Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging

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Houmard, J. Joseph A., Melinda L. Weidner, Karen E. Gavigan, Gilian L. Tyndall, Matthew S. Hickey, and Aymen Alshami. Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging. J. Appl. Physiol. 85(4): 1337–1341, 1998.—The purpose of this study was to determine whether enzymatic and histochemical characteristics of human skeletal muscle are altered with aging. Tissues from the vastus lateralis (VL) and gastrocnemius were analyzed for citrate synthase (CS) activity and fiber type in 55 sedentary men (age range 18–80 yr). In this population, CS activity in the gastrocnemius was negatively related to age ($r = -0.32, P < 0.05$); there was no relationship in the VL. Treadmill-determined maximal oxygen consumption was positively related ($r = 0.40, P < 0.05$) to CS in the gastrocnemius but not in the VL. CS activity in the gastrocnemius was 24% lower in the oldest (≥60 yr; $n = 10$) vs. the youngest (<30 yr; $n = 12$) men; there was no change in CS activity in the VL with aging. No changes in fiber type were evident with age in either muscle. These data suggest a reduction in oxidative enzyme activity in human skeletal muscle with the aging process; this relationship may be muscle-group specific.

METHODS

Subjects. Sedentary Caucasian men who were 18–80 yr of age were studied. Inclusion criteria for all subjects included no regular exercise in the previous 2 yr and no evidence of cardiovascular disease or diabetes (fasting plasma glucose concentration >7.7 mmol/l on 2 occasions). On the basis of verbal questioning and a medical history, we determined that the aged subjects had led a largely sedentary lifestyle with no prolonged periods (>3 yr) of a regular exercise program; this was supported by the maximal oxygen consumption ($V_{O2\text{max}}$), body composition, and muscle (oxidative enzyme activity, fiber type) data (see RESULTS). All subjects were nonsmokers; a total of 55 men were studied. The experimental protocol was approved by the University Policy and Review Committee on Human Research.

Cardiorespiratory fitness. Fitness was determined by the $V_{O2\text{max}}$ elicited during an incremental treadmill test. The Bruce protocol (6) was utilized in subjects up to age 40 yr. The Balke protocol (3) was used in subjects >40 yr; a modified Balke protocol, which utilized a slower speed (67.1 m/min), was used in the oldest subjects (>60 yr of age). Different protocols were used so that exhaustion was reached within ~8–15 min, as recommended for a valid $V_{O2\text{max}}$ test (7, 19). Criteria for data inclusion were a respiratory exchange ratio >1.0, voluntary fatigue, and attainment of at least 85% of maximal heart rate; similar criteria have been used in other studies (7, 19).Expired gases were monitored continuously (Beckman Horizon MMC, SensorMedics, Anaheim, CA) for determination of oxygen uptake. Twelve-lead electrocardiogram (ECG) tracings were acquired every minute, and a resting ECG was obtained before treadmill testing.

Adiposity. Body density was determined by hydrostatic weighing, and body fat percent, fat mass, and fat-free mass were computed as described previously (17). Height was measured to the nearest 0.1 cm, and mass was measured to the nearest 0.1 kg.

Muscle characteristics. A sample from the lateral gastrocnemius and vastus lateralis was obtained with the percutaneous needle-biopsy technique; a portion was immediately frozen in liquid nitrogen for subsequent analysis of citrate synthase activity at 22°C (30). Part of the biopsy was mounted...
in a trigacanth gum-OCT (Miles Elkhart, IN) mixture and frozen in isopentane cooled over liquid nitrogen. Mounted muscle was subsequently sectioned (10 µm) at −20°C and stained at pH 10.3 and 4.54 for determination of fiber type (S). Fiber type [vastus lateralis, 331 ± 23 (SE) fibers counted; gastrocnemius, 326 ± 21 fibers counted] was determined from the vastus lateralis of 46 subjects and from the gastrocnemius of 45 subjects; citrate synthase activity from the gastrocnemius was determined in 52 subjects and from the vastus lateralis of 53 subjects.

