Effect of Exercise on Turnover and Fate of [4-¹⁴C]-Cholesterol Administered Intraperitoneally and Orally to Rats[†]

Nobuhiro FUKUDA, Yasuyuki TSUGE* and Michihiro SUGANO

Laboratory of Nutrition Chemistry, Kyushu University School of Agriculture, Fukuoka

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The fate of [4-¹⁴C]-cholesterol administered intraperitoneally or orally was compared in exercised (treadmill running for 14 days) and sedentary rats.

Plasma triglyceride, phospholipid and cholesterol decreased in exercised rats and this reduction lasted at least for 10 days after exercise was terminated.

When rats received [4-¹⁴C]-cholesterol intraperitoneally or orally, the turnover rate of serum cholesterol was considerably higher in exercised rats at the time shortly after the administration of the label. The radioactivity remaining in the liver was consistently lower in exercised rats, whereas that in extrahepatic tissues was the same between two groups.

Excretion into feces of the label as total steroids was moderately enhanced by exercise. This effect was almost entirely ascribed to the increase in output of the label shortly after the administration.

These results suggest that the mechanism responsible for cholesterol lowering effect of exercise is mainly attributable to the increase in turnover of cholesterol in the hepato-plasmic system. The moderate increase in fecal output of endogenous steroids may be the reflection of the increased turnover.

We have previously reported that, in rats, treadmill running causes not only the decrease in plasma cholesterol, but also increases in hepatic cholesterolgenesis, lipoprotein-lipase activity in the cardiac muscle and neutral and acidic steroid excretion into feces.^{1~4)}

Sodhi⁵⁾ has predicted that, when plasma cholesterol decreases under the condition in which hepatic cholesterol synthesis is enhanced, the turnover rate of plasma cholesterol should be increased. Therefore, exercise may cause an increase in the turnover rate of plasma cholesterol. Information concerning the effects of exercise on turnover in the plasma compartment appears very scanty, although there is evidence that specific activity of serum cholesterol in exercised rats which received intraperitoneal administration of [1-¹⁴C]-acetate is higher than that in the sedentary animals.⁶⁾

In the present study, effects of treadmill running on the turnover rate of serum chole-

sterol, fecal excretion as neutral and acidic steroids and the distribution of the radioactivity in tissues were studied in rats which received [4-¹⁴C]-cholesterol intraperitoneally or orally.

MATERIALS AND METHODS

Materials. [4-¹⁴C]-Cholesterol (57.7 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, England and purified by thin-layer chromatography on silica-gel G with *n*-hexane-chloroform (1:1, v/v) prior to use.

Animals. Male Wistar rats weighing 140~150 g (Kyudo Co., Kumamoto) were housed individually in an air-conditioned room (22~25°C, light on 6:00 a.m. ~6:00 p.m., 65% relative humidity) and maintained on a commercial pellet ration (Oriental Yeast Co., Type NMF).

The animals were randomly divided into exercise and pair-feeding sedentary groups. The exercise group was subjected to a mechanical treadmill running for 1 hr everyday (1:00 p.m. to 2:00 p.m.); 11 m/min for the first one week and 16 m/min for the next one week as described previously.^{1~4)} Control animals were handled in the same manner except that they did not run.

Administration of label. On the next day of the

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* Present address: Meiji Milk Product Company Ltd., Chigasaki, Kanagawa.

termination of running for 14 days, rats received [$4\text{-}^{14}\text{C}$]-cholesterol intraperitoneally or orally at 10:00 a.m.

Labeled cholesterol was dissolved in 0.1 ml of 4% Tween 20 in methanol. After removal of methanol in a stream of nitrogen under 40°C , the residue was dissolved in 10 ml of 0.15 M sodium chloride ($2\text{ }\mu\text{Ci/ml}$). One milliliter of the solution was injected intraperitoneally. In experiment with oral dose, labeled cholesterol dissolved in diethyl ether was mixed with 4 ml of safflower oil, the ether was removed in a stream of nitrogen under 40°C and two-tenth ml ($1\text{ }\mu\text{Ci}$) was given orally by stomach tube.

