Effects of Graded Levels of Dietary Casein and Corn Oil on Total Cholesterol and Triacylglycerol in Plasma and Liver of Rats

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Summary With the intention of examining the effects of dietary protein and oil levels on total cholesterol (T-CHOL) and triacylglycerol (TG) concentrations in the plasma and liver, male Wistar rats, weighing about 170 g, were fed diets containing graded levels of casein and corn oil for 2 wk. At the 5, 20, and 30% levels of dietary corn oil, plasma T-CHOL concentrations were generally enhanced in proportion to the rise of dietary casein level, but plasma TG contents were scarcely influenced by the level. At the 8 to 35% casein levels, plasma T-CHOL and TG concentrations were the highest at the 5% corn oil level, followed in order by the 20 and 30% levels of oil. At the 5 and 20% oil levels, hepatic T-CHOL contents were hardly changed at the 8 to 30% casein levels, but enhanced at the 35% casein level. At the 30% oil level, the T-CHOL contents tended to be changed proportionally to casein levels. At all levels of casein, hepatic T-CHOL contents tended to be relatively high at the 30% corn oil, middle at the 20% oil, and low at the 5% one. At each corn oil level, TG contents in the liver tended to be elevated at the 8 to 15% casein levels and highly preserved at the 15 to 25% ones. Then, the raised TG contents declined at the 5 and 20% levels of corn oil and remained constant at the 30% oil. At each casein level, the contents of hepatic TG were generally high at the 30% oil level, followed in order by the 20 and 5% oil levels. These results indicated that plasma and liver T-CHOL concentrations were proportionately enhanced by the increase in casein level, and plasma TG contents were hardly affected by the level and hepatic TG ones were lowered by relatively lower or higher casein level, and the rise in corn oil level generally reduced plasma T-CHOL and TG concentrations, but raised hepatic ones.

Key Words dietary casein level, dietary corn oil level, plasma cholesterol and triacylglycerol, liver cholesterol and triacylglycerol, rat plasma and liver

The concentrations of total cholesterol (T-CHOL) in the plasma and liver inclined to be enhanced by the increase in dietary protein level in experimental animals (1–3). When the biological value of protein was raised by adding methionine or sulfur-containing amino acids (s-amino acids) to casein or soy protein isolate, blood T-CHOL concentrations were elevated in rats (4–6). Serum CHOL level was also enhanced by feeding excess amino acids (6). On the other hand, a high carbohydrate (low oil) markedly enhanced the enzyme activities relating to triacylglycerol (TG) synthesis, and the concentrations of T-CHOL and TG in the plasma and liver were raised (7, 8). The increases in dietary corn oil suppressed the activities of liver lipogenic enzymes, and reduced the 14C-palmitic acid incorporation into TG in rats and the levels of serum and liver TG (9). The activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase known to be the rate limiting enzyme of cholesterol synthesis in animal tissues was proportional to the increment in dietary oil level, and plasma T-CHOL contents were increased (10–13). Though the above reports suggest that the increase in dietary protein level elevates the concentrations of plasma and liver lipids, and enhanced levels of dietary oils reduce the contents of T-CHOL and TG in the plasma and liver, there is little information available concerning the effects of dietary protein and oil levels on the concentrations of plasma and liver lipids. In the present study, the changes in the concentrations of T-CHOL and TG in the plasma and liver of rats fed the diets containing various levels of casein and corn oil were systematically investigated.

MATERIALS AND METHODS

Materials. Corn oil, which mainly consisted of 13.3% palmitic acid, 34.6% oleic acid, and 50.2% linoleic acid, and corn starch (α) were supplied by Hohnen Corporation Inc. (Shimizu, Japan) and Fuji Seifun Co. (Shimizu, Japan), respectively. Mineral and vitamin mixtures (AIN-76™) (14) and cellulose were obtained from Nihon Nosan Kogyo, Ltd. (Yokohama, Japan). Casein, sucrose, and the other chemicals were purchased from Nacalai Tesque, Inc. (Kyoto, Japan), Fuji Seito Co. (Shimizu, Japan), and Wako Pure Chemical Industries (Osaka, Japan), respectively. The composition of the basal diet is shown in Table 1.

