EFFECT OF VOLUNTARY EXERCISE AND DIETARY PROTEIN LEVELS ON SERUM LIPOPROTEIN DISTRIBUTIONS AND LECITHIN:CHOLESTEROL ACYLTRANSFERASE (LCAT) ACTIVITY OF MICE

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Summary The effect of voluntary exercise on serum lipoprotein distributions, lecithin:cholesterol acyltransferase (LCAT) activity and serum electrophoretic patterns of mice fed different levels of dietary protein were investigated.

Serum cholesterol of all exercise groups (E) showed a lower value than that of the non-exercise groups (NE). Ratios of cholesteryl ester to serum total cholesterol tended to be higher in the exercise groups than in non-exercise groups. High density lipoprotein (HDL)-cholesterol/serum total cholesterol ratios and HDL-cholesterol/low density lipoprotein (LDL)-cholesterol ratios were increased by voluntary exercise. With regard to low density lipoprotein cholesterol levels, there were significant differences between 20% E and 20% NE groups, 4% E and 4% NE groups, respectively. It was found that HDL fractions in serum lipoprotein patterns of exercise groups differed from those of non-exercise groups. This seemed to be prominent in low protein diet groups. LCAT activity showed decreasing values as dietary protein levels decreased and its activity was raised by voluntary exercise in all groups.

Keywords voluntary exercise, protein-deficient diet, serum lipoprotein, high density lipoprotein, lecithin:cholesterol acyltransferase (LCAT), serum electrophoretic patterns

Many investigations concerning the effects of exercise on serum lipids have been reported from a preventive point of view; for instance, hyperlipemia and atherosclerosis have often been discussed with regard to the relationship between exercise and health (1–4). Furthermore, the relation between physical exercise and serum lipoprotein or lecithin:cholesterol acyltransferase has also been reported by Lopez-S et al. (5). They found an increase in the activity of the lecithin:cholesterol

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acyltransferase in four of the exercising subjects for which the activity was assayed before and at the end of a 7-week exercise program.

We have previously reported an investigation (6) concerning the effects of voluntary exercise on liver and serum lipids of mice fed four levels of dietary protein. It was expected that the changes in these lipids through exercise and dietary protein levels would reflect the greater metabolic changes in serum lipoprotein and LCAT activity. A previous study (6) also showed that an increase of lipids in liver is easily brought about in animals fed low protein diets. In liver disease, the esterification of cholesterol may be impaired and alterations in plasma LCAT activity have been reported (7, 8). It is thought, therefore, that an impairment of liver function due to the increase of lipids in liver (9, 10) may have effect on LCAT activity or serum lipoprotein. There are still very few reports concerned with the nutritional investigation of the relation between exercise and lipoprotein or LCAT activity (11).

The purpose of the present study was to investigate the effect of voluntary exercise on serum lipoprotein distributions, LCAT activity and serum electrophoretic patterns of mice fed three levels of dietary protein.

**EXPERIMENTAL**

*Animals and diets.* Weaning, male JCL:ICR mice (Japan Clea Inc., Tokyo), weighing 11 to 13 g, were used in this experiment. The experimental diets are shown in Table 1. All mice were housed individually in wire net cages and fed a 20% casein diet for one week. After this period, they were divided into six groups, designated non-exercise group (20% NE) or exercise group (20% E) on a 20% casein diet, non-exercise group (6% NE) or exercise group (6% E) on a 6% casein diet and non-exercise group (4% NE) or exercise group (4% E) on a 4% casein diet. There were 14 mice in each group. All exercise groups were housed individually in wire net cages with a revolving treadwheel. The animals were kept individually on the experimental diets for about 90 days. Throughout the experiment, fresh diet and water were supplied ad libitum. Lighting was regulated to provide 12 hr of light, from 8:00 a.m. to 8:00 p.m. and 12 hr of darkness, from 8:00 p.m. to 8:00 a.m. Room temperature was maintained at 24 ± 1°C.

*Measurement of the amount of voluntary exercise.* The revolving treadwheel (circumference: 44 cm, width: 6 cm) allowed the animals to exercise voluntarily. The amount of voluntary exercise (running distance) was measured by an electromagnetic counter which records the number of rotations of the revolving treadwheel.