Statistical analysis. Univariate and multivariate linear regression analysis determined relationships between variables of interest in the population (age range 18–80 yr) studied. To determine differences between the maximal age ranges of the population (young vs. aged), data were also compared with a factorial analysis of variance in subgroups of the youngest (≤30 yr) vs. oldest (≥60 yr) individuals. Statistical significance was accepted at the P < 0.05 level.

RESULTS

Subject characteristics. Descriptive data for the 55 subjects are presented in Table 1. Body fat percentage (r = 0.46, P < 0.001) was positively associated with age in this population. Descriptive characteristics of the young (≤30 yr, n = 12) vs. aged (≥60 yr, n = 10) subgroups, respectively, were as follows: age, 23.7 ± 0.8 vs. 70.8 ± 2.2 yr; \( V_{O_{2\max}} \) (ml·kg\(^{-1}\)·min\(^{-1}\)), 41.6 ± 1.2 vs. 23.6 ± 1.7; \( \Delta V_{O_{2\max}} \) (0.40 ml·kg\(^{-1}\)·min\(^{-1}\)·yr\(^{-1}\) and -0.033 l·min\(^{-1}\)·yr\(^{-1}\)) agreed with other data (7, 19, 31). A similar pattern was evident when the subgroups of the youngest and oldest men were compared (Fig. 3); citrate synthase activity was significantly lower (P < 0.05) in the younger men only in the gastrocnemius, and the value was lower by 24%. In the younger men, citrate synthase activity was significantly (P < 0.05) lower in the vastus lateralis compared with the gastrocnemius. As presented in Fig. 4, in this population relative \( V_{O_{2\max}} \) on the treadmill was significantly related to citrate synthase activity in the gastrocnemius (r = 0.43, P < 0.01) but not in the vastus lateralis (r = 0.09, P = 0.54); there were similar relationships (data not shown) between absolute \( V_{O_{2\max}} \) and citrate synthase activity in the gastrocnemius (r = 0.35, P < 0.05) and the vastus lateralis (r = 0.02, P = 0.88).

Table 1. Descriptive characteristics of the men studied

| Variable              | Mean ± SE
|-----------------------|-----------
| Age, yr               | 45.4 ± 2.2
| \( V_{O_{2\max}} \), ml·kg\(^{-1}\)·min\(^{-1}\) | 37.7 ± 1.0
| \( V_{O_{2\max}} \), l·min\(^{-1}\) | 2.98 ± 0.09
| Body fat, %           | 25.4 ± 0.8
| Fat mass, kg          | 22.6 ± 1.1
| Fat-free mass, kg     | 66.7 ± 1.1
| BMI, kg/m\(^2\)       | 27.9 ± 0.5

Values are means ± SE for 55 men. \( V_{O_{2\max}} \), maximal oxygen consumption; BMI, body mass index.

Fig. 1. Relationships of age with maximal oxygen consumption (\( V_{O_{2\max}} \)). Decline in relative \( V_{O_{2\max}} \) (A) was -0.40 ml·kg\(^{-1}\)·min\(^{-1}\)·yr\(^{-1}\); rate of decline in absolute \( V_{O_{2\max}} \) (B) was -0.033 l·min\(^{-1}\). n = 55 Men.
with the gastrocnemius containing more of these oxidative fibers. There were no relationships between muscle fiber composition and age in the population studied; muscle fiber type was also not significantly different between the young and aged subgroups in either muscle (data not shown).