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Table 1. Composition of the basal diet.

| Ingredient         | Basal (%) |
|--------------------|-----------|
| Casein             | 25.0      |
| Corn oil*          | 5.0       |
| Mineral mixture**  | 3.5       |
| Vitamin mixture**  | 1.0       |
| Vitamin C & K mixture*** | 0.2   |
| Choline bitartrate | 0.2       |
| Corn starch (α)    | 41.6      |
| Sucrose            | 18.5      |
| Cellulose          | 5.0       |

* Mainly consisted of 13.3% palmitic acid, 34.6% oleic acid, and 50.2% linoleic acid.
** AIN-76™ (16).
*** Ascorbic acid (L), 2,500mg and menadione, 22.5mg/100g of the mixture (15), which were added to prevent a lack of vitamins C and K.

Animals and diets. Male rats of the Wistar strain (Japan SLC, Inc., Hamamatsu), weighing about 150 g, were used. They were randomly housed in suspended individual cages in an air conditioned room with controlled temperature (24±1°C) and humidity (50±5%) and a 12-h light (06:00-18:00) and dark cycle, and fed a basal diet for 4 to 5 d. The animal care protocol was approved by the Experimental Animal Care Committee of the Faculty of Agriculture at Shizuoka University. Animals having nearly 170 g of average body weight were divided into eighteen groups of 5 rats each, and had free access to test diets and water for 2 wk. The test diets were prepared by replacing the protein and oil in the basal diet with 8, 15, 20, 25, 30, or 35% of casein and 5, 20, or 30% of corn oil at the expense of corn starch and sucrose, keeping the corn starch/sucrose ratio, 9:1. The 8% casein diet was arranged on the assumption of a diet containing the lowest level of dietary protein needed to barely preserve body weight.

The body weight and food intake were recorded every other day. The urine in each dietary group was collected into a vat containing 10 mL of 0.1 N sulfuric acid for 3 d immediately before the end of the experiment, and neutralized. The animals were killed by decapitation after 5 h (8:30-13:30) deprivation of food from their cages at the end of the feeding period, and instantly the blood was collected into heparinized polyethylene tubes and the liver was removed. The plasma was separated from the blood samples by centrifugation (900×g, for 10 min). These samples were stocked at −20°C until analysis.

Analysis. The concentrations of T-CHOL and TG in the plasma were estimated using clinical kits based on enzymatic methods {Cholesterol C-test (16) and Tri-glyceride G-test (17), Wako Pure Chemical Industries, Osaka, Japan}. Liver T-CHOL and TG contents were determined by the modified method of Zak-Henley (18) and Fletcher’s method (19), respectively, after the extraction of lipids with the mix solvent of chloroform–methanol (2:1, v/v) from the liver freeze-dried.

Statistical analysis. Data were expressed as means±SE for 5 rats. Effects of dietary casein and corn oil and the interaction were evaluated by two-way variance (ANOVA). Differences among means were examined using Duncan’s multiple range test (20).

RESULTS

Body weight gain, food intake, and liver weight

The changes in body weight gain, food intake, and liver weight are shown in Table 2. By two-way ANOVA, no effects of dietary levels of casein and corn oil on food intake and liver weights were detected except for the effect of casein level on weight gain, which was significant (p<0.01), but there was no significant interaction between dietary casein and corn oil levels in determining body weight gain, food intake, or liver weight. At the 5% corn oil level, there was hardly any difference in weight gain among casein levels except for the 8% casein, at which the lowest gain was observed. At the 20 and 30% levels of corn oil, weight gains were relatively high at the 25 and 35% casein levels, followed by the 15, 20, and 30% ones, which gains showed scarcely any difference, and low at the 8% casein. The weight gains were scarcely changed among any corn oil levels at the 20 to 30% levels of casein, but at the 8 and 35% casein levels inclined to be lower at the 5% corn oil than at the 20 and 30% oil, among which the gains were not different. At the 5 and 30% levels of corn oil, food intakes tended to be high at the 15% casein level, medium at the 20 to 35% levels of casein, and low at the 8% casein. At the 20% corn oil level, relatively high food intake was shown at the 15% casein level, followed in order by the 25 to 35% levels of casein, and low at the 8 and 20% levels. Independently of corn oil levels, liver weights were generally high at the 15% casein level, followed by the 20 to 35% levels of casein, and low at the 8% casein. At all casein levels, the liver weights tended to be larger at the 20 and 30% levels of corn oil than at the 5% level.

