Age-Related Differences in the Effect of Running Training on Cardiac Myosin Isozyme Composition in Rats

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We examined the effect of running training on age-related changes in cardiac myosin isozyme composition in rats. Female Fischer 344 rats (6, 12, 20, and 27 months old) were divided into two groups: sedentary control and trained. The trained group rats were trained by treadmill running for up to 60 minutes per day, 5 days per week for 8 weeks at up to 30 m per minute. In sedentary control rats, the proportion of V1 myosin, that is, α-myosin heavy chain (MyHC) isoform, decreased progressively from 6 to 27 months of age. In the younger age groups (6 or 12 months old), there was a shift from V1 myosin to V3 myosin (β-MyHC isoform) in trained hearts. However, the training program did not induce a cardiac myosin isozyme transition in older rats (20 or 27 months old). These results suggest that the mechanisms mediating the responses of cardiac muscle to running training alter during aging.

METHODS
Animal Care and Experimental Groups
Female Fischer 344 rats aged 4 weeks were obtained from Japan SLC, Inc. (Hamamatsu, Japan). They were housed individually in sedentary cages and were not exercised until the physical training period began. At the end of the training period, the rats were 6, 12, 20, or 27 months old. The rats in each age group were divided randomly into sedentary and running-training groups. The numbers of sedentary and running-trained rats were as follows: 6 months old, 7 and 5, respectively; 12 months old, 7 and 9, respectively; 20 months old, 7 and 7, respectively; 27 months old, 6 and 5, respectively. All the animals were kept under controlled conditions throughout the experiment. Temperature was...
23 ± 1°C, and the rats were subjected to a 12-hour light–12-hour dark cycle. Chow (CE-2; CLEA, Japan) and water were provided ad libitum. These experiments conformed with the Guiding Principle for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

Running Training

The rats in the running-training groups were trained on a rodent treadmill (KN-73; Natsume Seisakusyo Co. Ltd., Tokyo, Japan). An electric grid at the rear of the belt was used to supply a running stimulus. All rats ran 5 days per week for 8 weeks. Speed and duration were progressively increased until the rats ran at 30 m per minute for 60 minutes per day on 0% incline.

Muscle Sampling

After the 8-week training program, hearts were removed under pentobarbital sodium anesthesia. The atria and great vessels were dissected away from the rest of the heart, and the ventricular muscle was weighed and then separated into left ventricle and interventricular septum plus right ventricle. The left ventricular (LV) muscles were quickly frozen in liquid nitrogen, and they were stored at −80°C until assayed.

Cardiac Myosin Isozyme Analysis

The cardiac myosin isozymes were analyzed according to the pyrophosphate polyacrylamide gel electrophoresis technique, as described previously (6). The myosin was extracted from each LV sample according to the method of Farrar and colleagues (11), and the extracted myosins were stored at −20°C for electrophoretic analysis. The myosins were loaded onto a 3.88% acrylamide and 0.12% N,N’-methylene-bis-acrylamide gel (T = 4%; C = 3%). The electrophoresis buffer was 20 mM tetrasodium pyrophosphate, 1 mM ethylenediamine tetra-acetic acid (EDTA), 10% glycerol, and 2 mM L-cysteine, pH 8.9. Electrophoresis was performed for 24 hours at 70 V (constant), using a GE-4 apparatus (Pharmacia, Uppsala, Sweden), which allows circulation of the upper and lower chambers with buffer. The temperature was maintained at 2°C. After the run, the gels were stained (0.1% R-250 and G-250 Coomassie blue, 25% methanol, and 10% acetic acid) and destained (30% ethanol and 4%; C) -MyHC isoform between control and trained rats in either age group was similarly increased by training in all age groups, although the effect of training was not significant in the oldest rats (27 months old).

Cardiac Myosin Isozyme Composition

Figure 1 shows representative pyrophosphate gel patterns for the various cardiac myosin isozymes, together with densitometric scans of the gels; these were obtained from LV samples from control and trained 6- and 20-month-old rats. Table 2 shows the effects of aging and running training on myosin isozyme composition in the left ventricle of rats of various ages. In sedentary control rats, the proportion of V1 myosin decreased progressively from 6 to 27 months of age. Only in the younger age groups (6 or 12 months old) did the training have any effect on cardiac myosin isozyme composition; in these groups, there was a shift from V1 myosin to V3 myosin in trained hearts. In contrast, training had no effect on cardiac myosin isozyme composition in the older groups (20 or 27 months old). Figure 2 shows the proportion of β-MyHC isoform in the ventricles from control and trained groups at each age. In both of the younger groups, the proportion of the β-MyHC isoform was significantly greater in trained than in control rats of the same age. However, there was no difference in the proportion of the β-MyHC isoform between control and trained rats in either of the older groups (20 or 27 months old).

