animals (21). Dietary fructose causes no increase of plasma insulin and elevates blood glucose much more less than the equivalent amount of sucrose (22). Insulin plays an effective role in the metabolism of glucose, whereas fructose metabolism is insulin-independent (22). The present observation that rats fed a fructose diet had markedly lower concentration of serum amino acids, in comparison with that of rats fed glucose or sucrose diets, is inexplicable on the basis of above metabolic interrelationships. The reducing effect of fructose on the growth rate may be the probable factor responsible for leading such a decrease. However, cholesterol feeding did not influence weight gain of rats of this group, but increased the concentration of serum amino acids, this possibility appears unlikely. Rather, a drastic fall in the concentration of a number of amino acids due to feeding fructose seems to be relevant to the fact that hepatic lipogenesis from this compound proceeds much more rapidly than from glucose (22). In addition, hepatic lipogenic activities are elevated when rats are given fructose-containing diets (23), though the results are not all in agreement (24). In this context, more amino acids should be required for lipogenic enzymes in rats fed fructose than in the animals fed glucose. This assumption can be at least partly supported by the observation that fructose and cholesterol in combination caused apparent normalization of serum amino acid levels, as dietary cholesterol depresses lipogenesis and cholesterogenesis (2–4) and influences the metabolic process of amino acids (7–9).

Parenteral administration of a large dose of fructose to rats causes a transit but strong inhibition of incorporation of labeled leucine into liver proteins and this is closely correlated with the depression of hepatic ATP levels (25). A single large dose of glucose also produces fluctuations in the pattern of plasma amino acids (21). As an adaptation of enzymes involved in catabolism of fructose is found in rats which have had a high fructose diet over three weeks (22), effects of feeding fructose on above parameters seem to be very complex.

At present, more information is required until exact mechanism whereby fasting serum aminograms are modified by dietary sugars. Effects of dietary cholesterol also remain to be elucidated. However, since dietary sugar-dependent fluctuation of the serum aminogram appeared to be relevant to the magnitude of hypercholesterolemia, the present observation provides at least an alternate aspect.
of information to different responses of serum cholesterol levels to dietary manipulations.

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