*Sampling of the blood and liver.* All mice were deprived of food for 10 hr and then killed by dissection of the axillary artery under light ethyl ether anesthesia. Blood was immediately collected in a test tube. Then the liver was removed and weighed. Serum was prepared by a routine method.

*Chemical analysis.* Serum cholesterol was determined by the Cholesterol B-
Table 1. Diet composition.

| Diet                      | 4% casein | 6% casein | 20% casein |
|---------------------------|-----------|-----------|------------|
|                           | g/100 g diet |          |            |
| Potato starch             | 85        | 83        | 69         |
| Soybean oil              | 5         | 5         | 5          |
| Casein                    | 4         | 6         | 20         |
| Salt mixture\(^a\)       | 4         | 4         | 4          |
| Water-soluble vitamin mixture\(^b\) | 1         | 1         | 1          |
| Fat-soluble vitamin mixture\(^c\) | 0.8       | 0.8       | 0.8        |
| Choline chloride         | 0.2       | 0.2       | 0.2        |

\(^a\) Harper's salt mixture purchased from Oriental Yeast Co., Ltd.
\(^b\) Harper's water-soluble vitamin mixture purchased from Oriental Yeast Co., Ltd.
\(^c\) Fat-soluble vitamin mixture: vitamin A, 1,000 IU/100 g diet; vitamin D, 100 IU/100 g diet; vitamin E, 10 mg/100 g diet.

Test (Wako Pure Chemical Industries, Ltd.). Serum free cholesterol was measured by the Free-cholesterol B-Test (Wako Pure Chemical Industries, Ltd.). Assay of lecithin:cholesterol acyltransferase activity was carried out by the method of Stokke and Norum (12). (7-\(^3\)H(N))-Cholesterol with specific activity of 10–25 Ci/mmol (obtained from New England Nuclear, Boston) was added to serum. The radioactivity was measured with a liquid scintillation spectrometer (Packard, Tri-carb; Packard Instrument Co., Downers Grove, Illinois). The activity of the lecithin: cholesterol acyltransferase reaction is given by the percentage of labeled cholesterol acylated per hour to labeled cholesterol in serum.

Isolation of lipoprotein fractions. One hundred and seventy-five microliters of serum were centrifuged (Beckman Airfuge) for 2.5 hr at 100,000 rpm. Serum lipoproteins were quantified by cholesterol content. Cholesterol was measured in serum and the bottom fractions of tubes centrifuged at a density of serum and 1.060 g/ml. Top and bottom fractions were separated by air aspiration (Beckman Airfuge Tube Fractionator); LDL cholesterol was obtained by subtracting HDL cholesterol (bottom fraction of tube centrifuged at density 1.060 g/ml) from LDL+HDL cholesterol (bottom fraction of tube centrifuged at serum density).

Electrophoretic procedure and incubation of serum for electrophoresis. The serum was prestained with Sudan black B and electrophoretically separated at a polyacrylamide gel concentration of 3.75%. The electrophoretic procedure for Sudan B-prestained samples has been described in detail by Narayan et al. (13).

The incubation was performed in a thermostat for 24 hr at 37°C. Prior to use
for incubation test tubes with polycarbonate screw caps were sterilized by heating at 121°C for 20 min. The equipment used in the present experiments were a gel electrophoresis apparatus GE-4 (Pharmacia Fine Chemicals) and a dual-wavelength TLC scanner CS-900 (Shimadzu Seisakusho, Ltd., Kyoto, Japan).

RESULTS

**Body weight gains and amount of voluntary exercise**

As shown in Table 2, body weight gains were suppressed as dietary protein levels decreased. The suppression in the 4% casein diet group was particularly marked. It is interesting that the body weight gains of the 6% E group increased to levels approaching those of the 20% E group.

Running distances were slightly longer in the 20% E group than in the 4% and 6% E groups, but no significant differences were observed.

