The Effect of Aging on Anaerobic and Aerobic Enzyme Activities in Human Skeletal Muscle

Jan J. Kaczor,1,2 Wieslaw Ziolkowski,1 Jedrzej Antosiewicz,1 Stanislaw Hac,4 Mark A. Tarnopolsky,2,3 and Jerzy Popinigis1

1Department of Biochemistry, J. Sniadecki Academy of Physical Education and Sport, Gdansk, Poland. Departments of 2Pediatrics and 3Medicine, McMaster University, Hamilton, Ontario, Canada. 4Department of General Endocrine and Transplant Surgery, Medical University of Gdansk, Poland.

The effect of aging on metabolic enzyme activity remains controversial, possibly due to physical activity differences. We examined the effect of aging on the enzyme activity for anaerobic and aerobic pathways in nonweight-bearing human skeletal muscle from relatively sedentary males. The muscle obliquus internus abdominis was analyzed for anaerobic (creatine kinase, adenylate kinase, and lactate dehydrogenase) and aerobic (2-oxoglutarate dehydrogenase and carnitine palmitoyltransferase) enzyme activities in two groups: middle-aged (29–54 years) and older (61–74 years) adults. All enzyme activities were lower in older versus middle-aged adults when results were expressed as muscle wet weight (p < .05). When activity was expressed relative to the protein content, only lactate dehydrogenase remained significantly lower in older versus middle-aged adults (p < .001). In conclusion, some of the reduction in muscle performance in older adults may be due to lower activity of the anaerobic and aerobic enzymes as well as protein content, not solely due to a decrease in physical activity.

HUMAN aging is a gradual process taking place over decades. Age-related changes are associated with a progressive decline in skeletal muscle mass (sarcopenia) and muscle performance, characterized by decreased muscle strength and function and increased muscle fatigue (1,2). The functional and metabolic consequences of sarcopenia lead to morbidity and mortality (3). There have been several hypotheses regarding the pathophysiological mechanisms responsible for age-related sarcopenia. These include: decreased muscle protein synthesis (4,5), increased reactive oxygen species (ROS) and nitrogen species generation (6,7) originally based on the free radical theory of aging (8), reduced or increased enzyme activity (9–11), impaired glucose metabolism (12,13), and an imbalance between degradation and removal of “damaged” muscle proteins (14). Much attention has focused on mitochondrial capacity, with studies showing lower activity of enzymes involved in the respiratory chain, possibly related to an age-associated increase in deletions and point mutations in mitochondrial DNA (mtDNA) (15). Recently, it has been shown that aging is associated with alterations in messenger RNA (mRNA) levels, which may indicate changes in skeletal muscle gene expression, mRNA stability, or both (16). Using microarray technology it has been documented that 113 genes were regulated (55 decreased and 58 increased) with aging. The 55 genes involved in energy metabolism were reduced in their expression level more than 2-fold during the aging process. In general, these genes were associated with mitochondrial function and turnover, glycolysis, and glycogen metabolism (16).

The activity of several mitochondrial enzymes (citrate synthase, complex I-III, II-III, and cytochrome c oxidase) have been reported to decrease (9,17,18) or remain unchanged (19–22) with aging in weight-bearing muscle. However, there is little known about the effect of aging on 2-oxoglutarate dehydrogenase (OGDH), the rate-limiting enzyme of tricarboxylic acid (TCA) cycle. Recent reports have shown that OGDH activity is inhibited by ROS and 4-hydroxy-2-nonenal, a product of lipid peroxidation, in rat heart mitochondria (23,24). Given the increase in ROS damage to proteins with aging (11), we chose to assess the activity of this particular enzyme and others in a nonlocomotor muscle from sedentary persons.

There is conflicting evidence regarding an age-associated alteration in lipid oxidation with some studies showing a reduction (25,26), while others finding no effect (27). A possible age-related reduction in fat oxidation could be explained by lower habitual physical activity. Although aging may be associated with lower fat oxidation in older as compared to younger adults (28), it has been reported that regular physical training prevents the decline in fat oxidation during the aging process (29). The changes in the capacity of muscle to oxidize fatty acids have often been evaluated by measurements of β-hydroxy-acyl-coenzyme A (CoA) dehydrogenase activity as an indicator of the mitochondrial β-oxidation and less often by carnitine palmitoyltransferase (CPT; acyl-CoA transport). However, there are still contradictory results concerning both β-hydroxy-acyl-CoA dehydrogenase and CPT, with some studies showing decreases in activity (30,31), while other investigators finding no effect (20,29) on fatty acid oxidation capacity consequent to the aging process.