Stepwise regression. Results of stepwise regression analysis in the men studied are presented in Table 2. Variables entered were fat-free mass, percent fat, fat mass, muscle fiber composition, and citrate synthase activity of both muscle groups; only subjects with all of these data \((n = 35)\) were entered into the regression analysis. Regression analysis indicated that percent fat accounted for 53% of the variance in relative \(\dot{V}O_2\)max, \(\dot{V}O_2\)max (ml · kg\(^{-1}\) · min\(^{-1}\))

**Table 2.** Stepwise regression analysis of body composition, muscle citrate synthase activity, and muscle fiber composition with \(\dot{V}O_2\)max.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial (r^2)</th>
<th>Multiple (r^2)</th>
<th>Adjusted (r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}O_2)max (ml · kg(^{-1}) · min(^{-1}))</td>
<td>0.53</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>0.07</td>
<td>0.60</td>
<td>0.57</td>
</tr>
<tr>
<td>Gastrocnemius citrate synthase activity (µmol · g wet wt(^{-1}) · min(^{-1}))</td>
<td>0.07</td>
<td>0.67</td>
<td>0.62</td>
</tr>
<tr>
<td>Vastus lateralis (%IIb)</td>
<td>0.07</td>
<td>0.67</td>
<td>0.63</td>
</tr>
<tr>
<td>(\dot{V}O_2)max (l/min)</td>
<td>0.34</td>
<td>0.34</td>
<td>0.32</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>0.19</td>
<td>0.53</td>
<td>0.50</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>0.07</td>
<td>0.60</td>
<td>0.55</td>
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<td>0.62</td>
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All variables were significant at the \(P < 0.05\) level; \(n = 35\). Variables entered into the regression were fat-free mass, %fat, fat mass, muscle fiber composition, and citrate synthase activity.
followed by citrate synthase activity of the gastrocnemius (7%) and percentage of type IIb of the vastus lateralis (7%). For predicting absolute VO2max, regression analysis indicated that fat-free mass accounted for 34% of the variance followed by percent fat (19%), citrate synthase activity in the gastrocnemius (7%), and percentage of type IIb of the vastus lateralis (7%).

**DISCUSSION**

The impact of aging on enzymes that reflect respiratory capacity in human skeletal muscle is not clear. The data from the present study suggest that, in humans, distinct leg muscles may respond differently to the aging process. More specifically, muscle oxidative capacity appeared to decrease in the gastrocnemius but not in the vastus lateralis with advancing age. This conclusion was deduced from our findings of 1) a negative relationship between age and citrate synthase activity in the gastrocnemius but not in the vastus lateralis in a population of men (Fig. 2) and 2) a decline in citrate synthase activity (−24%) in the lateral gastrocnemius but not in the vastus lateralis when a subgroup of young (≤30 yr) vs. aged (≥60 yr) men was directly compared (Fig. 3).

This finding is not in agreement with the data of Rooyackers et al. (28), who reported a negative relationship between citrate synthase activity in the vastus lateralis and age in a population of men and women. Our data do agree with other work (1, 2, 12, 15, 16, 22, 27) that found no change in oxidative enzyme activities in the vastus lateralis with aging. Studies that have sampled the gastrocnemius have reported a similar decline (−25%) in oxidative enzyme activity (9, 10). No previous work, however, has directly compared the biochemical characteristics of these two muscle groups in sedentary young vs. aged individuals. Grimby et al. (16) reported a decrease in muscle fiber area with aging in the vastus lateralis but not in the biceps brachii; muscle citrate synthase activity was not, however, altered in either muscle group. These data and the present findings suggest that citrate synthase activity and oxidative capacity either decreases or is not altered with the aging process in human skeletal muscle; this depends, however, on the muscle group studied.

