EFFECT OF DIFFERENT LEVELS OF DIETARY 
\(\alpha\)-TOCOPHEROL AND LINOLEATE 
ON PLASMA AND LIVER LIPIDS IN RATS

Shuji Cho* and Michihiro Sugano

Laboratory of Nutrition Chemistry, Department of Food Science and Technology, 
Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan
(Received October 19, 1977)

Summary  The influences of dietary \(\alpha\)-tocopherol, in combination with three different levels of linoleate (low, moderate or high levels), on the concentrations of plasma and liver lipids in rats were examined. Male Wistar rats were fed diets ad libitum for 2 weeks. dl-\(\alpha\)-Tocopheryl acetate was added to the diets in 0, 20 or 200 mg tocopherol/100 g diet. The following results were obtained.

(1) Higher dietary levels of \(\alpha\)-tocopherol tended to cause higher plasma lipid levels in proportion to the amounts added. This phenomenon is most remarkable in rats fed a diet containing cholesterol and low linoleate (0.8% of calorie).

(2) The concentrations of plasma lipids were influenced significantly than those of liver lipids by the levels of \(\alpha\)-tocopherol under the conditions of this experiment.

Despite numerous studies concerning the relationship between dietary \(\alpha\)-tocopherol and plasma cholesterol levels (1–3), available information is rather controversial and no consistent results have yet been achieved. In a previous report (4), in which the influences of dietary \(\alpha\)-tocopherol on plasma and liver lipids of rats fed diets containing different sugars were examined, the concentrations of plasma cholesterol and triglyceride were not necessarily paralleled with dietary tocopherol levels. It is thus assumed that the effect of tocopherol is influenced not only by its dietary levels, but also by other nutrients ingested simultaneously.

It is well known tocopherol requirements vary with the quality as well as the quantity of dietary fatty acids, and feeding fats high in linoleic acid increases the need for dietary tocopherol (1).

In the present study, effects of dietary tocopherol levels, in combination with
different quantities of linoleate in dietary fats, on the concentrations of plasma and liver lipids in young rats were studied.

MATERIALS AND METHODS

Male rats of the Wistar strain, weighing 75–83 g, were housed individually in stainless steel cages in an air-conditioned room at a temperature of approximately 23°C. They were fed experimental diets for two weeks. The composition of the basal diet was (%): casein (vitamin-free, NBC Co.) 20, salt mixture 4 (5), vitamin mixture in lactose 1 (5), cellulose 2, choline chloride 0.15, fat 5 and sucrose to 100. The diets contained vitamin A 2,400 I.U. and vitamin D 200 I.U. per 100 g.

The basal diet was supplemented with different levels of dl-α-tocopherol acetate (Tokyo Kasei Kogyo Co.) as follows (mg/100 g diet): group A; 0, group B; 20 and group C; 200. Cholesterol (1%) and cholic acid (0.25%) were added at the expense of sucrose.

The mixtures of fatty acid ethyl esters (Tokyo Kasei Kogyo Co.), which were free of tocopherol, were used as a source of dietary fat. The fatty acid compositions of these mixtures are as follows (%).

Experiment 1 (low linoleate diet, 0.8% calorie): 16:0; 35, 18:0; 15, 18:1; 38, 18:2; 7, others (14:0 and 16:1 etc.); 5.

Experiment 2 (moderate linoleate diet, 5.3% calorie): 16:0; 15, 18:0; 3, 18:1; 30, 18:2; 47, others (14:0 and 16:1 etc.); 5.

Experiment 3 (high linoleate diet, 10.4% calorie): 18:1; 8, 18:2; 92.

The compositions of these mixtures in experiments 1 and 2 are similar to those of lard and corn oil, respectively. Each group was composed of five rats. The diets and water were given freely, and the animals were sacrificed by decapitation after fasting overnight (16 hr).

The procedures for analyzing lipid components (triglyceride, phospholipid and total cholesterol) were the same as described elsewhere (5). The significance of results was analyzed by the Student’s “t” test and 2 × 3 factorial analysis of variance (6).

RESULTS

1. Food intake, body weight gain and liver weight (Table 1)

There were no significant differences in food intake and body weight gain of rats fed diets either free of or containing cholesterol, respectively, irrespective of dietary levels of tocopherol, throughout experiments. The extent of increases in liver weights due to feeding cholesterol were also similar among three experiments.