Lipid analysis. Rats were exanguinated by decapitation. Serum and tissue lipids were extracted and purified according to the method of Folch *et al.*⁷⁾ Triglyceride, phospholipid and cholesterol were determined by the method of Fletcher,⁸⁾ Gomori⁹⁾ and Sperry and Webb,¹⁰⁾ respectively. Epididymal adipose tissue was directly saponified in an ethanolic KOH solution and nonsaponifiable matter was extracted three times with petroleum ether.

Aliquots of these lipid extracts were transferred to counting vials, dried and dissolved in toluene scintillation fluid (2 g of 2,5-diphenyloxazole and 50 mg of 1,4-di-2-(5-phenyloxazolyl)-benzene per liter of toluene).

Feces were collected daily. Lyophilized feces were homogenized in chloroform-methanol (1:1, v/v) and steroids were extracted in the same solvent mixture at 50°C for 4 hr. Aliquots of the extract were decolorized with H_2O_2 ¹¹⁾ and counted in 1 ml of methanol and 9 ml of scintillation fluid. Neutral and acidic steroid fractions were separated by thin-layer chromatography.¹²⁾ Radioactivity in the former fraction was counted in toluene scintillation fluid and the mixture of 1 ml of methanol and 9 ml of toluene scintillation fluid was used for the latter fraction. Radioactivity was counted in an Intertechnique Spectrometer SL-32 and corrected for quenching by an external standard.

RESULTS

Body weight gain was the same between exercised and pair-feeding sedentary rats throughout each experiment.

Plasma lipids

Figure 1 shows effects of exercise on plasma lipids. Rats were subjected to treadmill running for 14 days and sacrificed after 1, 3, 6 and 10 days of termination of exercise. The concentration of plasma triglyceride and phospholipid in the exercise group was lower than that in the sedentary group at any time after

FIG. 1. Time Course of the Concentration of Plasma Lipids.

Sedentary group $\bullet\cdots\bullet$, exercise group $\bullet\text{---}\bullet$. Each point represents mean $\pm$ SE of 5 rats. Average initial body weight was 140 g.

*** Significantly different from the exercise group at $p < 0.05$ and $p < 0.01$, respectively.

FIG. 2. Turnover of [$4\text{-}^{14}\text{C}$]-Cholesterol in Serum after Intraperitoneal (A) and Oral (B) Administration.

Sedentary group $\bullet\cdots\bullet$, exercise group $\bullet\text{---}\bullet$. Each point represents mean $\pm$ SE of 6 rats. After a day of treadmill running for 14 days, rats were dosed with labeled cholesterol. Average initial body weights were 160 g (A) and 153 g (B), respectively.

* Significantly different from the exercise group at $p < 0.05$.

exercise load for 14 days and the difference between exercise and sedentary groups was statistically significant in the days 3, 6 and 10.

The plasma cholesterol lowering effect of exercise also lasted at least for 10 days, the difference in the days 1, 6 and 10 being significant.

Turnover of [4-¹⁴C]-cholesterol in serum

Time course of radioactivity decay in serum cholesterol is shown in Fig. 2. Rats were subjected to running for 14 days and received [4-¹⁴C]-cholesterol intraperitoneally or orally on the next day of the last running. Blood was periodically withdrawn from the tail vein. Since there were no demonstrable differences in body weights at any time between exercise and sedentary groups, the data were presented as dpm/mg cholesterol in both experiments.