Plasma and liver lipids

Figure 1 shows the concentrations of T-CHOL and TG in the plasma. By the ANOVA, the levels of dietary casein and corn oil had significant effects on the concentrations of T-CHOL and TG in the plasma and liver, but no interactions of dietary casein and corn oil on them were detected. Regardless of dietary corn oil levels, plasma T-CHOL concentrations generally rose in proportion to the increase in casein level in a diet. At any casein level except for the 8% casein level, at which plasma T-CHOL contents were scarcely changed for any corn oil level, the T-CHOL contents inclined to be the highest at the 5% corn oil level, medium at the 20% corn oil, and the lowest at the 30% oil. Plasma TG concentrations, irrespective of corn oil level, were not greatly different among casein levels. The concentrations of plasma TG at any casein level tended to be the highest at the 5% corn oil level, followed in order by the 20 and 30% corn oils.
Fig. 1. Effects of graded levels of dietary casein and corn oil on the concentrations of plasma total cholesterol (T-CHOL) and triacylglycerol (TG). Male Wistar rats having average body weight, 170 g, were fed the diets containing graded levels of casein and corn oil for 2 wk. Values represent means ± SE for 5 rats. Points without vertical bars indicate that the bars fall within the size of the points. Different large and small superscript-letters show the significant difference at p<0.05 among the diets with different casein levels at each dietary corn-oil level and among the diets with different levels of corn oil at each dietary casein level, respectively.

As shown in Fig. 2, significant effects of casein and corn-oil levels in a diet but no interaction between them were detected in determining the contents of liver T-CHOL and TG by the ANOVA. At the 5 and 20% levels of corn oil, T-CHOL contents in the liver were enhanced at the 35% casein level compared to the other casein levels, among which contents were hardly changed, but tended to be proportionally raised according to the increase in the levels of corn oil in each dietary casein level. No significant effects of casein and corn-oil levels were detected in determining the contents of liver TG. The liver TG contents in the control rats were not changed by the graded levels of casein and corn oil. The liver TG contents in the control rats were not changed by the graded levels of casein and corn oil.

Table 2. Body weight gain, food intake, and liver weight of rats fed diets containing graded levels of casein and corn oil for 2 wk.

| Dietary casein level (%) | 8 | 15 | 20 | 25 | 30 | 35 | Casein | Corn oil | Casein×Corn oil |
|-------------------------|---|----|----|----|----|----|--------|-----------|-----------------|
| Body weight gain (g/d)  | 5 | 2.17±0.37^a,b | 5.51±0.31^a,b | 3.89±0.36^a,b | 5.40±0.39^a,b | 6.11±0.29^a,b | NS** | p<0.05 | NS |
| Food intake (g/d)       | 5 | 11.4±0.4^c | 14.0±0.3^a,b | 12.9±0.5^b | 13.2±0.4^a,b | 12.5±0.3^b | NS | NS | NS |
| Liver weight (g/100 g)  | 5 | 3.48±0.11^b,c | 4.48±0.10^a,b | 3.94±0.15^a,b | 4.11±0.12^a,b | 3.66±0.09^a,b | NS | NS | NS |
| Body wt (g)             | 5 | 4.17±0.13^c | 4.97±0.07^a,b | 4.10±0.11^a,b | 4.52±0.09^a,b | 4.37±0.15^a,b | NS | NS | NS |

* Mean±SE of 5 rats. Values in horizontal and vertical rows with different large and small superscript-letters, respectively, in measuring terms are significantly different at p<0.05.

** Not significant.
Effects of Dietary Protein and Oil Levels on Lipids and Urea

Fig. 2. Effects of graded levels of dietary casein and corn oil on the concentrations of liver total cholesterol (T-CHOL) and triacylglycerol (TG). Male Wistar rats having average body weight, 170g, were fed the diets containing graded levels of casein and corn oil for 2 wk. Values represent mean±SE for 5 rats. Points without vertical bars indicate that the bars fall within the size of the points. Different large and small superscript-letters show the significant difference at p<0.05 among the diets with different casein levels at each dietary corn-oil level and among the diets with different levels of corn oil at each dietary-casein level, respectively.

crease in casein level at the 30% corn oil level. Regardless of corn oil levels, liver TG contents were raised at the 8 to 15% casein levels and remained constant at the 15 to 25% caseins. At levels of casein over 30%, the TG contents declined to the concentrations shown at the 8% casein at the 5 and 20% corn oils, and scarcely changed at the 30% oil. At each casein level, the contents of liver TG tended to be the highest at the 30% corn oil, followed in order by the 20 and 5% levels of oil.

