Do Exercise-induced Changes in Distensibility and Elastic Components of Rat Aorta Last for Long after the Cessation of Training?

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We examined the detraining effects on the exercise-induced changes of the distensibility and elastic components of the rat aorta. Exercised (E) and sedentary (S) rats were divided into two groups respectively (i.e., E1, E2, S1, and S2). The E1 and E2 ran in a wheel cage spontaneously for 16 weeks from 9 to 25 weeks old. The E1 and S1 were sacrificed at the end of the exercise period. The E2 and S2 were bred sedentarily for 15 weeks until 40 weeks old. The aortic incremental elastic modulus at extension ratio of 1.5 and the contents of polar amino acids and calcium in aortic elastin were significantly lower in E1 than in S1. The aortic incremental elastic modulus and polar amino acids content were increased with aging significantly in S2 but not significantly in E2, and accordingly, those values were significantly lower in E2 than in S2. The content of elastin calcium, however, was increased significantly not only in S2 but also E2. The increase was especially marked in E2, and then, there was no significant difference in the values between two groups. These results suggest that the aortic wall in exercise-trained rats may keep more distensible structure with lower elastic fiber degeneration for a long time compared with sedentary rats even after detraining. The reason why elastin calcium content in the exercise-trained rats was markedly increased after the detraining was remain to be investigated.

Keywords: rat, aorta, incremental elastic modulus, elastin, detraining

1. Introduction

Distensibility of central arteries (aortas and relatively large arteries bonded to aortas) decreases with age [Nichols & O’Rourke, (1998)], whereas the possibility exists that regular aerobic exercise is effective in suppressing the lowering of arterial distensibility [Mohiaddin, et al., (1989); Vaikevicius, et al., (1993); Kakiyama, et al., (1995); Kingwell, et al., (1997); Kakiyama, et al., (1998); Tanaka, et al., (2000); Kakiyama, et al., (2001)]. A study that examined the efficacy of aerobic exercise in young subjects [Kakiyama, et al., (1999)] has found that relatively short-term exercise enhances arterial distensibility. However it has been reported that cessation of training neutralizes the effect, which was once achieved, within a short period. Studies have shown that exercise, commenced in middle age, helps enhance arterial distensibility in middle-aged and elderly persons, however significant effects do not remain intact if they lead a sedentary lifestyle in their late middle age, even if they had exercised early in life [Kakiyama, et al., (1995)]. Therefore it can be inferred that the effects of exercise on arterial distensibility are transient and that exercise needs to be done continuously in order to sustain the effects.
Arterial distensibility is greatly influenced by elastic components of the tunica media of the arterial wall [Nichols & O'Rourke, (1998)]. Sedentary aging is associated with a decrease in elastic fibers and increase in collagenic fibers in the tunica media of the arterial walls. Also, degeneration of elastin, which is constituent protein of elastic fibers, as well as calcium deposition occur, and these changes induce lower arterial distensibility [Partridge & Keeley, (1974); Yoshimura, et al., (1978)]. However, in addition to these structural factors, functional factors, as in the tone of vascular smooth muscles, affect arterial distensibility. From the results of previous studies showing that exercise has effects on arterial distensibility within a short period of time and that the effect is transient, it can be assumed that the effect of exercise in large part comes from the impact on arterial distensibility. However, animal experiments have shown that chronic exercise in young rats has also has an effect on structural factors that influence arterial distensibility [Matsuda, et al., (1988); Matsuda, et al., (1993); Matsuda, et al., (1993); Kingwell, et al., (1997); Kingwell, et al., (1998)].

These studies have demonstrated that rat aortas subjected to aerobic exercise exhibit higher level of distensibility as well as lower level of deformability and calcium deposition. These types of structural changes may remain intact for longer period of time after the cessation of training. However no research has been done to determine whether exercise-induced effects in elastic components of the tunica media can be sustained after the cessation of training.

The purpose of this study is to examine how exercise-induced effects in arterial distensibility and elastic components of rats undergo a change with age after the cessation of training.

2. Subjects and methods

Forty-two male Wister-Kyoto rats (Charles River Japan), 9 wks old, were obtained as subjects. Fourteen of them were assigned to an exercise group, housed individually in cages (12 x 14 x 38cm) with a free wheel and provided with an environment where they can exercise spontaneously between 9 and 25 wks old. The remaining 28 rats were assigned to a sedentary group and kept in individual cages (12 x 14 x 22cm) for the same duration. Six rats from the exercised group (exercised group 1) and 13 rats from the control group (control group 1) were sacrificed at 25 wks old and used to examine the effects of exercise. The remaining 8 exercise-trained rats (exercised group 2) were kept, but prevented from access to a free wheel. They, along with the remaining 15 rats in the control group (control group 2) were sacrificed at 40 wks old to find the effects of the cessation of exercise.