The rate of disappearance of [¹⁴C]-cholesterol from serum was more rapid in running rats than in sedentary rats at early times (1~3 days) after isotope dosing. However, the contours of the decay curves were essentially the same thereafter. Semi-logarithmic analysis of the disappearance curve by successive subtractions indicated that the curve could be subdivided into at least 2 compartments. From these constants, half-lives ($t_{1/2}$) were computed. The fastest constant was tentatively referred to as pool A and the slower, pool B. The results are summarized in Table I. Half-life in pool A was significantly shorter in exercised rats than in sedentary rats, both after intraperitoneal and oral administration, but that in

TABLE I. THE RATE CONSTANT OF SERUM CHOLESTEROL DISAPPEARANCE CURVE^{1,2)}

Groups	$t_{1/2}$ of the exponential	
	Pool A	Pool B
	(Days)	
	After intraperitoneal administration	
Sedentary	1.04±0.09 ^b	4.41±0.22
Exercise	0.70±0.03	4.33±0.19
	After oral administration	
Sedentary	0.79±0.03 ^a	2.45±0.24
Exercise	0.59±0.04	2.23±0.21

1) Values are the means±SE for 6 rats.

2) The half-life of circulating cholesterol was calculated from the rate constants obtained by the graphic resolution of the disappearance curve (see also Fig. 2).

^{a, b} Significantly different from the exercise group at $p<0.05$ and $p<0.01$, respectively.

pool B was the same between two groups.

Steroid excretion

Effects of exercise on fecal excretion of total, neutral and acidic steroids are illustrated in Fig. 3. Fecal excretion of total [¹⁴C]-steroids was to some extent greater in rats subjected to running than in sedentary rats in both experiments. The cumulative values for 11 days after intraperitoneal injection in exercise and sedentary groups were 48% and 45% and those for 6 days after oral dose were 84% and 81%, respectively. Excretion of neutral and acidic steroids was also slightly greater in exercised rats than in sedentary animals. Thus, the increase in the excretion of total steroids was ascribed to an equivalent increase in that of neutral and acidic steroids. Judging from the contours of the cumulative excretion curves, it was obvious that the effect of exercise was exerted at the time shortly after administration of the isotope. In fact, statistically significant differences were found after 1~2 days of administration of labeled cholesterol. No demonstrable differences in the slope could be observed thereafter.

FIG. 3. Cumulative Percentage Excretion of the Radioactivity as Total, Neutral and Acidic Steroids into Feces after Intraperitoneal (A) and Oral (B) Administration of [4-¹⁴C]-Cholesterol.

Sedentary group —○—, exercise group —●—. Each point represents mean±SE of 6 rats. (See also Fig. 2). ** Significantly different from the exercise group at $p<0.01$.

Tissue distribution

Rats were sacrificed after 11 days and 6 days

TABLE II. DEPOSITION OF [4-¹⁴C]-CHOLESTEROL IN TISSUES¹⁾

Groups	Liver	Heart ($\times 10^3$ dpm/tissue)	Testes	Adrenal	Adipose tissue (dpm/g)
After intraperitoneal administration ²⁾					
Sedentary	95.8 $\pm$ 3.7	5.02 $\pm$ 0.08	12.2 $\pm$ 0.8	3.74 $\pm$ 0.37	8244 $\pm$ 2079
Exercise	82.7 $\pm$ 6.3	4.68 $\pm$ 0.23	12.6 $\pm$ 1.1	4.28 $\pm$ 0.43	7053 $\pm$ 1329
After oral administration ³⁾					
Sedentary	35.7 $\pm$ 3.5 ^a	2.50 $\pm$ 0.25	2.47 $\pm$ 0.17	1.84 $\pm$ 0.24	925 $\pm$ 71
Exercise	23.4 $\pm$ 3.9	2.17 $\pm$ 0.40	2.05 $\pm$ 0.27	1.31 $\pm$ 0.35	740 $\pm$ 112

1) Values are the means $\pm$ SE for 6 rats.

2) Rats were killed at 10:00 a.m. after 11 days of intraperitoneal administration of [4-¹⁴C]-cholesterol (see also Fig. 2).

3) Rats were killed at 10:00 a.m. after 6 days of oral administration of [4-¹⁴C]-cholesterol (see also Fig. 2).