In muscle from older adults, the activity of glycolytic enzymes such as lactate dehydrogenase (LDH) and hexokinase (HEK) are markedly decreased (32,33). In
contrast, others have reported no changes in glycolytic (LDH) and high-energy phosphate enzyme activities such as creatine kinase (CK) and adenylate kinase (AK) in human skeletal muscle of older versus younger adults (20,30).

One explanation for changes in muscle function and enzyme capacity with aging is a change in fiber type composition. Although a lower proportion of fast-twitch muscle fibers has been reported with aging (32,34), others have found minimal changes or no differences in fiber type composition with age (30,35). Consequently, it is not possible to use directional changes in fiber type to explain a reduction of both glycolytic and mitochondrial enzyme capacities. Because most of the studies looking at the effect of aging on skeletal muscle have used a weight-bearing muscle such as the vastus lateralis (20,22,32,33) or others (17,18,30), variations in physical activity may explain some of the conflicting data. However, it is well known that muscle protein content is determined by the balance between protein synthesis and breakdown. Some (17,36,37), but not all (38,39), studies in humans have shown that muscle protein synthesis decreases with age.

The purpose of this study was to quantify the effects of aging on the anaerobic and aerobic capacities in a nonlocomotor human skeletal muscle from sedentary participants of different ages. The high-energy phosphate (CK, AK), glycolytic (LDH), and mitochondrial (OGDH and CPT) enzyme activities were measured in obliquus internus abdominis muscle obtained from sedentary and older adults. Our hypotheses were that all of the above enzyme activities, which represent the major systems of adenosine triphosphate (ATP) resynthesis in skeletal muscle, would be lower in older as compared to middle-aged adults.

METHODS

Participants
Twenty-four sedentary Caucasian males aged 29–74 years participated in this study. All of the participants provided informed consent for the investigation. The experimental protocol was approved by the local Ethics Committee of the Medical University of Gdansk, where all of the data collection and analysis was completed. Statistical analysis, interpretation, and manuscript preparation were completed at McMaster University. Samples of the obliquus internus abdominis muscle were collected from patients undergoing hernia surgery at the Medical University Clinic in Gdansk. This muscle was chosen for it is nonweight-bearing, and a hernia surgery has no effect on enzyme activity in the adjacent skeletal muscle (40). Furthermore, any potential effects of a hernia on enzyme activity should be similar between investigated patients of different ages. Participants with neuromuscular or other chronic diseases known to lead to changes in muscle structure and function were excluded. All of the patients were relatively sedentary nonathletes and were not performing exercise aside from occasional activities. The physiological characteristics are shown in Table 1. The participants were divided into two groups: middle-aged (29–54 years) (N = 13) or older (61–74 years old) (N = 11) adults. Age-associated changes in human skeletal muscle are relatively small until the person reaches age 60–70 years and accelerate after that age. We could not recruit patients older than 74 years, and it was difficult to find older adults older than 70 years who were relatively free from comorbidities. In addition, official data of the Polish Health Ministry indicate that the average life span is about 70 years, which is approximately 6–7 years shorter than that in Western European countries. Consequently, age-related changes occur faster in Polish men, and the 61- to 74-year age range may in fact be 6–7 biological years greater when compared to participants’ age ranges in studies conducted in North America or Western Europe.

Muscle Samples and Maximal Enzyme Activities
Muscle samples (30–50 mg) were dissected free of visible fat and connective tissue, weighed, and immediately frozen in liquid nitrogen and stored at −80°C until analysis. Muscle specimens were then minced and homogenized in a glass-Teflon Potter-Elvehejm homogenizer in a 1:25 (wt/vol) dilution of buffer containing 50 mM potassium phosphate, 1 mM EDTA, 1 mM dithiothreitol, and 0.05% Triton X-100 (pH 7.4). The homogenates were then centrifuged at 4°C for 10 minutes at 600 g. The resulting supernatant was divided into serial 200-μl aliquots, frozen in liquid nitrogen, and than stored at −80°C until assayed. To characterize the anaerobic and aerobic capacity, several pathways were examined. Specifically, enzyme activities were evaluated for: (i) anaerobic pathway: CK, AK and LDH and (ii) aerobic pathway: OGDH and CPT. For each of the subsequent assays, all of the samples were measured in duplicate and the average activity over the linear portion of the absorbance-versus-time relationship was used to represent the enzyme activity. The maximum rates (Vmax) of the following enzyme activities were measured spectrophotometrically using a spectrophotometer (Cecil 9200 Super Aquarius; Cambridge, U.K.) with a thermostatic holder at 30°C as previously described (41).