2. Concentrations of plasma and liver lipids (Table 2)

In experiment 1, the concentrations of plasma and liver lipids were not altered by tocopherol supplementation when the diets free of cholesterol were given. When 1%
Table 1. Effect of different levels of dietary tocopherol and linoleate on food intake, body weight gain and liver weight in rats.a

| Group             | Food intake (g/day) | Body weight gain (g/2 weeks) | Liver weight (g/100 g body weight) |
|-------------------|---------------------|------------------------------|-----------------------------------|
| Cholesterol-free  |                     |                              |                                   |
| A(5)b             | 14.3 ± 0.7          | 96.0 ± 5.9                   | 5.4 ± 0.2                         |
| B(5)              | 13.8 ± 0.5          | 92.4 ± 2.9                   | 5.5 ± 0.1                         |
| C(5)              | 14.2 ± 0.7          | 93.6 ± 2.9                   | 5.7 ± 0.3                         |
| Expt. 1           |                     |                              |                                   |
| Cholesterol 1%    |                     |                              |                                   |
| A(5)              | 13.8 ± 0.5          | 89.6 ± 3.4                   | 6.8 ± 0.2                         |
| B(5)              | 13.2 ± 0.4          | 84.6 ± 4.4                   | 6.7 ± 0.4                         |
| C(5)              | 13.9 ± 0.4          | 89.2 ± 2.7                   | 7.1 ± 0.1                         |
| Expt. 2           |                     |                              |                                   |
| Cholesterol-free  |                     |                              |                                   |
| A(5)              | 14.8 ± 0.5          | 96.4 ± 4.4                   | 5.5 ± 0.1                         |
| B(5)              | 14.5 ± 0.2          | 94.2 ± 2.8                   | 5.5 ± 0.1                         |
| C(5)              | 14.8 ± 0.7          | 95.6 ± 4.8                   | 5.6 ± 0.2                         |
| Cholesterol 1%    |                     |                              |                                   |
| A(5)              | 14.4 ± 0.3          | 87.2 ± 4.8                   | 6.7 ± 0.2                         |
| B(5)              | 14.1 ± 0.6          | 89.6 ± 5.0                   | 6.6 ± 0.2                         |
| C(5)              | 14.3 ± 0.3          | 86.8 ± 4.0                   | 6.5 ± 0.2                         |
| Expt. 3           |                     |                              |                                   |
| Cholesterol-free  |                     |                              |                                   |
| A(5)              | 13.2 ± 0.8          | 81.2 ± 5.5                   | 5.3 ± 0.1                         |
| B(5)              | 13.9 ± 0.9          | 86.6 ± 6.0                   | 5.2 ± 0.3                         |
| C(5)              | 14.4 ± 0.6          | 89.4 ± 2.4                   | 5.2 ± 0.2                         |
| Cholesterol 1%    |                     |                              |                                   |
| A(5)              | 14.0 ± 0.3          | 84.2 ± 3.4                   | 6.7 ± 0.1                         |
| B(5)              | 13.9 ± 0.4          | 81.8 ± 5.0                   | 6.3 ± 0.2                         |
| C(5)              | 14.4 ± 0.9          | 86.4 ± 5.3                   | 6.5 ± 0.2                         |

a Values are the means ± S.E. b Numbers of rats in parenthesis. Expt. 1: low linoleate diet (0.8% of calorie). Expt. 2: moderate linoleate diet (5.3% of calorie). Expt. 3: high linoleate diet (10.4% of calorie). dl-α-Tocopheryl acetate were supplemented to the basal diet as follows (mg/100 g diet): group A; 0, group B; 20, group C; 200.

Cholesterol was included in the diet, plasma triglyceride and phospholipid were significantly increased and total cholesterol tended to be elevated as the tocopherol level increased. On the other hand, no effects could be seen in liver lipids.

In the study with moderate linoleate in the diet (experiment 2), there were also no demonstrable changes in the liver lipids of rats fed the diets free of cholesterol, regardless of the levels of tocopherol. However, in the animals fed cholesterol, hepatic triglyceride level was the highest in the group B (a moderate tocopherol group). In rats fed no cholesterol, plasma triglyceride was the highest in the group C (an excess tocopherol group), while in the animals fed cholesterol, plasma triglyceride tended to increase with increasing levels of tocopherol supplementation. Again, in the cholesterol diet, plasma cholesterol level was the highest in the group C.