**Serum cholesterol**

As shown in Table 3, serum total cholesterol values tended to increase with decreasing levels of dietary protein. The values of exercise groups were lower than those of the non-exercise groups. In the 20% casein diet groups, a significant difference between the exercise and non-exercise groups was found (p<0.01). Free cholesterol values of the 20% NE group were lower than those of the 4% and the 6% NE groups. When the ratios of cholesteryl ester to serum total cholesterol of the 20% NE group was compared with those of the 4% and 6% NE groups, the 20% NE group was found to have significantly higher values than the 4% and 6%

| Group | Initial body weight (g) | Final body weight (g) | Average daily amount of exercise (m) |
|-------|------------------------|-----------------------|-------------------------------------|
| 4%    | NE 20.0±0.7 (14)       | 18.5±0.8 (13)         | —                                   |
|       | E 21.2±0.2 (14)        | 20.9±0.7 (14)         | 3,344±398 (10)                      |
| 6%    | NE 21.4±0.3 (14)       | 28.8±1.3 (14)         | —                                   |
|       | E 21.3±0.2 (14)        | 30.5±0.9 (14)         | 3,186±562 (9)                       |
| 20%   | NE 21.3±0.3 (14)       | 35.7±0.9 (14)         | —                                   |
|       | E 21.3±0.3 (14)        | 33.2±0.6 (14)         | 3,902±357 (10)                      |

*p<0.05 Between non-exercise and exercise groups.
The number of mice is in parentheses. Values are means±SE.
4%, 4% casein diet; 6%, 6% casein diet; 20%, 20% casein diet; NE, non-exercise group; E, exercise group.

*J. Nutr. Sci. Vitaminol.*
Table 3. Effect of voluntary exercise on serum lipoprotein distributions of mice fed three levels of dietary protein.

| Diet group | 4% |       | 6% |       | 20% |       |
|------------|----|-------|----|-------|-----|-------|
|            | NE | E     | NE | E     | NE  | E     |
| TC<sup>d</sup> | 165.5 ± 11.1 (5) | 134.0 ± 4.0 (5) | 159.0 ± 6.0 (6) | 140.2 ± 2.9 (6) | 149.0 ± 3.1 (7) | 107.6 ± 8.6<sup>e</sup> (7) |
| FC<sup>g</sup> | 31.1 ± 2.7 (5) | 21.8 ± 1.3<sup>f</sup> (5) | 33.7 ± 2.1 (6) | 26.0 ± 1.3 (6) | 16.2 ± 1.9 (7) | 10.1 ± 1.4<sup>f</sup> (7) |
| ER<sup>f</sup> | 80.9 ± 2.0 (5) | 83.3 ± 1.1 (5) | 78.8 ± 1.1 (6) | 81.4 ± 0.9 (6) | 89.1 ± 1.1 (7) | 91.6 ± 1.0 (7) |
| HDL-C<sup>g</sup> | 113.3 ± 6.9 (4) | 112.5 ± 3.1 (5) | 122.6 ± 3.6 (5) | 114.0 ± 3.7 (6) | 109.8 ± 4.3 (6) | 89.5 ± 7.2 (6) |
| LDL-C<sup>h</sup> | 31.2 ± 4.3 (4) | 18.8 ± 1.1<sup>f</sup> (5) | 31.4 ± 3.4(5) | 22.7 ± 2.4 (6) | 32.4 ± 2.6 (6) | 17.0 ± 3.2<sup>f</sup>(6) |
| HDL/TC | 0.681 ± 0.008<sup>g</sup> (4) | 0.826 ± 0.022 (5) | 0.774 ± 0.017 (5) | 0.801 ± 0.025 (6) | 0.707 ± 0.029 (6) | 0.799 ± 0.018<sup>g</sup>(6) |
| HDL/LDL | 3.52 ± 0.41<sup>g</sup> (4) | 5.22 ± 0.21 (5) | 4.04 ± 0.37 (5) | 4.54 ± 0.34 (6) | 3.36 ± 0.94 (6) | 4.78 ± 0.88<sup>g</sup>(6) |

<sup>a</sup> p < 0.01, <sup>b</sup> p < 0.02, <sup>c</sup> p < 0.05  Between non-exercise and exercise group. <sup>d</sup> Total cholesterol, <sup>e</sup> free cholesterol, <sup>f</sup> ester ratio, <sup>g</sup> high density lipoprotein-cholesterol, <sup>h</sup> Low density lipoprotein-cholesterol.