An anaerobic enzyme activity.—CK (EC 2.7.3.2) was assessed using Test Kit 45 (Sigma, St. Louis, MO) (CK, AK, glucose-6-phosphate dehydrogenase [G-6-PDH] assay). Briefly, the reaction was started with 10 μl of diluted supernatant in potassium phosphate buffer (1:5) at pH 7.4. AK (EC 2.7.4.3) was measured according to Russell and colleagues (42). Briefly, the medium contained 50 mM Tris–HCl at pH 7.6, in the presence of 0.27 μM pyruvate kinase, 1.5 U LDH, 230 μM NADH, 1.3 mM ATP, 1.3 mM adenosine monophosphate (AMP), and 10 μl of supernatant. The substrates, NADH, ATP, and AMP were added.
immediately before measurement of enzyme activities, and the reaction was started. LDH (EC 1.1.1.27) was measured according to Leger and Taylor (43) in 50 mM potassium phosphate at pH 7.4, 250 mM mannitol, 10 mM KCl, 5 mM MgCl$_2$, 1 mM dithiothreitol, 10 mM potassium phosphate with 0.05% Triton X-100, 2 mM NAD$^+$, 0.63 mM coenzyme A (CoASH), and 10 mM 2-oxoglutarate. Eighty microliters of supernatant and the substrates NAD$^+$ and coenzyme A were added immediately before measurement of enzyme activities, and the reaction was started.

**Aerobic enzyme activity.**—OGDH (EC 1.2.4.2) activity was determined according to Cooney and colleagues (44) by measuring the production of NADH when 2-oxoglutarate is converted to succinyl-CoA. Briefly, the reaction mixture was composed of 100 mM Tris–HCl at pH 8.0, 1.5 mM EDTA, with 0.05% Triton X-100 and 0.25 mM DTNB, and 1.67 mM carnitine. One hundred microliters of supernatant and the substrates DTNB and carnitine were added immediately before measurement of enzyme activities. The reaction was started with the addition of 0.025 mM palmitoylCoA. The only descriptive variable that was different between the groups was height, which was significantly less in the older group than in the middle-aged adults (Figure 1A). AK activity was 26% lower in older adults than in middle-aged adults (Figure 1A, Figure 1B). LDH activity was 43% lower in older than in middle-aged adults (Figure 1C, Table 1).

The intra-assay coefficients of variation (CV) for all of the enzyme activities were less than 10%. The analysis of protein content was performed in the supernatant according to Lowry and colleagues (47).

**Statistical Analysis**

Statistical analysis was performed using a software package (Statistica, V. 5.0; Tulsa, OK). Results are expressed as mean ± standard deviation (SD). Differences between means were tested using an unpaired *t* test. The statistical significance was established at *p* < .05.

**RESULTS**

**Participant Data**

The only descriptive variable that was different between the groups was height, which was significantly less in the older than in the middle-aged adults (*p* < .01). Importantly, no statistically differences in weight and body mass index were observed between the groups (Table 1).

**Enzyme Activities Expressed as Wet Weight**

**Anaerobic enzymes.—**CK activity in the obliquus internus abdominis muscle was 616.6 ± 56.7 and 484.9 ± 116.3 μmol · min$^{-1}$ · g$^{-1}$ w.w. in middle-aged and older adults, respectively. The CK activity was 21% lower in older adults than in middle-aged adults (*p* < .002; Figure 1A). AK activity was 93.4 ± 20.0 and 68.9 ± 16.9 μmol · min$^{-1}$ · g$^{-1}$ in middle-aged and older adults, respectively. The AK activity was 26% lower in older adults than in middle-aged adults (Figure 1B). In older adults, LDH activity was lower (67.7 ± 14.1 μmol · min$^{-1}$ · g$^{-1}$ w.w.) than that in middle-aged adults (117.8 ± 19.3), respectively (Figure 1B). The LDH activity was 43% lower in older than in middle-aged adults (*p* < .0001; Figure 1B).