In experiment 3, as in experiments 1 and 2, liver lipids were not influenced by the levels of tocopherol irrespective of presence or absence of cholesterol in the diets,
Table 2. Effect of different levels of dietary tocopherol and linoleate on the concentrations of plasma and liver lipid.

| Experiment     | Group | Plasma (mg/dl) | Liver (mg/g) |
|----------------|-------|----------------|--------------|
|                |       | TG             | PL           | TC           | TG            | PL           | TC           |
| Cholesterol-free| A     | 135.5±13.8     | 200.8±9.8    | 105.0±4.7    | 29.8±2.6      | 29.4±0.6     | 2.7±0.1      |
|                | B     | 177.6±15.9     | 227.8±13.5   | 111.7±9.6    | 29.3±1.6      | 28.8±1.0     | 2.8±0.1      |
|                | C     | 168.3±12.0     | 199.9±5.3    | 98.7±3.4     | 30.7±2.1      | 27.1±1.0     | 2.8±0.1      |
| Expt. 1        |       |                |              |              |              |              |              |
| Cholesterol 1% | A     | 92.9±6.2       | 234.4±7.3    | 557.6±92.8   | 41.5±3.5      | 23.6±0.6     | 53.1±3.8     |
|                | B     | 130.5±9.9      | 271.0±6.4    | 674.6±57.0   | 44.1±2.5      | 23.6±0.5     | 52.4±2.4     |
|                | C     | 142.9±11.1     | 305.7±15.6   | 686.0±80.6   | 42.7±3.8      | 22.9±0.6     | 57.5±2.7     |
| Expt. 2        |       |                |              |              |              |              |              |
| Cholesterol-free| A     | 156.9±2.1      | 193.1±5.7    | 91.5±5.2     | 16.9±1.0      | 27.5±0.7     | 2.5±0.1      |
|                | B     | 147.6±13.9     | 191.2±18.4   | 99.4±11.0    | 15.5±0.5      | 29.6±0.3     | 2.5±0.1      |
|                | C     | 201.8±9.7      | 201.0±3.4    | 105.2±7.0    | 16.2±1.0      | 30.1±0.7     | 2.5±0.2      |
| Cholesterol 1% | A     | 192.9±19.7     | 253.3±15.4   | 369.3±17.9   | 28.7±2.1      | 28.1±1.3     | 43.9±2.3     |
|                | B     | 205.2±14.0     | 259.0±29.7   | 368.9±22.4   | 35.4±0.9      | 26.4±0.5     | 45.5±1.4     |
|                | C     | 232.0±9.2      | 258.5±7.5    | 414.6±4.0    | 30.5±1.3      | 27.2±0.8     | 48.9±3.6     |
| Expt. 3        |       |                |              |              |              |              |              |
| Cholesterol-free| A     | 96.2±10.3      | 207.6±4.6    | 99.7±2.7     | 14.7±1.1      | 31.2±0.8     | 2.2±0.1      |
|                | B     | 98.4±7.2       | 196.5±7.4    | 102.2±8.0    | 17.3±1.1      | 31.9±1.2     | 2.1±0.1      |
|                | C     | 82.1±13.1      | 228.2±9.7    | 103.2±2.0    | 17.1±1.3      | 32.1±0.8     | 2.2±0.1      |
| Cholesterol 1% | A     | 92.9±5.5       | 248.3±10.9   | 395.0±63.2   | 24.7±1.3      | 24.1±0.4     | 38.3±0.9     |
|                | B     | 111.5±10.3     | 254.1±7.8    | 381.3±49.8   | 28.0±0.7      | 27.5±0.9     | 44.1±1.0     |
|                | C     | 135.8±4.8      | 240.3±4.3    | 386.5±53.1   | 24.0±2.0      | 27.1±0.7     | 43.3±2.0     |

Values are the means ± S.E. TG, triglyceride; PL, phospholipid; TC, total cholesterol. * Significantly different from the corresponding group A at p<0.05. b Significantly different from the corresponding group B at p<0.05.
while in cholesterol-fed rats plasma triglyceride was markedly elevated by increasing levels of tocopherol.