In the raising phase, rats were provided with normal dry pellet type rat chow (CE-2, Clea-Japan) and tap water ad libitum. The rat rooms were controlled with a 12 : 12-h light-dark cycle at a constant temperature of 24°C. Pentobarbital solution (50mg/ml) was administered (7-12mg/100g BW) in the abdominal cavity to euthanize the rats and then the aortas were removed. With two parallel blades, each approx. 2mm in thickness, ring specimen were prepared by dissecting the origin site and the respective descending site of the removed aortas to be used for tensile testing and were stored in 4°C saline solution for 24 h, avoiding smooth-muscle activities. The thoracic descending aortas were refrigerated to measure elastic components.

Using an Instron-type testing machine (Toyo Baldwin), the ring specimen were stretched in a temperature controlled (24±1°C) saline solution, at the rate of 2mm/min until broken, and then Stress-Strain curve was determined. The initial length and cross-sectional area had been measured beforehand using a stereo microscope. Stress was defined as tensile load per initial cross-sectional area and extension ratio was defined as the percent increase length above the initial length. As indicators of aorta distensibility, the incremental elastic modulus, where extension ratio was 1.5, was determined as increments of stress, equivalent to extension ratio increase of 0.1. The extension ratio of 1.5 is equivalent to arterial extension on approximately 100mmHg of arterial pressure.

The thoracic descending aortas and aortic arches were boiled with NaOH(0.1N) for 50 min. Then various deposits were isolated as alkali insoluble elastin preparations [Kramsch & Chan, (1978); Kramsch, et al., (1980)] and their dry weight was measured. After 6N of HCl was added to the elastin preparations from the descending thoracic aortas and was hydrolyzed by boiling in a vacuum oven for 24h at 110°C, then the amino-acid composition was determined using an amino-acid analyzer (Hitachi). As an indicator of elastin degeneration, the total sum of polar amino acids in the amino-acid composition was determined using an amino-acid analyzer (Hitachi).
After elastin preparations from aortic arches were incinerated overnight at 600 °C and the residue was dissolved with 6N HCl, the calcium content was measured using an ICP (Inductively Coupled Plasma) emission spectrometry method to calculate the calcium content of the elastin.

Two-way ANOVA with exercise-induced and age-related factors was conducted on various data in the four groups, and Scheffe’s multiple comparisons were conducted in cases where significance was observed. Statistical significance set a priori at \( p < 0.05 \), and all data were expressed as mean ±SD.

3. Results

The incremental elastic modulus for each group is shown in Figure 1. Exercise-induced and age-related main effects were found to be significant. The result of the study has found no significance in interactions between exercise and age-related factors, confirming independence of the two factors. The incremental elastic modulus in the exercised group was significantly lower in comparison to that in the control group both immediately after the exercise period \( (p < 0.05) \) and after the detraining period \( (p < 0.01) \). Significant age-related increase \( (p < 0.01) \) was observed in the incremental elastic modulus in control group 2. In exercised group 2, age-related increase was observed in the incremental elastic modulus as well, although not significant \( (p = 0.061) \).

The amount of polar amino acids for each group is shown in Figure 2. Significant exercise and age-related effects have been observed. No significance has been observed in interaction between exercise and age-related factors. The amount of polar amino acids was significantly lower in exercised group 1 in comparison to that in control group 1 immediately after the exercise period \( (p < 0.05) \). Also after the detraining period, significantly lower values were observed. The same trend was observed in the second group. The amount of polar amino acids was significantly lower in the exercised group in comparison to the control group immediately after the exercise period \( (p < 0.01) \) in both groups. The age-related increase was observed in both groups, although not significantly different between the groups after the detraining period.
were observed in exercised group 2 in comparison to that in control group 2 ($p<0.01$). Significant age-related increase ($p<0.05$) was observed in polar amino acids in control group 2. In exercised group 2, increased polar amino acids were observed as well, although not statistically significant ($p=0.266$).

The calcium content in elastin for each group is shown in Figure 3. Significant exercise and age-related effects have been observed. No significance has been observed in interaction between exercise and age-related factors. The calcium content in elastin was significantly lower in exercised group 1 compared to that in control group 1 ($p<0.01$). Also after the detraining period ($p<0.01$), significantly higher values were observed in exercised group 2 ($p<0.001$) and control group 2 ($p<0.05$), compared with immediately after the exercise period. No significant difference between exercised group 2 and control group 2 was observed ($p=0.497$), since the magnitude of age-related increase was especially marked in the exercise-trained group.