Values are mean ± SE. The number of mice is in parenthesis.

d, e, g, h: Values are expressed as mg/100 ml.
NE groups. In all three casein diet groups, the ratios of cholesteryl ester to serum total cholesterol of the exercise groups tended to be higher than those of non-exercise groups.

Serum lipoprotein distributions

The results are summarized in Table 3. In all casein diet groups, LDL-cholesterol level of exercise groups showed a tendency to be lower than that of non-exercise groups. Notably, there were significant differences between the 20% E and 20% NE groups, 4% E and 4% NE group, respectively (20% E vs. 20% NE; p<0.01, 4% E vs. 4% NE; p<0.05). With regard to HDL-cholesterol level, no marked differences between the exercise groups and non-exercise groups were found. When the HDL-cholesterol/serum total cholesterol ratio and HDL-cholesterol/LDL-cholesterol ratio of exercise groups were compared with those of non-exercise groups, it was found that the ratios of exercise groups were higher than those of non-exercise groups. Particularly significant differences were found between 20% E, 4% E and 20% NE, 4% NE, respectively (HDL/serum total cholesterol, 20% E vs. 20% NE; p<0.02, 4% E vs. 4% NE; p<0.01, HDL/LDL, 20% E vs. 20% NE; p<0.02, 4% E vs. 4% NE; p<0.01).

Serum lipoprotein patterns

As shown in Fig. 1, similar patterns of serum lipoproteins in Sudan B-prestained samples were observed in all non-exercise groups. Namely, HDL fractions were separated into two bands. Notably, an upper layer of two bands exhibited greater intensity in low protein diet groups. It is considered that the upper layer and lower layer of HDL fractions may be identical to HDL$_3$ and HDL$_2$ fractions, respectively (22). On the other hand, the HDL fraction of all exercise groups appeared as only one band, which may be identical to the HDL$_2$ fraction (22). Thus, when the HDL fractions of non-exercise groups were compared with those of exercise groups, their electrophoretic patterns were markedly different. As shown in Fig. 2, the incubation of serum for 24 hr at 37°C resulted in changes in the electrophoretic patterns of HDL fractions. Namely, HDL fractions of the incubated serum showed only one band, whereas fractions of the non-incubated serum appeared as two bands.

Lecithin: cholesterol acyltransferase (LCAT) activity

The results are shown in Table 4. LCAT activity showed decreasing values as dietary protein levels decreased. In all groups, the activity of the exercise groups was significantly higher than that of non-exercise groups (20% E vs. 20% NE; p<0.01, 6% E vs. 6% NE; p<0.05, 4% E vs. 4% NE; p<0.01).
Fig. 1. Polyacrylamide disc gel electrophoretic patterns in 3.75% gel. Migration is from top to bottom. Twenty % NE to 4% E are representative Sudan black B-prestained serum lipoprotein patterns from the six groups. The lead band in these patterns is albumin. Sample load, 20μl of serum per gel. VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

Fig. 2. Representative densitometer traces and serum electrophoretic patterns of mice fed a 20% casein diet. The incubation was performed under N₂. A, nonincubated serum; B, incubated serum; a, albumin; b, HDL₂; c, HDL₃; d, LDL; e, VLDL.

DISCUSSION

The decrease in serum total cholesterol level due to exercise was similar to that found in a previous study (6) except for the 4% E group. The cholesterol ester ratio of exercise groups tended to be higher than that of non-exercise groups. The
Table 4. Effect of voluntary exercise on lecithin:cholesterol acyltransferase activities of mice fed three levels of dietary protein.

| Group | Number of mice | Lecithin:cholesterol acyltransferase (LCAT) activities (%) |
|-------|----------------|----------------------------------------------------------|
| 4%    | NE 5           | 6.4 ± 0.6                                                |
|       | E 6           | 10.0 ± 0.4*                                              |
| 6%    | NE 6           | 8.3 ± 0.7                                                |
|       | E 7           | 11.2 ± 0.6*                                              |
| 20%   | NE 6           | 11.2 ± 0.9                                               |
|       | E 6           | 15.5 ± 0.7*                                              |

Each test tube contained 100 μl of serum, 30 μl of the albumin-stabilized cholesterol emulsion, and 20 μl of 10 mM Ellman reagent. The reaction mixture was preincubated at 37°C. The esterification reaction was started by adding 20 μl of 0.1 M mercaptoethanol. The activity of LCAT is given as percentage of labelled cholesterol acylated per hour to labelled cholesterol in serum.