3. *Factorial analysis of variance* (Table 3)

Concentrations of plasma and liver lipids are analyzed by $2 \times 3$ factorial analysis (two levels of cholesterol and three levels of tocopherol in the diets) of variance. In these calculations, cholesterol feeding resulted in significant changes in plasma and hepatic lipid levels compared with those of rats fed no cholesterol except for liver phospholipid in experiment 2. In experiment 1, the concentrations of plasma triglyceride and phospholipid were changed by tocopherol supplementation irrespective of dietary cholesterol levels. There are interactions in the concentrations of plasma phospholipid between the levels of tocopherol and cholesterol. In experiment 2, liver cholesterol and plasma triglyceride, and cholesterol were affected by tocopherol regardless of the presence or absence of cholesterol in the diet. Interaction between tocopherol and cholesterol are observed in liver triglyceride. In experiment 3, the concentrations of liver triglyceride and cholesterol were altered by dietary tocopherol. There is an interaction in plasma triglyceride.

Table 3. Effect of dietary tocopherol and cholesterol levels on the plasma and liver lipid concentrations in rats.

| Experiment | Variables | Plasm | Liver |
|------------|-----------|-------|-------|
|            |           | TG    | PL    | TC    | TG    | PL    | TC    |
| 1          | Toc       | S     | S     | N     | N     | N     | N     |
|            | Chol      | S     | S     | S     | S     | S     | S     |
|            | Toc × Chol| N     | S     | N     | N     | N     | N     |
| 2          | Toc       | S     | N     | S     | N     | N     | S     |
|            | Chol      | S     | S     | S     | S     | N     | S     |
|            | Toc × Chol| N     | N     | N     | S     | N     | N     |
| 3          | Toc       | N     | N     | N     | S     | N     | S     |
|            | Chol      | S     | S     | S     | S     | S     | S     |
|            | Toc × Chol| S     | N     | N     | N     | N     | N     |

* Plasma and liver lipids were analyzed by $2 \times 3$ factorial analysis of variance (6). S, significant ($p<0.05$); N, not significant; Toc, tocopherol; Chol, cholesterol.

**DISCUSSION**

Dietary requirement for essential fatty acids of male rats is estimated to be 1–2 calorie % as linoleic acid (7). Dietary levels of this acid in the present studies were about 0.8, 5.3 and 10.4 calorie % in experiments 1, 2 and 3, respectively. Judging from these figures, it is not clear whether rats in experiment 1 are sufficient or insufficient to the supply of essential fatty acids.
The relationship between intake of polyunsaturated fatty acids and requirement for tocopherol has been extensively investigated. According to Jager (8), tocopherol requirements for rats are 2.4 to 3.2 mg per 1,000 kcal of food so far as dietary levels of linoleic acid are up to 10 calorie %. When the ratio of tocopherol (mg): polyunsaturated fatty acid (g) in a diet is lower than 0.6 (9), the experimental animals are regarded as in the status of tocopherol deficiency. In our experiments, the amounts of dietary tocopherol in group B (20 mg/100 g diet) and C (200 mg/100 g diet) are enough to satisfy above two propositions but rather excessive.

The present study shows that higher supplementary levels of tocopherol tend to result in higher plasma lipid levels. Gray and Loh have demonstrated that ingestion of 100 mg tocopherol per day brings about an increase in phospholipid and cholesterol in the plasma of healthy human subjects (10). Male Albino rats, maintained on a standard diet and fed 100 mg tocopherol per day have been shown to have significantly higher levels of liver phospholipid and cholesterol than those of control rats fed no additional tocopherol (11). In these two cases, dietary tocopherol levels are regarded as excess. Although Gray (11) have suggested that dietary tocopherol may enhance lipid synthesis in the liver, the exact mechanism responsible for elevation of plasma or liver lipid levels due to large doses of this vitamin is obscure at present.

In our experiments, the influence of tocopherol was most remarkable in rats fed a diet containing cholesterol and low linoleate. It is well known that feeding cholesterol or saturated fat (low linoleate) to rats causes fatty liver and hyperlipidemia (12). In addition, the rate of tissue lipid peroxidation is known to be accelerated by feeding cholesterol to rats (13). From these observations, it is thus likely that tocopherol, when included at higher levels in the diet, may exert this effect when lipids in the tissue are oxidized or are susceptible to oxidation.

In some experiments with adequate levels of dietary tocopherol (2, 3) plasma or tissue cholesterol levels of rats were significantly lower than those of tocopherol-deficient group. Alfin-Slater and Morris have assumed that the effectiveness of dietary tocopherol in lowering plasma cholesterol may be mediated through its antioxidant effect, reservation of polyunsaturated fatty acid required for cholesterol transport (1). In our studies, however, plasma cholesterol levels tended to be elevated by excessive tocopherol supplementation. It seems therefore likely that the effect of tocopherol on plasma lipid is not explained simply only by an antioxidant hypothesis. As proposed by Green (14), this vitamin may have an additional unknown specific function(s).

