EFFECTS OF PHYSICAL ACTIVITY ON COLLAGEN CONTENT IN MICE

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Abstract

Male mice of dd-strain, at 4 and 8 week-ages were used at the start of the trained. The trained continued for successive during 4 to 34 week-ages and 8 to 70 week-ages. The trained group was exercised on a treadmill, and the untrained group used as control under normal laboratory condition. The skin of the right dorsum near the tail was sampled and its collagen content was examined by means of concentration of hydroxyproline. The collagen content in the skin of the trained group is lower than in that of the untrained one at each week-age, 14-18, 27-34 and 63-70 week-ages. The trained group was depressed hydroxyproline concentration of defatted skin, compared with untrained one at each week-age. The continuous exercise reduced the accumulation of collagen in the skin of mice.

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key words : aging, collagen content, hydroxyproline concentration, physical activity, skin

I. Introduction

By aging, mammalian is generally known to decrease parenchymatous cell, and to increase interstitial cell or connective tissue. The phenomenon is represented in an accumulation of collagen content. Takeda, et al.¹⁴⁾ have observed the collagen content in a skin gradually increased during growth and development. Numerous reports have clarified that the amount of collagen changes to increase in the connective tissue of several organs as the animal grows⁴⁾¹²⁾¹³⁾¹⁷⁾. It is generally accepted that the collagen occurs abundantly in older animals.

Recently, distribution and chain composition of various types of collagen which is a main constituent of connective tissue of animal have been extensively studied¹⁾²⁾⁹⁾. The interstitial collagen is now divided into five types such as type I, II, III, IV and V³⁾⁹⁾. We were under-

taken to see the changes of collagen content of the skin, type I collagen, in mice which were subjected to the continuous exercise of the definite duration. And we were also objected to see the effects of the exercise by means of collagen content as the indicator. Because very little is known of the effects of physical activity on the metabolism of collagen. We are imagined that the metabolism of collagen is influenced indirectly by exercise in a skin compared with a tendon and skeletal muscle, and that a skin is indicated the effects of exercise on the whole.

II. Materials and Methods

Male mice of dd-strain were used in this work. They were divided into two groups (trained and untrained) and reared 5 animals in one small cage (17.5 cm in width, 24.5 cm in length and 12.5 cm in height at 20 $\pm$ 2 $^{\circ}$ C). When started this experiment, animals of both

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groups were allowed to eat *ad libitum* with solids (Oriental Yeast Co., Chiba) and water. Forty-six individual of untrained group were left under this condition for the definite duration of time that corresponded with that in the trained group. Forty-six animals of trained group were subjected to the treadmill exercise program with consisted of 4 times of running at a speed of 15.8 cm/s for 20 min per a week. Goto et al.⁵⁾ have reported to the running speed (cm/s) had relation to the logarithm value of all-out time. Our exercise program was determined by considering their relation and did not give the trained group a stress. The experiment was started when the animals became 4 and 8 week-ages. Mean weight of them was obtained from the all individual which have live in the day of weighting. Every individual was weighted before the training during course of the experiments each day.

At the 14, 27 and 63 week-ages of 3 animals were sacrificed for the chemical analysis. The skin of the right dorsum near the tail were taken either from 3 trained and/or 3 untrained animals after the hair was removed with scissors. About 100mg of a skin piece was used as the sample for each time of experiments. The collagen content of the skin was

examined by means of measuring of their hydroxyproline content. It is generally known to amino acid that hydroxyproline only existed in collagen. The methods were as follows. First, the fat in the sample was extracted with a 1:1 mixture of acetone and ethyl ether in a soxhlet apparatus over a periods of 28 hours. The weight of fat-free residue of the skin was measured. Protein hydrolysate was prepared by autoclaving 10mg of the fat-free residue with 2ml of 6N HCl in a sealed tube for 7 hours at 1.2 kg/cm² pressure. Two ml of protein hydrolysate was filtered and transferred to a 25 ml volumetric flask. After neutralized with ammonium hydroxide and it was diluted to the volum of 25 ml. The measurment of hydroxyproline concentration was carried out according to Neuman and Logan¹⁰⁾. The absorbancy at 560 nm was measured using a spectrophotometer (Hitachi Model 100-10).

III. Results

Mean weight of the trained and untrained group was measured every day, but they did not show any noticeable difference between. Therefore, mean weight of them shown in each week-age, when this experiment was performed (Table 1).

Table 1. Mean body weight and dry weights of defatted skin of trained and untrained groups.

