Adaptation of guinea pig diaphragm muscle to aging and endurance training

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Lieberman, David A., Leo C. Maxwell, and John A. Faulkner. Adaptation of guinea pig diaphragm muscle to aging and endurance training. Am. J. Physiol. 222(3): 556-560. 1972.—The percentage of red and white fibers in diaphragm muscle of guinea pigs 6, 14, and 40 weeks of age was determined by succinic dehydrogenase activity. The percentage of red fibers decreased with increase in age. The percentages were: 86% at 6 weeks, 70% at 14 weeks, and 65% at maturity. Mean fiber area increased with age in proportion to increases in body and diaphragm weights. Following 8 weeks of exercise, the percentage of red fibers in young animals was maintained while it increased by 20% in mature animals. These effects may be associated with the role of the guinea pig diaphragm in temperature regulation and ventilation.

In most mammalian species the diaphragm is composed of red and white fiber types (7, 8, 14), but some very small animals (shrew) have almost exclusively red fibers in the diaphragm and some very large animals (cow) have almost exclusively white fibers (7). From their data on diaphragms of several mammalian species, Gauthier and Padykula (7) conclude that the cytochemical characteristics of the muscle fibers are related to the functional requirements of the diaphragm.

Previously we studied changes in guinea pig plantaris muscle that occur with aging and with endurance training (6). An increase in age resulted in a decrease in the percentage of red fibers, fiber hypertrophy, and a loss in the total number of fibers. With endurance training, there was an increase in the percentage of small red fibers and no loss in total number. These changes indicated a loss of oxidative capacity in the plantaris with aging and an increase in oxidative capacity with endurance training. Although no data on adaptation of diaphragm fibers are available, the heterogeneity in fiber types and in fiber areas in the guinea pig diaphragm indicates a potential for adaptation similar to that observed in the plantaris.

Our objectives were to classify the fibers of the diaphragm muscle and to determine whether changes in relative proportions of the types of fibers were associated with increased age, increased exercise, or both. Fibers were classified as red or white on the basis of succinic dehydrogenase (SDH) activity, the percentage of each type of fiber present was determined, and the mean cross-sectional area of each fiber type was calculated. The effects of age were determined by comparison of 6-week, 14-week, and mature animals (older than 40 weeks). The effects of endurance training were determined by comparison of guinea pigs exercised on a motor-driven treadmill for 8 weeks with age-matched controls.

Methods

Twenty-eight young (6 weeks) guinea pigs and 10 mature (>40 weeks) animals were used in the study. Since no significant differences were observed in body weight, diaphragm size, or diaphragm fiber area among guinea pigs older than 40 weeks, they are all considered as mature. Twelve of the 6-week-old animals were used for determination of fiber types and fiber area of young animals. The remaining young and mature guinea pigs were divided into age- and weight-matched pairs. During an 8-week period, one of each pair was restricted to a 30 x 50 cm cage, and the other was trained to run on a motor-driven treadmill (6). All animals were fed ad libitum.

The young guinea pigs were trained easily; the mature guinea pigs were trained with much more difficulty. However, four mature animals exercised successfully for 8 weeks; these were able to run at 30 m/min for 40-50 min. One mature animal in the forced exercise group died in his cage of unknown causes, and a second could not be trained to run. These two and their matched controls were dropped from the study.

Animals were sacrificed in their original age-matched pairs of control and trained animals. The diaphragm was excised and weighed and small biopsy sections were removed from ventral and dorsal sites (Fig. 1). Preliminary research had showed that samples from sternal, ventral, and lateral regions were not significantly different from one another. Because there are already extensive data on the ventral site (7), this site was chosen to represent these parts of the muscle. Samples from the dorsal region exhibited characteristics somewhat different from those at the ventral sites.

The main portion of the guinea pig diaphragm is only about 1 mm thick, and the fibers deteriorate rapidly; therefore the muscle must be excised quickly once it is exposed. Quick removal of the total undamaged diaphragm is complicated by the extensive fibrous attachments to the thoracic wall and by the proximity of other organs. A complete diaphragm was never obtained and in some cases severely damaged diaphragm muscles resulted; these are not included in our weight data. Samples excised from the dia-


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from each sample were mounted and 14-μ cross-sections of the diaphragm were quick frozen in acetone and Dry Ice. Slices from each sample were mounted and 14-μ cross-sections were cut in a cryostat. The sections were placed on a cover slip and incubated with succinate and nitro-blue tetrazolium to localize SDH activity (13). In a few sections, ice crystals formed in fibers, or the sections crumbled when cut and histochemical data could not be obtained. Most of the missing data are from the ventral site, probably because of the thinness of the muscle at this site.

To ensure adequate sampling, each cross-section of the diaphragm muscle (Fig. 2) was divided into quadrants and a sample site was selected randomly from each quadrant. A Leitz Prado microprojector was used to project the site at X1,500. Approximately 50 fibers at each site were classified as red or white. The perimeter of individual fibers was traced and the fiber area determined by planimetry. We are aware that three fiber types, red, intermediate, and white, are generally recognized and that these three fiber types are identifiable by histochemical (1, 5, 7, 14, 20) and by ultrastructural characteristics (16). White fibers are readily classified and counted.

Means and standard deviations were calculated for percent composition and muscle fiber area in diaphragms of young and mature trained animals compared with their age-matched controls (Table 1); young trained guinea pigs had 15% (ventral site) and 15% (dorsal site) more red fibers per site than the age-matched controls. At both the ventral and dorsal sites, the diaphragm of the mature trained guinea pigs had approximately 20% more red fibers per site than controls. Although the percentage of red fibers increased with training, mean fiber area was not affected. The percentage increase in red fibers was accompanied by a slight, but insignificant, increase in fiber area in both ventral and dorsal sites in trained animals (Table 1). This resulted in no overall change in mean fiber area with training.