Table 1. Aging and Running-Training Effects on Body and Ventricular Weights

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>BW (g)</th>
<th>VW (mg)</th>
<th>VW BW⁻¹ (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>191 ± 7</td>
<td>518 ± 40</td>
<td>2.70 ± 0.15</td>
</tr>
<tr>
<td>T</td>
<td>5</td>
<td>205 ± 3</td>
<td>594 ± 16**</td>
<td>2.90 ± 0.08*</td>
</tr>
<tr>
<td>12 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>226 ± 16</td>
<td>553 ± 44</td>
<td>2.44 ± 0.05</td>
</tr>
<tr>
<td>T</td>
<td>9</td>
<td>216 ± 7</td>
<td>602 ± 38*</td>
<td>2.78 ± 0.13**</td>
</tr>
<tr>
<td>20 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>275 ± 22</td>
<td>626 ± 42</td>
<td>2.28 ± 0.17</td>
</tr>
<tr>
<td>T</td>
<td>7</td>
<td>242 ± 8</td>
<td>649 ± 30</td>
<td>2.68 ± 0.07***</td>
</tr>
<tr>
<td>27 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>255 ± 49</td>
<td>738 ± 80</td>
<td>2.98 ± 0.53</td>
</tr>
<tr>
<td>T</td>
<td>5</td>
<td>237 ± 9</td>
<td>738 ± 11</td>
<td>3.16 ± 0.11</td>
</tr>
</tbody>
</table>

Notes: Values are means ± standard deviation. n = number of experimental animals; BW = body weight; VW = ventricular weight; C = sedentary control; T = trained.

*p < .05 versus the C group; **p < .01 versus the C group.
training than previously reported (11) could alter the rate of
set out to determine whether a higher intensity of running
colleagues (11) in male Fischer 344 rats. In our study, we
ported by Boluyt and colleagues (27) in female Fischer 344
rats and by both Capasso and colleagues (2) and Farrar and
V1 myosin isozyme (\(V_1\)) and V3 myosin isozyme (\(V_3\)) are
months of age to 40.5% at 20 months was observed in fe-
and Capasso and colleagues (2) and Farrar and
classification is based on electrophoretic mobility in the native state, in
which the migration rate reflects the combined properties of both of
the myosin heavy chains (MyHCs). \(V_1\) and \(V_3\) are
homodimers, respectively, whereas \(V_2\) is an \(\alpha\)-\(\beta\)-MyHC heterodimer.
C = sedentary control; T = trained.

**DISCUSSION**

In this study, a profound reduction in the proportion of
the \(V_1\) myosin isozyme (\(\alpha\)-MyHC isoform) from 82.5% at 6
months of age to 40.5% at 20 months was observed in fe-
male Fischer 344 rats. This marked shift from the \(V_1\) to the
\(V_3\) myosin isozyme (\(\beta\)-MyHC isoform) is similar to that re-
ported by Boluyt and colleagues (27) in female Fischer 344
rats and by both Capasso and colleagues (2) and Farrar and
colleagues (11) in male Fischer 344 rats. In our study, we
set out to determine whether a higher intensity of running
training than previously reported (11) could alter the rate of
this age-related change in cardiac myosin isozyme com-
position. This training program affected cardiac myosin
isoform with a concomitant cardiac hypertrophy is remark-
able because exercise accelerated an effect of maturation. Usually
physical activity compensates for decrements oc-
curring with aging; for example, exercise attenuated the
age-related decline in the intrinsic mechanical performance
of the heart (14–16). In this case, the maturation-associated
shift to \(V_3\) myosin isoform was accelerated by exercise
training.