Judging from the data of other investigators (15), the rate of tocopherol absorption and the concentrations of liver and plasma tocopherol seem to be significantly different among three tocopherol groups. It is therefore likely that the differences in the levels of dietary \( \alpha \)-tocopherol cause observed changes in plasma and liver lipids.

From the results of factorial analysis of variance, significant interactions
between the levels of dietary tocopherol and cholesterol are observed only in a few cases.

In experiment 1, irrespective of the levels of dietary tocopherol, the concentrations of plasma triglyceride in rats fed cholesterol were lower than those of cholesterol-free group. This observation was in contrast to the results of experiments 2 and 3. In addition, in the cholesterol diet, the levels of plasma triglyceride in the tocopherol-free group was extremely low compared with the moderate or excess tocopherol group. These data suggest that the levels of plasma triglyceride is not fully influenced by dietary cholesterol under the conditions of experiment 1.

Finally, the concentrations of plasma lipids were affected much more markedly than those of liver lipids by the levels of tocopherol.

REFERENCES

1) ALFIN-SLATER, R. B., and MORRIS, R. S. (1963): Vitamin E and lipid metabolism. *Adv. Lipid Res.*, 1, 183-210.
2) ALFIN-SLATER, R. B., SHIMMA, Y., HANSEN, H., WELLS, P., and AFTERGOOD, L. (1972): Dietary fat composition and tocopherol requirement: III Quantitative studies on the relationship between dietary linoleate and vitamin E. *J. Amer. Oil Chem. Soc.*, 49, 395-402.
3) CHEN, L. H., LIAO, S., and PACKETT, L. V. (1972): Interaction of dietary vitamin E and protein level or lipid source with serum cholesterol level in rats. *J. Nutr.*, 102, 729-732.
4) CHO, S., YAMAMOTO, K., IDE, T., and SUGANO, M. (1977): The effects of the dietary α-tocopherol and different sugars on plasma and liver lipids in rats. *Eiyo To Shokuryo (J. Jpn. Soc. Food Nutr.)*, 30, 275-281.
5) YANAGITA, T., and SUGANO, M. (1975): Liver and plasma lipids in rats fed casein reacted with oxidized ethyl linoleate. *Agric. Biol. Chem.*, 39, 63-69.
6) SNEDECOR, G. W., and COCHRAN, W. G. (1956): Statistical methods, The Iowa State University Press, Ames, Iowa.
7) HOLMAN, R. T. (1960): The ratio of trienoic: tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J. Nutr.*, 70, 405-410.
8) JAGER, F. C. (1972): Linoleic acid intake and vitamin E requirement in rats and ducklings. *Annal. N.Y. Acad. Sci.*, 203, 199-211.
9) HARRIS, P. L., and EMBREE, N. D. (1963): Quantitative consideration of the effect of polyunsaturated fatty acid content of the diet upon the requirements for vitamin E. *Am. J. Clin. Nutr.*, 13, 385-392.
10) GRAY, D. E., and LOH, S. H. (1958): Influence of α-tocopherol acetate on some lipids and nitrogen compounds of plasma in human subjects. *Can. J. Biochem. Physiol.*, 36, 269-273.
11) GRAY, D. E. (1959): Influence of α-tocopherol acetate on cholesterol and phospholipid synthesis in rat liver homogenates. *J. Vitaminol.*, 5, 19-23.
12) NARAYAN, K. A., McMULLEN, J. J., BUTLER, D. P., WAKEFIELD, T., and CALHOUN, W. K. (1974): The influence of a high level of dietary corn oil on rat serum and liver lipids. *Nutr. Rep. Int.*, 10, 25-33.
13) TSAI, A. C. (1975): Lipid peroxidation and glutathione peroxidase activity in the liver of cholesterol fed rats. *J. Nutr.*, 105, 946-951.
14) GREEN, J. (1972): Vitamin E and the biological antioxidant theory. *Annal. N.Y. Acad. Sci.*, 203, 29-44.
15) BIERI, J. G., and FARRELL, P. M. (1976): Vitamin E, *Vitam. Horm.*, 34, 31-75.