* p < 0.01 Between non-exercise and exercise groups. Values are means ± SE.

cholesterol ester ratio of the 20% NE group was significantly higher than that of the 4% and 6% NE groups. There is evidence showing that LCAT plays a considerable role in the formation of plasma cholesterol ester (14). In the present study, it was found that LCAT activity rises as dietary protein levels increase and that its activity is also elevated by exercise. Therefore, it is surmised that the increase in the cholesterol ester ratio is caused by the effects of LCAT. This enzyme appears to exist in both the HDL and LDL fractions and to react with both lipoproteins, but the activity is considerably higher in the HDL fraction than in the LDL fraction (15, 16). The present investigation showed that HDL/serum total cholesterol ratio and HDL/LDL ratio of exercise groups are higher than those of non-exercise groups. Accordingly, it is thought that HDL-cholesterol levels of exercise groups increase relatively more than those of non-exercise groups, though no difference between absolute HDL cholesterol levels of exercise and non-exercise groups was found. The results mentioned above appear to be in agreement with the results obtained by Lopez-S et al. (5) and Wood et al. (17) in studies on man. In addition, since only esterified cholesterol can be exchanged between tissue and plasma lipoprotein, and since HDL by virtue of its protein structure is a favorable substrate for LCAT, an interesting role of HDL in cholesterol migration from the tissue pools to liver for catabolism and excretion is postulated (18). In the present results, LDL-cholesterol of exercise groups was lower than that of non-exercise groups. Reducing serum triglyceride levels induced by exercise found in a previous study (6) may be, in part, due to its effect on low density lipoprotein. It has been thought that LCAT synthesized in liver is secreted into the blood (19), and

1. Lopez-S et al. (5)
2. Wood et al. (17)
3. J. Nutr. Sci. Vitaminol.
functional damage of the liver results in decreased concentration and/or activity of the plasma LCAT (20). The present results show that the LCAT activity of the 4% NE group decreased markedly as compared with other groups. In our previous study (6), it was found that lipids in the liver of animals fed a low protein diet increase more than those of animals fed normal protein diets. Enwonwu et al. (21) have reported that histological evaluation of liver from protein-calorie-deficient animals indicates an extensive fatty metamorphosis and loss of cytoplasmic basophilia. Thus, in animals fed a low protein diet, particularly the 4% NE group, an impairment of liver function may be brought about. However, the activity of LCAT in low protein diet groups was elevated by voluntary exercise. It is interesting that functional damage of the liver caused by nutritional deficits may be restrained to some extent by voluntary exercise. In the present study, the HDL fraction in serum lipoproteins of all non-exercise groups was separated into two bands by electrophoresis, whereas those of all exercise groups showed only one band. Generally, total HDL can be separated into HDL2 and HDL3 fractions (22). Nichols et al. (23) have reported that, during the incubation of human serum for 24 hr at 37°C, the serum concentrations of HDL3 decreased, while those of HDL2 increased. This indicates that HDL3 exists as a favorable substrate of LCAT. In the present investigation, it was observed that HDL fractions in the electrophoretic pattern of non-incubated serum are separated into two bands, but those of incubated serum show only one band. This may be because HDL3 is converted into HDL2 by LCAT. In the present experiment, we observed in the electrophoretic patterns that the non-exercise groups with low activity of LCAT appear to have two bands corresponding to the HDL2 and HDL3 fractions, while the exercise groups with high activity of LCAT show only one band corresponding to the HDL2 fraction. This phenomenon indicates a possibility that the conversion of HDL3 catalyzed by LCAT to HDL2 is markedly accelerated in exercise groups. Thus, these experiments seem to support the idea that LCAT activity increases with exercise.

The evidence concerning LCAT activity and lipoprotein distributions described above indicates an important effect of voluntary exercise on the transport of serum cholesterol. It is also thought that functional impairment of the liver of animals fed low protein diets may be improved to some extent by voluntary exercise.

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