Effect of Voluntary Exercise and Dietary Protein Levels on Incorporation of ^<14>C-Leucine into Protein by Mice Liver Slices in Vitro

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Effect of Voluntary Exercise and Dietary Protein Levels on Incorporation of ^{14}C–Leucine into Protein by Mice Liver Slices in Vitro

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Summary

The effect of voluntary exercise on incorporation of ^{14}C-leucine into protein by mice liver slices in vitro were examined with mice fed 4%, 6% and 20% protein diets.

The incorporation of ^{14}C-leucine increased as dietary protein levels decreased and was significantly higher in liver slices of exercise groups than in slices of non-exercise groups.

Our previous investigations have clarified the effects of voluntary exercise on lipid metabolism of the serum and liver of mice fed different levels of dietary protein (1, 2, 3). From the previous experimental results showing that the activity of serum glutamic pyruvic transaminase and the levels of liver lipids in animals fed a severe low protein diet were considerably decreased by voluntary exercise, it appears likely that the animals experiencing long-term exercise training may adapt to a severe low protein diet. With regard to the effect of exercise on the adaptation to low protein diet in animals, there are few reports on ability to synthesize protein.

The purpose of the present investigation was to examine the effect of voluntary exercise on incorporation of ^{14}C-leucine into protein of sliced liver of mice fed three levels of dietary protein.

Experimental procedure

Experimental conditions in animals, diets, measurement of the amount of voluntary exercise and sampling of the liver were same as those described in the previous report (3). The experimental diets are shown in Table 1. The animals were each kept on the experimental diets for about 90 days. All exercise groups were housed individually in wire net cages with a revolving treadwheel. After

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 mxiture[b]	1	1	1
Fat-soluble vitamin mxiture[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 mxiture purchased from Oriental Yeast Co., Ltd.
[c]Fat-soluble vitamin mxiture: vitamin A, 1,000*IU*/100*g* diet, vitamin D, 100*IU*/100*g* diet, vitamin E, 10 *mg*/100*g* diet.

decapitating animals, their livers were rapidly removed, placed in cold 0.9% NaCl solution. Liver slices were made with a slicer of Stadie-Riggs type.

One hundred to 150 mg of liver slices were incubated at 37°C for 2 hours under O_2–CO_2 (95%–5%) in 2.5 ml of Krebs-Ringer phosphate buffer (pH 7.4) containing 0.6 μCi of L-leucine-U-^{14}C (specific activity; 339 mCi/mmol). After incubation, an equal volume of 10% Trichloroacetic acid (TCA) was added to the flasks which contained the radioactive leucine. Precipitated materials with 10% TCA were regarded as protein fraction. The incorporation was expressed as cpm/mg protein.

Results and Discussion

As shown in Table 2, body weight gains were supprssed as dietary protein levels decreased. The suppression in the 4% protein diet group was particularly makred. That the body weight gain of the 6% exercise (abbreviation; E) group increased to levels approaching those of the 20% E group was in agreement with the previous report (2). Running distances were slightly longer in the 20% E group than in the 4% and 6% E groups, but no significant differences were observed.

The incorporation of ^{14}C-leucine increased as dietary protein levels decreased. The incorporation of 4% non-exercise (abbreviation; NE) and 6% NE group was three or four times that of the 20% NE group. There are many reports concerning adaptation to dietary restriction. For example, Waterlow (6), from his experimental results on the effect of protein depletion on protein synthesis, suggested the possibility that in protein depletion the synthesis of protein is concentrated in the more essential organs such as liver and brain at the expense of less essential ones such as muscles and skin. Similarly, Akamal Khan and Bender (7) reported that administration of labelled methionine resulted in greater recovery rate of radio-

TABLE 2. *The Body Weight Gains, Amount of Voluntary Exercise and Incorporation of ^{14}C-leucine into Protein by Liver Slices.*

		Initial body weight (*g*)	Final body weight (*g*)	Average daily amount of exercise (*m*)	Liver weight (*g*)	^{14}C-leucine incorporation *cpm/mg* protein ($\times 10^3$)
4%	NE	20.0±0.7 (14)	18.5±0.8 (13)		0.54±0.04 (7)	1.89±0.29 (5)
	E	21.2±0.2 (14)	20.9±0.7 (14)	3,344±398 (10)	0.64±0.03 (7)	3.10±0.32[b] (5)
6%	NE	21.4±0.3 (14)	28.8±1.3 (14)		0.92±0.05 (7)	1.10±0.18 (5)
	E	21.3±0.2 (14)	30.5±0.9 (14)	3,186±562 (9)	0.94±0.04 (7)	2.19±0.12[b] (5)
20%	NE	21.3±0.3 (14)	35.7±0.9 (14)		1.30±0.04 (7)	0.44±0.05 (5)
	E	21.3±0.3 (14)	33.2±0.6[b] (14)	3,902±357 (10)	1.29±0.05 (7)	0.72±0.03[a] (5)

Experiment for incorporation of amino acid was undertaken as follows: One hundred to 150 *mg* of liver slices were incubated at 37°C for 2 hours under O_2–CO_2 (95%–5%) in 2.5 ml of Krebs-Ringer phosphate buffer (pH 7.4) contained 0.6 μCi of L-leucine-U-^{14}C(specific activity: 339 *mCi/mmol*). After incubation an equal volume of 10% TCA was added to the flasks which contained the radiaoactive leucine. The incorporation was expressed as *cpm/mg* protein.
$^{a}p<0.01$, $^{b}p<0.05$ Between non-exercise and exeircse groups
Values are means±SE. The number of mice is in parenthesis.
4%, 4% casein diet; 6%, 6% casein diet; 20%, 20% casein diet; NE, non-exercise group; E, exercise group.

activity in the liver of rats fed a low protein diet than those fed normal protein diet and the reverse was found in the muscles. Although the incorporation of amino acid by muscle was not examined and there is a distinction between in vivo and in vitro, it seems that the present results might reflect the fact mentioned above. At the same time, it could also be anticipated that the incorporated ^{14}C-leucine would be more diluted by the amino acid pool of liver in the normal protein diet group than in low protein diet group. Furthermore, the noteworthy phenomenon of this investigation is that the incorporation of ^{14}C-leucine by liver of the exercise groups increased considerably compared with the non-excercise groups. The present results might support the adaptation to low protein diet and the improvement of liver impairment in exercise groups previously reported (3). Such effect might be mediated through cortisol and insulin which, in some respects, have reciprocal actions on protein metabolism (7). Namely, cortison appears to promote synthesis in the liver at the expense of peripheral tissues while insulin promotes the uptake of amino acids by muscle but not by liver (8, 9, 10, 11). Such hormonal action might be strengthened by exercise training (12). The maintenance of more essential tissues at the expense of muscle may be partly due to enzymic adaptation (7, 13).

In any event, the present study suggests the possibility that exercise training may promote the animal's adaptation to protein deficient diet.

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