**Aerobic enzymes.—**The activity of OGDH in middle-aged and older adults was 1.0 ± 0.3 and 0.7 ± 0.2 μmol · min$^{-1}$ · g$^{-1}$ w.w, respectively (Figure 1C). OGDH activity was 93.4 ± 20.0 and 68.9 ± 16.9 μmol · min$^{-1}$ · g$^{-1}$ in middle-aged and older adults, respectively.
activity was 31% lower in older adults than in middle-aged adults (p < .005). Total CPT activity was 0.4 ± 0.1 and 0.3 ± 0.1 μmol · min⁻¹ · g⁻¹ w.w for middle-aged and older adults, respectively. CPT activity was 25% lower in older than in middle-aged adults (p < .05; Figure 1C).

**Protein content.**—Total protein content was 102.3 ± 16.2 (n = 11) and 130.3 ± 19.5 (n = 12) mg/g w.w in older and middle-aged adults, respectively. In older adults, mean protein content was 21% lower than that in middle-aged adults (p < .001).

**Enzyme Activities Expressed as Milligrams of Protein**

The results of anaerobic CK, AK, LDH, and aerobic OGDH and CPT enzyme activities measured in the obliquus internus abdominis muscle in middle-aged and older adults are summarized in Table 2. Due to protein differences between groups, only LDH activity remained significantly lower between the two groups (p < .0003; Table 2). The negative correlation (γ = 1212.9 – 7.770; n = 23) between LDH activity and age was significant (p < .05; Figure 2). OGDH activity had a tendency to be lower (15%) in older participants than in middle-aged participants, but the difference did not reach statistical significance (p = .12; Table 2).

**Discussion**

The current study represents the first data concerning the activities of enzyme involved in high-energy phosphate, anaerobic and aerobic pathways in a nonlocomotor skeletal muscle from humans (29–74 years). The three main findings of the current study are that: (i) high-energy phosphate (CK and AK), anaerobic (LDH), and aerobic (OGDH, CPT) enzyme activities are lower in older adults; (ii) total protein content in the muscle is lower in older adults; and (iii) only LDH activity remained lower in older versus middle-aged adults when data were expressed per total protein content in the muscle. Together, these data suggest that expression of enzyme activity as wet weight or per net protein can influence the interpretation of data regarding the effect of age on enzyme activity. Furthermore, lower LDH activity in older adults is a very robust finding still present after accounting for the lower total protein content associated with aging.

In this study we showed that maximal activity of OGDH was 31% lower in skeletal muscle of older as compared to middle-aged adults. Moreover, when enzyme data were expressed relative to total protein content in skeletal muscle, OGDH activity still tended to be lower (15%) in older versus middle-aged adults. Recently, it has been reported that OGDH activity in isolated muscle (soleus) was decreased by exogenously produced superoxide anions (O₂⁻), whereas the activity of citrate synthase was unaffected (48). It has also been shown that OGDH activity is fully activated during exercise in skeletal muscle (49). Consequently, the production of superoxide anions would prevent maximal activity of the TCA cycle and therefore decrease the maximal oxygen consumption by the tissue. The inhibition of OGDH activity by O₂⁻ and peroxinitrite (ONOO⁻) or 4-hydroxy-2-nonenal may have an important physiological role in regulating the rate of the TCA cycle, and can be one factor involved in aerobic capacity. Given the changes in oxidative stress with aging (11), OGDH is likely to be particularly sensitive to the effects of aging. We did not evaluate the level of hydrogen peroxide (H₂O₂) and antioxidant enzyme activities in the current study, but it has been reported that both H₂O₂ production and antioxidant enzyme activities (compensatory) are higher in skeletal muscle with aging (22,50).

The present study found that CPT activity was lower in older than in middle-aged adults, and this finding is in agreement with results from other groups (30,31). The oxidation of long-chain fatty acids in mitochondria plays an important role in energy production, especially in skeletal muscle. Long-chain fatty acids are shuttled across the mitochondrial membrane by two CPTs (CPT I and CPT II). Therefore, a lower oxidation of fatty acids in the muscle of older versus middle-aged adults may be responsible for the higher intramyocellular lipid content observed in muscle from older adults (12,13). However, when lower protein content in skeletal muscle from our participants was taken into account, the activity of CPT was not longer different.