DISCUSSION

Age and endurance training both affect the development of muscle fibers of the diaphragm, but in different ways. With aging, the diaphragm fibers hypertrophy and the percentage of red fibers decreases. Training does not alter the fiber area, but causes an increase in percentage of red fibers in both the ventral and dorsal sites.

As body size increases with age, basal metabolic rate (BMR) per unit mass decreases (12, 18). The decrease in BMR results from diminished energy requirements as rapid growth subsides, as well as from a disproportionate increase of tissues with low metabolic rate, such as fat, bone, and connective tissue (18). Greater airway resistance and increased compliance have been observed in older rabbits (4) and older humans (2), compared with mature but younger individuals. When airway resistance and compliance are increased, optimal breathing frequency for a given alveolar ventilation decreases (15). Thus, small young animals have a much higher breathing frequency than large mature animals, even though their absolute pulmonary ventilation is less.

Among our guinea pigs, as breathing frequency decreased with age, from 120 to 80 breaths/min, the percentage of red fibers in the diaphragm decreased from 87 to 66%. Decrease in breathing frequency with age has been reported pre-
FIG. 2. Photomicrographs of selected sample sites from diaphragms of 6-week-old guinea pigs (A), 14-week-old controls (B), mature controls (C), and 14-week-old trained (D). Linear magnification X245.
and percentage of red fibers in diaphragm for control and trained guinea pigs of various ages

<table>
<thead>
<tr>
<th>Body Wt, g</th>
<th>Sample Site</th>
<th>Fiber Area, ( \mu^2 )</th>
<th>Percentage of Red Fibers by Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Red</td>
<td>White</td>
</tr>
<tr>
<td>6-week controls (n = 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral</td>
<td>1,030 ± 180</td>
<td>1,660</td>
<td>87 ± 11</td>
</tr>
<tr>
<td>Dorsal</td>
<td>1,170 ± 207</td>
<td>1,770</td>
<td>86 ± 10</td>
</tr>
<tr>
<td>14-week controls (n = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral</td>
<td>1,510 ± 220</td>
<td>2,330</td>
<td>71 ± 4*</td>
</tr>
<tr>
<td>Dorsal</td>
<td>1,700 ± 210</td>
<td>2,890</td>
<td>70 ± 4*</td>
</tr>
<tr>
<td>14-week trained (n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral</td>
<td>1,650 ± 410</td>
<td>3,000</td>
<td>87 ± 4*</td>
</tr>
<tr>
<td>Dorsal</td>
<td>1,810 ± 520</td>
<td>3,200</td>
<td>89 ± 5*</td>
</tr>
<tr>
<td>Mature controls (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral</td>
<td>1,680 ± 220</td>
<td>3,230</td>
<td>66 ± 3*</td>
</tr>
<tr>
<td>Dorsal</td>
<td>2,360 ± 170</td>
<td>4,490</td>
<td>62 ± 6*</td>
</tr>
<tr>
<td>Mature trained (n = 4)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ventral</td>
<td>2,190 ± 330</td>
<td>2,920</td>
<td>86 ± 2*</td>
</tr>
<tr>
<td>Dorsal</td>
<td>2,750 ± 680</td>
<td>4,010</td>
<td>86 ± 8*</td>
</tr>
</tbody>
</table>

Values are means ± sp. *Mature or 14-week-old controls, significantly different from 6-week-old controls; 14-week-old trained significantly different from 14-week-old controls; and mature trained significantly different from mature week-old controls by t test difference between the means. \( P < .05 \). ↑Dorsal site of mature trained and controls significantly different from ventral site of mature trained and controls by paired t test difference between the means. \( P < .05 \).

The percentage of red fibers in the diaphragms of young and mature trained guinea pigs was approximately the same (86%), and both groups of trained animals had more red fibers than their age-matched controls. In spite of these similarities, the adaptation of muscle fibers to endurance training was quite different in the two age groups. In young animals, endurance training prevented the shift of 15 to 20% of the fibers from red to white which occurred in control animals. In mature animals, endurance training resulted in the transformation of 20% of the fibers from white to red. With a greater proportion of red fibers, the dia-

**FIG. 3.** Relationship between diaphragm weights and guinea pig body weights. ● Controls; ○, trained.

**FIG. 4.** Relationship between diaphragm muscle fiber cross-sectional areas and guinea pig body weights.
phragm of young and mature trained guinea pigs has a greater mitochondrial density (16) and a greater oxidative capacity (10) per unit mass than the diaphragm of controls. The transformation from red to white and from white to red indicates that the muscle fibers of the diaphragm have adaptive potential similar to that observed in other skeletal muscle fibers (1, 5, 6).

In summary, growth of the diaphragm muscle of guinea pigs with age resulted in part from fiber hypertrophy, but fibers in different portions of the diaphragm showed different amounts of hypertrophy. The increase in fiber area was proportional to the increase in body weight, but the mechanism by which fiber growth was stimulated is not clear.

Aging was accompanied by a loss of mitochondrial density from the diaphragm fibers. Daily endurance training maintained the oxidative capacity of the diaphragm fibers of young guinea pigs and enhanced the oxidative capacity of the diaphragm fibers of mature guinea pigs. These observations support the concept that breathing frequency is a major determinant of the oxidative capacity of the diaphragm. The adaptability of the fibers of the diaphragm to both aging and training may be of clinical significance in certain types of obstructive lung disease.

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REFERENCES