DISCUSSION

In the present study, the changes in body weight gain and food intake (Table 2) met our expectations from the previous reports (21–23). Liver weights nearly corresponded to the changes in weight gain (Table 2). Particularly, energy intakes were greater at the 20 and 30% levels of dietary corn oil than at the 5% level despite the decline in food intake (no calculation shown).

As shown in Fig. 1, plasma T-CHOL concentrations inclined to rise in proportion to the increase in dietary casein level independently of the level of dietary corn oil, which was similar to the earlier reports (1–6). This observation supports the view that the increase in dietary s-amino acids are involved in the enhancement of plasma CHOL concentration (4–6), since the amount of s-amino acids in a diet are proportional to the increase in protein level. At any casein level, T-CHOL contents in the plasma were the highest at the 5% corn oil, followed in order by the 20 and 30% corn oils, which did not coincide with our presumption from the previous reports (10–13). Plasma TG concentrations, irrespectively of dietary corn oil level, were hardly different among dietary casein levels, but the concentrations at each casein level were higher at the 5% oil than at the 30% one, which was basically consistent with the previous reports (7, 8), though the results exceeded our conjecture like plasma T-CHOL. This observation seemed to be due to the decrease in the activities of enzymes participating in fatty acid synthesis caused by the increase in dietary fat level as previously described (9).

Hepatic T-CHOL contents were scarcely changed at the 8 to 30% levels of dietary casein, but the contents at the 5 and 20% levels of dietary corn oil were higher at the 35% casein level than at the other levels of casein (Fig. 2), and those at the 30% oil level were nearly proportional to the casein level, which was as anticipated from the previous reports (4–6). At most casein levels, liver T-CHOL contents were significantly higher at the 30% corn oil than at the 5% oil. This result also came true as predicted, since the increment in oil level was reported to accelerate the activity of liver HMG-CoA reductase and enhance the CHOL amounts (10–13). Regardless of casein levels, the contents of TG in the liver were also significantly higher at the 30% corn oil level than at the 5% one as liver T-CHOL, which was as predicted, since a good deal of energy was ingested in spite of decreased food intake at the 30% corn oil level compared to the other levels of corn oil. Independently of corn oil levels, liver TG contents tended to be higher at the 15 to 30% casein levels than at the 8 and 35% ones, the cause of which remains to be made clear.

The decline in plasma T-CHOL and TG concentrations and the enhancement of hepatic ones by the feeding of high fat-containing diets to rats may be involved in the protein sparing effect of fats (24, 25), the appearance of which was suggested from the reductions of plasma urea concentrations and urinary urea amounts excreted into the urine observed in the present study.
(no data shown), though this needs to be further investigated.

In conclusion, the concentrations of plasma and liver T-CHOL were not a little influenced by dietary casein level, plasma TG concentrations were scarcely affected by the level of dietary casein, and liver TG contents were relatively low at the low and high casein levels in a diet and high at the middle level. Plasma T-CHOL and TG concentrations were nearly always higher at the low corn oil level than at the high level of oil, but the contents of liver T-CHOL and TG were contrary to those in the plasma.

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REFERENCES

1) Leveille GA, Sauberlich HE. 1964. Plasma and liver lipids of mice as influenced by dietary protein and sulfur-containing amino acids. J Nutr 84: 10–14.

2) Kato N, Tani T, Yoshida A. 1980. Effect of dietary level of protein on liver microsomal drug-metabolizing enzymes, urinary ascorbic acid and lipid metabolism in rats fed PCB-containing diets. J Nutr 110: 1686–1694.

3) Sugiyama K, Kanamori H, Takeuchi H. 1992. Effect of cholesterol-loading on plasma and tissue taurine levels in rats. Biosci Biotechnol Biochem 56: 676–677.