The increase in \(V_3\) myosin isoform has been associated
with an intermittent increase in hemodynamic load only
during running exercise (24). It is generally accepted that
chronic pressure or volume overload induces a cardiac hy-
pertrophy, with a decrease in mechanical performance and
an increase in the proportion of \(V_3\) myosin (2,28,29). It is
thus possible that a transient pressure or volume overload
during exercise would induce a myosin isozyme transition
in young rats. Therefore, the increased myocardial contrac-
tile activity that was due to intense and prolonged exercise
induced a fast (\(V_1\) isozyme)-to-slow (\(V_3\) isozyme) myosin
transformation. This would be analogous to the well-known
fast-to-slow fiber type of transformation observed in skele-
tal muscle after endurance training (30,31). It is well known
that \(V_3\) myosin has low ATPase activity and a greater econ-
yomy of force production than \(V_1\) myosin (8). Fitzsimons
and colleagues (24) demonstrated that LV contractile per-
formance (i.e., pressure generation) was maintained in
trained rats, even though the proportion of \(V_1\) myosin was
decreased. Perhaps, in the present study, the changes in my-
osin isoform composition seen in relatively young trained

**Table 2. Aging and Running-Training Effects on LV Myosin
Isozyme Composition**

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>(V_1) (%)</th>
<th>(V_2) (%)</th>
<th>(V_3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>C 6</td>
<td>82.5 ± 3.8</td>
<td>12.3 ± 2.5</td>
<td>5.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>T 5</td>
<td>71.7 ± 3.6**</td>
<td>19.1 ± 2.4**</td>
<td>9.2 ± 1.3**</td>
</tr>
<tr>
<td>12 mo</td>
<td>C 7</td>
<td>65.0 ± 5.7</td>
<td>22.5 ± 2.5</td>
<td>12.5 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>T 9</td>
<td>54.1 ± 8.4**</td>
<td>26.1 ± 3.2*</td>
<td>19.8 ± 5.4**</td>
</tr>
<tr>
<td>20 mo</td>
<td>C 7</td>
<td>40.5 ± 4.1</td>
<td>31.2 ± 2.2</td>
<td>28.3 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>T 7</td>
<td>41.6 ± 8.3</td>
<td>30.0 ± 1.4</td>
<td>28.4 ± 7.1</td>
</tr>
<tr>
<td>27 mo</td>
<td>C 6</td>
<td>36.9 ± 5.4</td>
<td>31.7 ± 1.4</td>
<td>31.4 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>T 5</td>
<td>38.5 ± 5.0</td>
<td>30.5 ± 0.9</td>
<td>31.0 ± 4.4</td>
</tr>
</tbody>
</table>

**Notes:** Values are means ± standard deviation. \(n = \) number of experimental
animals; LV = left ventricular; C = sedentary control; T = trained.

\(^*p < .05\) versus the C group; \(^**p < .01\) versus the C group.
female rats (6 or 12 months old) allow the heart to economize on cross-bridge cycling without compromising the potential for pressure generation.

The accelerated shift to V3 myosin isozyme in young female rats who exercised confirms a previous report in which only young female rats were exercised (24), but this effect may be gender specific. We recently reported that in young male Sprague-Dawley rats, running training had little effect on cardiac myosin isozyme composition, regardless of the running intensity (32). The reason for the differential responses between the genders remains unclear.

In the present study, the training program did not induce a cardiac myosin isoform transition in older rats (20 or 27 months old). Farrar and colleagues (11) also reported that cardiac myosin isoform composition in older rats (24 months old) was not affected by running training at lower intensity (20 m per minute for 60 minutes per day). The question then arises as to why we did not observe any effect of the running training on cardiac myosin isoform composition in older trained rats. One possible factor is that the proportion of V3 myosin isoform in the older rats is far higher than it is in the younger rats. We noted an increase in the proportion of V3 myosin isoform with aging, from 5.2% at 6 months to 31.4% at 27 months. We had hypothesized that a higher intensity of running (30 m per minute for 60 minutes per day) would further decrease V1 myosin isoform in old rats, but the results did not support the hypothesis. It may be that in the hearts of older rats, which have predominantly V3 myosin, little or no further shift from V1 to V3 myosin isoform is possible in response to training. Indeed, the percentage of V3 myosin isoform in nontrained 20-month-old rats (28.3%) was already greater than in trained 12-month-old rats (19.8%).

In conclusion, running training had a significant effect on cardiac myosin isoform composition in young female Fischer 344 rats in our study. However, the training program failed to induce a cardiac myosin isoform transition in older rats. These results suggest that the mechanisms mediating the response of cardiac muscle to training alter during aging.

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References

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