The age-related changes were seen in LDH activity, with older adults demonstrating an almost 2-fold decrease as compared to middle-aged adults. Even, when the results
were expressed relative to total protein content, LDH activity was still significantly lower in older than in middle-aged adults. This relationship was also established by the finding of a negative correlation between LDH activities in skeletal muscle from the relatively sedentary patients across the age range. These findings are in agreement with the data of Pastoris and colleagues (33), who reported significantly lower LDH activity in m. rectus abdominis with age. However, they did not observe any changes in the glycolytic enzyme activities with aging in m. vastus lateralis (33). Consequently, a lower LDH activity may partially contribute to the lower anaerobic capacity observed in older adults (51,52); however, a loss of total protein may also be a factor in the decreased performance of older adults.

The age-associated reduction in muscle CK and AK activity in older adults further supports the hypothesis that older adults have lower anaerobic capacity than do middle-aged adults. Our results are in accordance with data from other investigators who have demonstrated significant age-associated decreases in CK and AK activities in human skeletal muscle (19,20). Lower AK activity would also result in decreased production of AMP and lead to inhibition of glycolysis in older adults. However, when the CK and AK data were expressed per milligram of total protein, there was no change in the enzyme activities between groups. Together, these findings are consistent with the decreases of anaerobic power demonstrated in older adults (51).

It is well known that protein metabolism is altered during aging, with a number of studies in humans showing that muscle protein synthesis decreases with age (17,18). The current study found 21% lower total protein in older adults. This finding may reflect an imbalance between protein breakdown and synthesis in older muscle, and is very similar to the 20% reduction in protein content reported in muscle from senescent rats (53). From a mechanistic standpoint, a lower protein content in muscle would also suggest an imbalance between reactive oxygen and nitrogen species (RONS) generation and antioxidant capacity in older muscle. It is well known that RONS cause protein oxidation and elevated levels of oxidatively modified proteins. These modified proteins are targeted for degradation via the ubiquitin proteosome pathway (54,55). Although we did not measure markers of oxidative stress in the current study, we have recently reported increased protein carbonyls in skeletal muscle from older adults (11). From a mechanistic standpoint, lower protein content may explain some of the discrepancies in the existing literature looking at age and enzyme activity. However, the overall “performance” of the muscle is likely a function of the enzyme capacity expressed relative to the total net mass of muscle and not to the protein.

Although there are many reports considering age-related changes, controversies remain regarding the effect of aging on biochemical, histochemical, and molecular indices in human skeletal muscle. These discrepancies in age-related changes in anaerobic and aerobic capacities perhaps are not altered by age per se but may be due to a decline in protein synthesis, impaired protein turnover, reduction of physical activity, lifestyle, fiber composition, and/or muscle function. Recently, it has been shown that a higher level of physical activity in elderly people could delay changes in mitochondrial enzyme activity, protein degradation, and mtDNA mutation and/or deletion (56–58). Thus, protein metabolism, enzyme activities, and mtDNA mutations in the muscle may be strongly affected by physical activity.

Conclusion

Creatine kinase, AK, and LDH activities involved in anaerobic energy production were significantly lower in older adults than in middle-aged adults. In addition, the activity of mitochondrial enzymes OGDH and CPT as well as total protein in skeletal muscle in older adults was also lower in middle-aged adults. However, when data were expressed per milligram of total protein, differences remained only in LDH activity. The lower anaerobic and aerobic capacities in older versus middle-aged adults may be due to their smaller muscle mass (decreases in number and size of muscle fibers), lower enzyme activity, higher RONS generation (6), or a combination of all factors. However, little change in enzyme activity occurs when normalized to the total protein. Thus, the loss in performance in older adults may be partially due to loss of muscle protein with age, among other factors.

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Address correspondence to Jan J. Kaczor, PhD, Department of Pediatrics, Room 4U4, McMaster University Medical Center, 1200 Main St. W., Hamilton, Ontario, Canada L8N 3Z5. E-mail: kaczorj@mcmaster.ca

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