4) Kato N, Tani T, Yoshida A. 1980. Effect of dietary quality of protein on liver microsomal mixed function oxidase system, plasma cholesterol and urinary ascorbic acid in rats fed PCB-containing diets. J Nutr 111: 123–133.

5) Sugiyama K, Kushima Y, Muramatsu K. 1984. Effects of methionine, cystine and taurine on plasma cholesterol level in rats fed a high cholesterol diet. Agric Biol Chem 48: 2897–2899.

6) Katayama T, Hayashi J, Kishida M, Kato N. 1990. Effects of dietary excess amino acids on the concentrations of cholesterol, α-tocopherol, ascorbic acid, and copper in serum and tissues of rats. J Nutr Sci Vitaminol 36: 485–495.

7) Nestel PJ, Carroll KF, Havenstein N. 1970. Plasma triglyceride response to carbohydrates, fats and calorie intake. Metabolism 19: 1–18.

8) Gisberg H, Olefsky JM, Kimmelring G, Crapo P, Reaven GM. 1976. Induction of hypertriglyceridemia by a low-fat diet. J Clin Endocrinol Metab 42: 729–735.

9) Iritani N, Fukuda E. 1980. Effect of corn oil feeding on triglyceride synthesis in the rat. J Nutr 110: 1138–1143.

10) Takase S, Morimoto A, Nakanishi M, Muto Y. 1971. Long-term effect of medium chain triglyceride on hepatic enzyme catalyzing; lipogenesis and cholesterogenesis in rats. J Nutr Sci Vitaminol 23: 43–51.

11) Craig MC, Dugan RE, Muesing RA, Slakey LL, Porter JW. 1972. Comparative effects of dietary regimens on the levels of enzymes regulating the synthesis of fatty acids and cholesterol in rat liver. Arch Biochem Biophys 151: 128–136.

12) Ide T, Okamatsu H, Sugano M. 1978. Regulation by dietary fats of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase in rat liver. J Nutr 108: 601–612.

13) Fernandez ML, McNamara DJ. 1991. Regulation of cholesterol and lipoprotein metabolism in guinea pigs mediated by dietary fat quality and quantity. J Nutr 121: 934–943.

14) American Institute of Nutrition. 1977. Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. J Nutr 107: 1340–1348.

15) Kakuota T, Sukane I, Takihara T, Ozaki Y, Takeuchi H, Kuroyanagi M. 1996. Hypoglycemic effects of extracts from Lagerstroemia speciosa L. leaves in genetically diabetic KK-Ay mice. Biosci Biotech Biochem 60: 204–208.

16) Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. 1974. Enzymic determination of total serum cholesterol. Clin Chem 20: 470–475.

17) Spayd RW, Bruschi B, Burdick BA, Dappen GM, Eikenberry JN, Esders TW, Figueras J, Goodhue CT, LaRossa DD. 1978. Multilayer film elements for clinical analysis: applications to representative chemical determinations. Clin Chem 24: 1343–1350.

18) Kitamura M. 1968. The modified method of Zak-Henley. In: Clinical Chemical Analysis (Niwa M, Kitamura M, Saito M, eds), Vol III, p 72–78. Tokyo Kagakudouin, Tokyo.

19) Fletcher MJ. 1968. A colorimetric method for estimating serum triglycerides. J Clin Chem Acta 22: 393–397.

20) Duncan DB. 1955. Multiple range and multiple F tests. Biometrics 11: 1–42.

21) Takeuchi H, Muramatsu K. 1971. Correlation between the nutrition value of dietary protein and the activity of kidney transaminase of growing rats. J Nutr 101: 495–500.

22) Tanaka H, Yamauchi M, Kametaka M. 1974. Body composition and utilization of protein and energy in growing rats at different dietary protein to energy ratios by use of purified whole egg protein. Agric Biol Chem 38: 1113–1120.

23) Fukuda N, Hlok I, Etoh T, Hidaka T, Ikeda I, Sugano M. 1992. Comparisons of the effects of dietary fats on serum and liver lipid levels. Biosci Biotechnol Biochem 56: 676–677.

24) Nakano K, 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.

25) Nakano K, Ashida K. 1972. Further studies on the effect of dietary carbohydrate and fat on protein metabolism in rats. J Nutr 102: 283–290.