4. Discussion

The incremental elastic modulus of the aorta has been found to be lower immediately after the exercise period in rats subjected to 16 wks of spontaneous exercise from an early age compared with that in sedentary rats. Also the calcium content in aortic elastin and polar amino acids in amino-acid composition exhibited low values. These results of the present study were similar to those in previous studies [Matsuda, et al., (1988); Matsuda, et al., (1989); Matsuda, et al., (1993); Kingwell, et al., (1997); Kingwell, et al., (1998)] suggest the possibility that spontaneous running and/or swimming exercise suppresses age-related changes in aortic distensibility in rats. However, other research has reported that aortic pulse wave velocity in rats subjected to 8 wks of treadmill running exhibited no difference from that in a control group [Niederhoffer, et al., (2000)]. Variations in types and/or duration of training might account for conflicting findings. In this research [Niederhoffer, et al., (2000)], however, aortic pulse wave velocity was measured in vivo under anaesthesia, and thus we are unable to rule out the possibility that the disagreement comes from aortic pulse wave velocity being evaluated under the influence of functional factors in addition to structural factors. The present study and our previous studies measured aortic distensibility in the samples removed from aortas and stored for 24h. Therefore it can be presumed that our studies evaluated the effects created by structural factors alone, almost totally ruling out the effects of functional factors on aortic distensibility. Findings presented in the present study suggest the possibility that spontaneous running in rats’ growth period suppresses age-related changes in structural factors that determine aortic distensibility, and that those effects may be further lasted after cessation of training.

Under physiological conditions, structural factors that affect aortic distensibility are mainly properties of elastic fibers that comprise elastic lamina [Berry, et al., (1975)]. It is known that elastin, a constituent protein of elastic fibers, experiences age-related degeneration to cause an increase in polar amino acids in amino-acid composition, and both calcium and lipids are deposited on elastin [Partridge & Keeley, (1974)]. Age-related changes such as calcium deposits in elastic fibers of aortas were observed in rats as well [Fleckenstein, et al., (1982)], resulting in deteriorated arterial distensibility [Niederhoffer, et al., (1997)]. The present study has shown that 16 wks of spontaneous exercise is effective in suppressing such deterioration of arterial distensibility, calcium
deposits on elastic fibers and increase in the amount of polar amino acids. These findings are similar to those in our previous studies [Matsuda, et al., (1988); Matsuda, et al., (1989); Matsuda, et al., (1993)], where rats were subjected to spontaneous running or swimming exercise. Furthermore, the present study found that the incremental elastic modulus of the aorta and the amount of polar amino acids of elastin were lower in the aorta of detrained rats in comparison to those in sedentary rats after a period of time equal to the exercise duration. Therefore, it can be inferred that chronic exercise in rats' growth period limits deterioration of arterial distensibility induced by age-related changes in elastic fibers of the aorta, and that differences are lasted after exercise is ceased.

Our previous study [Matsuda, et al., (1993)] has found positive correlation between the incremental elastic modulus of the aorta and calcium content in elastin, thus suggesting that the effects of chronic exercise on arterial distensibility might be correlate well with the reduction of calcium deposition on elastin. The present study also found that both the incremental elastic modulus and the calcium content of elastin in exercise-trained rats were lower immediately after the exercise period in comparison to those in sedentary rats. In 15 wks after the cessation of exercise, although the incremental elastic modulus remained low, the calcium content in elastin showed a significant increase, and therefore difference with the value in sedentary rats had diminished. That is, age-related calcium deposition on elastin may progress in larger scale in the aortas of detrained rats compared with those of sedentary rats. And yet, arterial distensibility was higher compared with that of sedentary rats. Calcium ion will be strongly bonded to polar amino acids in elastin [Tsushima, (2000)], and when binding of calcium ion occurs, it will induce binding of phosphoric acid ions, resulting in accumulation of calcium phosphate [Keeley & Partridge, (1974)]. Furthermore, there is the possibility that protein with ample polar amino acids might be bonded to elastin through calcium [Keeley & Partridge, (1974)]. However, our findings show that the increase in polar amino acids in the detrained group is not significant, thus significant difference with the sedentary group is sustained. Therefore, it can be inferred that calcium deposits in the detrained group might have progressed in sites other than polar amino acids. Calcium deposits on elastin can occur on pentapeptides (Val-Pro-Gly-Val-Gly) in non-bridged areas (Urry, et al., (1976); Rapaka, et al., (1983)). It is presumed that possibilities are low that increased calcium deposits, observed in the detrained group in the present study, actually generated on pentapeptide sites. Direct repeat of pentapeptides affects elastic function of elastic fibers [Okamoto, et al., (1991)] and calcium deposition in the region will limit the elasticity [Uemura, et al., (1994)]. Therefore, properties of pentapeptides appear to play a significant role in the incremental elastic modulus of the aorta. The present study has found that despite the increased calcium deposit, the incremental elastic modulus remained low in the detrained group. Finally, the present study findings did not reveal the reason and site of increased calcium in elastin in detrained rats, which remain to be determined in future studies.