The explanation for a reduction in citrate synthase activity in the gastrocnemius but not the vastus lateralis with aging is not clearly evident. Some data (4) suggest that the gastrocnemius is more tonically stimulated than is the vastus lateralis. It is possible that tonically active muscle groups are more susceptible to the impact of the aging process. Self-reported physical activity is thought to decrease with aging (7, 19, 31). Although all subjects were determined to be sedentary on the basis of questioning and their VO2max body composition, and muscle characteristics (fiber type, oxidative enzyme activity), it is possible that lower levels of spontaneous physical activity in the aged group contributed to the reduction in muscle oxidative capacity in the gastrocnemius. It is not evident, however, why the gastrocnemius would be more dramatically affected by a change in activity levels with aging than would the vastus lateralis. There is also evidence for a change in the neurogenic stimulus to skeletal muscle with aging that can lead to structural changes (1, 2, 12, 15, 16, 24, 25). Certain muscle groups, such as the gastrocnemius, could respond more profoundly to a change in neurogenic stimuli with aging and exhibit an accompanying reduction in mitochondrial content. The explanation for the different responses of specific leg muscles to aging observed, however, remains to be more clearly elucidated with further study.

The contractile and energy-producing characteristics of the gastrocnemius and vastus lateralis may influence functional capacity with aging (8, 14). Findings in the gastrocnemius are important because a reduction in plantar flexor strength and endurance in the elderly is related to an increased incidence of falls (29). In young individuals, it has been hypothesized that the pattern of tension development and energy production is closely synchronized between the vastus lateralis and gastrocnemius during activities such as locomotion (14). With aging, the reduction in oxidative capacity in the gastrocnemius noted in the present study may disrupt this coordination and contribute to a functional impairment. Such interactions indicate it is vital to explore potential mechanisms in skeletal muscle that may contribute to limitations in physical ability with aging.

In other work comparing the human vastus lateralis and gastrocnemius, Green et al. (14) reported increased lactate dehydrogenase activity in the vastus lateralis but no differences in other enzymes indicative of energy production (succinate dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase, total phosphorylase) or fiber type in young (20–24 yr), athletic individuals. In sedentary, young individuals there are trends for the gastrocnemius to be more oxidative than the vastus lateralis in terms of fiber type and enzyme activities, although the number of subjects studied has been small (11, 13). Because our young subjects were sedentary, we are in agreement with these latter findings (Fig. 3). These data suggest that fitness level and age are important factors that influence the characteristics of these two muscle groups within an individual; however, more study needs to be done to conclusively define these relationships.

Fiber type was studied because it can be indicative of the metabolic properties of muscle. Again, the literature is controversial as in the vastus lateralis an increase in the relative percentage of type I fibers (22, 27) or no change (1, 2, 12, 21, 24, 25) in fiber type has been reported with aging. The data from the present study support the premise that fiber type is not altered with aging; therefore, any alterations in functional capacity cannot be attributed to a change in muscle fiber composition. The present study did not, however, investigate the degree of muscle atrophy that occurs with advancing age; such atrophy would alter exercise tolerance and functional capacity (1, 2, 9, 10, 12, 21–25).

The decline in VO2max with aging is well documented and has been attributed to many factors (7, 19, 31). The decrease in citrate synthase activity in the gastrocnemius observed suggests that a reduction in skeletal muscle oxidative enzyme activity may parallel the
decline in \( V\dot{O}_2 \text{max} \) with the aging process. Citrate synthase activity was measured because it is found in direct proportion to muscle mitochondrial content (18) and correlates highly with in vivo oxidative metabolism in humans (9, 26). These data do not, however, provide solid evidence for a cause-and-effect relationship. It has been proposed that many of the detrimental effects of aging, including the decline in \( V\dot{O}_2 \text{max} \), increased body fat, and reduction in muscle functional capacity, are secondary to a reduction in physical activity (7, 19, 31). The decline in \( V\dot{O}_2 \text{max} \) and citrate synthase activity in skeletal muscle and the increased body fat in the present study could thus reflect concomitant changes with a reduction in physical activity with the aging process.

In conclusion, not all muscles in humans appear to be affected similarly by the aging process. In the present study there was a decrease of citrate synthase activity in the gastrocnemius but not in the vastus lateralis with advancing age. These data suggest that the decline in mitochondrial capacity in human skeletal muscle is not uniform and may be muscle-group specific.

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