Group	Week-ages	N	Mean body weight	Dry weights of defatted skin
			g	mg/100 mg wet weights
Trained	14-18	12	45.5±7.2	33.6±6.8
	27-34	13	54.3±8.2	20.0±3.7
	63-70	21	35.3±5.7	34.8±4.5
Untrained	14-18	13	51.8±4.8	30.3±6.8
	27-34	13	55.8±7.9	20.3±3.4
	63-70	20	35.9±6.5	33.4±6.8

A standard curve prepared with 0 to 20 μg of hydroxyproline. The standard curve can be expressed with a formula $Y=0.023x+0.008$. Of both trained and untrained groups in each week-age the mean values of dry weight and hydroxyproline concentration of defatted skin are shown in Table 1 and Fig. 1. The dry weights of the defatted skin are shown in Table 1, which gave the slightly weightier value in trained group at 14-18 and 63-70 week-ages than in untrained one, indicating to the amount of fat of the trained group is less than that of the untrained one. Two groups of 27-34 week-ages did not show difference between.

Hydroxyproline concentration are shown in Fig. 1 which clearly illustrated significant difference between two groups in each week-age. Hydroxyproline concentration of defatted skin ($\mu\text{g}/\text{mg}$ dry weights $\pm\text{SD}$) was 24.6 ± 3.8 ,

17.2 ± 2.8 and 24.8 ± 2.6 at 14-18, 27-34 and 63-70 week-ages, respectively, in trained group, and was 27.5 ± 2.8 , 24.9 ± 5.9 and 28.1 ± 1.9 at each week-age in untrained one. The collagen content in the skin of the trained group is lower than in that of the untrained one in each week-age. The collagen content at 14-18 week-ages gradually decreased in two groups. Moreover, the collagen content at 27-34 to 63-70 week-ages increased in two groups. Hydroxyproline concentration of dry weight at 63-70 week-ages in the trained group is as much as that at 27-34 week-ages in the untrained group. This result indicated that the continuous exercise reduced the accumulation of collagen content in the skin.

IV. Discussion

Numerous studies have reported about collagen content of several organs of some ani-

Fig. 1. Hydroxyproline concentration of defatted skin in each week-age of trained and untrained groups. Hydroxyproline concentration give significant difference in t-test. **, * ; Difference from untrained group : $p<0.01$, $p<0.05$.

mals. Sobel et al.¹³⁾ measured the collagen content of femurs of rats, and pointed out the increase of collagen with somatic growth. Elster and Lowry⁴⁾ also measured the collagen content of liver, kidney, lung, heart, skeletal muscle and spleen of guinea pig, and did not observed changes of collagen in skeletal muscle and spleen. This is due to influence by a nature of collagen in each tissue. In this work, we measured the collagen content of skin in mice.

In skin collagen, Heikkinen⁶⁾⁷⁾ have reported to increase of collagen in rat skin with growth, and noticed that the rate of metabolism of collagen became slow after cessation of the growth. Takeda et al.¹⁵⁾ showed the increase of the collagen content until 12 week-ages in the male mouse skin and until 10 week-ages in female one. They pointed out that the collagen content varied with the sex. Male mice were used in our work, which sacrificed after the increase of collagen content, that is, the collagen content saw subsequent to the process of growth.

On the influence of physical activity, we showed that the mice of the trained group have less amount of the skin collagen than those of the untrained group (cf. Table 1 and Fig. 1). By subjecting rats to the define training which is composed of sprint and endurance, the results of Tipton et al.¹⁶⁾ is well consistent with that of our case. That is, the collagen content in the skin of the trained group is lower than in that of the untrained one when compares with the same age mice. This fact clearly indicate that the skin of the trained mice is younger and more fresh than the untrained one (cf. Fig. 1). These results are the difference in collagen metabolism of the skin between trained and untrained mice. Physical activity seems to depress aging of a

skin.

Previous studies a five type of collagen have revealed that type I collagen is contained mainly in skin, tendon and aorta etc⁹⁾. It is necessary to study in relation the physical activity and the occurrence of some other type of collagen.

In hydroxyproline concentration, dividing $\mu\text{g}/\text{mg}$ dry weights by $\text{mg}/100 \text{ mg}$ wet weights in each week-age, the results obtained the high value at 27-34 week-ages in both group. And also, dry weights of defatted skin in 27-34 week-ages showed lower value than other week-ages (cf. Table 1). This difference, first, is need to consider hypodermic fat, triglyceride. Accumulation of triglyceride may influence the dry weights of defatted skin, owing to triglyceride attach to the skin. In 27-34 week-ages, the quantity of triglyceride seems to accumulate more than other week-ages. And next, it may be the participation of intercellular substance, in other word, it means that the water content is merely replaced by intercellular substance and polysaccharide. To decrease of water content, it assume to concern in swelling of fibrous protein, i. e., collagen and elastin, and in the quantity of polysaccharide. Moreover, Heikkinen⁸⁾ have reported to decrease of water content into collagen of skin with aging. These are suggested that the water content of skin occurs the difference in each week-age. It may be need to study metabolism of hypodermic fat and the water content of tissue.

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