One study [Kakiyama, et al., (1995)] has shown that in healthy middle-aged and older persons, exercise performed early in life can limit age-related changes in arterial distensibility, but the effect might be diminished by the cessation of exercise. It is presumed that the disparate findings in this previous study and the present study can be attributed firstly to the difference in the time period that changes are observed. That is, the present study examined rats for several months, whereas Kakiyama, et al., (1995) examined changes in humans over several years. Elastic fibers of arterial walls constantly receive mechanical stimulation from the pulse. As long-lived humans receive mechanical stimulation over a longer period of time, material fatigue will cause plasmotomy, flow disorder and straightening of arrangement, whereas rats do not experience such changes [Nichols & O’Rourke, (1998)]. It is therefore possible that the study conducted by Kakiyama, et al., (1995) covered a longer period of time and factored in effects of material fatigue due to mechanical stimulation as well as chemical changes, thus the latter effects outstripped the former, resulting in disparate findings. Secondly, arterial distensibility is affected not only by functional factors such as elastic fibers but also structural factors such as the tone of vascular smooth muscles. Chronic exercise may improve arterial distensibility in a relatively short time by changing autonomic nerve balance [Ekblom, et al., (1973); Scheuer & Tipton, (1977)] and endothelial function [Maeda, et al., (2001)], whereas changes in structural factors will be diminished after the cessation of exercise [Maeda, et al., (2001)].
Though it is true that even a short duration of exercise will create higher arterial distensibility [Kakiyama, et al., (1999); Tanaka, et al., (2000); Kakiyama, et al., (2001)], the effects will be diminished in a short period after detraining [Kakiyama, et al., (1999)]. It is, therefore, possible that the differences in structural factors created by varied exercising practices early in life may have been outstripped by those in functional factors created by exercising practices in late middle age.

The findings of the present study suggest that effects achieved by chronic training in rats’ growth period might be further sustained after detraining. Elastin has slow metabolic turnover, and will not be easily reproduced once degeneration or calcification has occurred. Normal elastin, without major degeneration or calcification, will inhibit migration of vascular smooth muscle into the endothelium, whereas denatured elastin, if degraded into soluble elastin-peptide by elastase that the macrophage secretes, will induce vascular smooth muscle to migrate [Ooyama, et al., (1987)]. It follows that degeneration of elastic fibers, once it has occurred, might be a risk factor of atherosclerosis as well as age-related irreversible degeneration. Meanwhile, our previous study has found that the magnitude of effects of exercise on degeneration of elastin in postgrowth persons, whose elastin is expected to be denatured, was slighter compared to those who exercised in their growth period [Nosaka, et al., (2003)]. Effects of exercise on degeneration of elastin cannot be compared among varied exercise durations in living organisms. Changes in structural factors (properties of elastic fibers) in human arteries play a significant role in age-related changes in arterial distensibility [Nichols & O'Rourke, (1988)]. One previous study of sectio cadaveris has shown that the calcification level of tunica media in arteries is positively correlated with antemortem aortic pulse wave velocity [Arai, et al., (1985)]. Supposing increased amount of exercise early in life creates a greater effect on properties of elastic fibers, and that the effect is sustainable, habituated exercise early in life will be effective in deterring deterioration of arterial distensibility and development of atherosclerosis. Although effects of habituated exercise early in life on arterial distensibility in middle-aged persons appear to be small [Kakiyama, et al., (1995)], still it is unassailable that they have some degree of positive significance.

5. Summary

Rats subjected to exercise in their growth period were used to examine types and degrees of age-related changes that exercise has created on arterial distensibility and elastic fibers after cessation of exercise. That is, elastic characteristics of aortas and properties of elastic fibers in rats immediately after exercise period were compared with those in rats subjected to training, and then detrained and kept in sedentary condition. The results have shown the implications that effects of chronic exercise on elastic structural factors of aortas might be sustained for a given period of time after exercise is ceased.

References


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