^a Significantly different from the exercise group at $p < 0.05$.

of intraperitoneal and oral administration of labeled cholesterol, respectively. Table II shows the distribution of radioactivities remaining in various tissues. Effects of exercise on the extrahepatic distribution of the label were not apparent. Hepatic radioactivity of exercised rats tended to be lower than in sedentary rats, and the difference observed after oral administration of [¹⁴C]-cholesterol was significant.

DISCUSSION

The plasma cholesterol lowering effect of exercise lasted at least for 10 days after exercise was terminated (Fig. 1). Hebert and Lopez-S¹³⁾ and Watt *et al.*¹⁴⁾ reported that the hypocholesterolemic effect of exercise in rats could be observed for 3 and 8 weeks after exercise load (running for 10 and 8 weeks), respectively. Rochelle¹⁵⁾ also reported that in human the effect lasted for 4 weeks after running for 5 weeks. These data indicate that exercise may be a useful and effective measure for maintenance of low levels of plasma cholesterol.

The mechanism responsible for plasma cholesterol lowering effect of exercise is not necessarily apparent at present. In the previous study,⁴⁾ exercise stimulated hepatic cholesterol synthesis, lipoprotein lipase activity in cardiac muscle and fecal excretion of neutral and acidic steroids. It is thus presumable that exercise enhances the turnover rate of plasma lipo-

proteins. As shown in the present study, the turnover of [4-¹⁴C]-cholesterol in serum shortly after administration was considerably rapid in exercised rats compared with that in sedentary rats, as judged from the semi-logarithmic analysis of cholesterol decay curves (Table I). Thus, half-life in that compartment was significantly shorter in exercised rats. On the contrary, there were no differences in the half-life at the later stages after administration of the label between two groups. These results suggest that exercise causes an increase in turnover of rapidly exchangeable cholesterol pool.

In addition, when rats were sacrificed after 11 days of intraperitoneal injection or 6 days of an oral dose of labeled cholesterol, the radioactivity remaining in the liver from exercised rats was considerably low. This is probably attributable to an initial loss of the label from the blood stream due to exercise. Meanwhile, the cumulative excretion of labeled total steroids in exercised rats was consistently, but not significantly, higher than that in the sedentary animals (Fig. 3), and this effect almost entirely accounted for the increase in the loss of the radioactivity immediately or shortly after the administration of the label. As shown in Table II, effects of exercise on the deposition of labeled cholesterol in extrahepatic tissues were not observed. Also, exercise did not show any effects on the concentration of carcass cholesterol.⁴⁾ These data also provide evidence that exercise causes the modification in the metabolism of cholesterol in the hepato-plasmic

system while it may not affect the redistribution of cholesterol from this compartment to other slowly exchangeable tissue pools.

Bile salts are necessary for absorption of cholesterol from the small intestine. Changes in the composition of bile acids may influence the rate of absorption of cholesterol, since the effectiveness varies depending on the type of bile acids. Taurochenodeoxycholate is most effective for absorption of cholesterol.¹⁶⁾ Among bile acids, the excretion of chenodeoxycholic acid and its derivatives was increased by exercise.⁴⁾ In this context, the decrease in the rate of cholesterol absorption may be a factor responsible for plasma cholesterol lowering effect of exercise. Although the experiment with an oral dose of labeled cholesterol employed herein may not be satisfactory enough to verify absorbability of that compound, the data obtained shortly after administration serve as an index for this parameter. Differences in fecal excretion of the orally administered label as neutral steroids between two groups were moderate but statistically significant. Thus, it is likely that the difference in the absorption may also be associated with plasma cholesterol lowering effect of exercise. More detailed information awaits further works on cholesterol-containing diets.

In conclusion, the hypocholesterolemic activity of exercise is affected entirely, but not

exclusively, through enhanced turnover of rapidly exchangeable pool of cholesterol. Increase in hepatic oxidation of cholesterol to bile acids accompanied by an acceleration of fecal output of steroids⁴⁾ supports this view.

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