Anaerobic and Aerobic Enzyme Activities in Human Skeletal Muscle from Children and Adults

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ABSTRACT

Literature has shown that children have lower anaerobic capacity and oxidize more lipids during aerobic activity compared with adults. The purpose of the present study was to examine the effects of age on the activity of marker enzymes for anaerobic and aerobic metabolism in human skeletal muscle from relatively sedentary children and adults. The *m. obliquus internus abdominis* was analyzed for anaerobic [creatine kinase, adenylate kinase, and lactate dehydrogenase (LDH)] and aerobic (carnitine palmitoyltransferase and 2-oxoglutarate dehydrogenase) enzyme activities in 32 male individuals. The subjects were divided into two groups: children (3–11 y; $n = 20$) and adults (29–54 y; $n = 12$). LDH activity was higher in adults (118.2 ± 20.1) compared with children (27.8 ± 10.1) $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight ($p < 0.0002$). Creatine kinase activity was 28% ($p < 0.0003$) lower in children than in adults, and adenylate kinase activity was 20% ($p < 0.006$) lower in children than in adults. In addition, we found higher 2-oxoglutarate dehydrogenase activity in adults compared with children ($p < 0.04$), with no effect of age

on carnitine palmitoyltransferase activity (NS). When samples were expressed relative to protein content, only LDH activity remained significantly lower in children compared with adults ($p < 0.0001$). In conclusion, the lower LDH activity observed in children compared with adults may partially explain decreased anaerobic and lactate generation capacity of the children studied. However, the mechanisms for the relatively deficient anaerobic enzyme activities of children are not clear. (*Pediatr Res* 57: 331–335, 2005)

Abbreviations

AK, adenylate kinase
CK, creatine kinase
CPT, carnitine palmitoyltransferase
CV, coefficient of variation
LDH, lactate dehydrogenase
OGDH, 2-oxoglutarate dehydrogenase
PFK, phosphofructokinase

The functional characteristics of human skeletal muscle are partially dependent on fiber type; however, modifications to the nature and the capacity of the energy-delivering metabolic pathways can occur independent of fiber type. It is widely known that metabolic enzymes in skeletal muscle have the ability to respond to physiologic stimuli such as exercise (1–4), as well as pathologic processes such as muscular dystrophy, acute respiratory failure, mitochondrial myopathy, denervation, and inactivity (5–9). Although there is some controversy regarding fiber type differences between children and adults (10–13), there are only minor increases in the proportion of type II fibers with age (10–12), with no difference in ultrastructure (13). Moreover, there is still disagreement over the

effect of age on the enzymatic capacity of skeletal muscle, particularly during the maturation from the pediatric to the adult age group.

It has been reported that phosphofructokinase (PFK) was 3-fold lower in the skeletal muscle of children (11–13 y) compared with adults (24–52 y); however, succinate dehydrogenase activity was not significantly altered by age (11,14). Other studies that have examined anaerobic enzyme activity in children have reported lower glycolytic [PFK, lactate dehydrogenase (LDH)] and higher aerobic (succinate dehydrogenase, fumarase) enzyme activities in children compared with younger adults (14,15). In contrast, one report demonstrated similar activity of PFK, LDH, and citrate synthase in children (13–15 y) compared with adults (22–42 y) (16).

Children's lower glycolytic enzyme capacity may also lead to decreased anaerobic power. Furthermore, a 2- to 3-fold lower blood lactate concentration after both submaximal and maximal exercise in children compared with untrained and trained adults (10,17–19) is consistent with lower anaerobic

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enzyme activity in children. These data suggest that age-related differences in anaerobic performance, from childhood to adulthood, may be attributed to the enzyme activities involved in anaerobic pathways in skeletal muscle. In addition to the glycolytic pathway, the creatine kinase (CK) and adenylate kinase (AK) pathways contribute to anaerobic energy transduction, but to our knowledge, AK enzyme activity has not been reported in children compared with adults.

The effect of pediatric to adult maturation on the maximal activity of aerobic enzymes is unclear (16,20), and no studies have measured 2-oxoglutarate dehydrogenase (OGDH), which is the rate-limiting enzyme of the tricarboxylic acid cycle. Similarly, there have been no reports of carnitine palmitoyl-transferase (CPT) activity in children compared with adults, although children are known to oxidize proportionately more total fat than adults during submaximal endurance exercise (21). CPT isoenzymes are involved in the transfer of long-chain acyl groups from the cytosol into the mitochondrial matrix. In addition, because CPT deficiency is the most common cause of exercise-induced myoglobinuria (22–24), it is important to determine whether there are age-related changes in skeletal muscle CPT activity.

The purpose of the present study was to examine the effects of age on nonglycolytic anaerobic enzyme capacity (CK and AK), an enzymatic marker enzyme for lactate generation (LDH), mitochondrial lipid transport (CPT), and the rate-limiting enzyme of the tricarboxylic acid cycle (OGDH) from human skeletal muscle obtained from healthy but relatively sedentary children and adults. Our hypotheses were that the activity of CK, AK, and LDH enzymes would be lower in children *versus* adults. Furthermore, we hypothesized that the activity of CPT would be higher and OGDH would be similar in children compared with adults.

METHODS

Thirty-two sedentary white male individuals aged 3–54 y participated in this study. Adult participants and the parents of child participants provided informed written voluntary consent for the investigation. The experimental protocol was approved by the Local Ethics Committee of the Medical University of Gdansk, and all data collection and analysis were completed there. Samples of the *obliquus internus abdominis* muscle were collected from patients who underwent hernia surgery at the Medical University Clinic in Gdansk. The current literature reports that a hernia has no effect on enzyme activity (25) in the adjacent skeletal muscle. Furthermore, any potential effects of a hernia on enzyme activity should be similar between children and adults. Individuals with neuromuscular or other chronic diseases that are known to lead to changes in muscle structure and function were excluded. The individuals were divided into two groups: children (3–11 y; $n = 20$) and adults (29–54 y; $n = 12$).

Muscle samples and maximal enzyme activities. The muscle samples (30–50 mg) were dissected free of visible fat and connective tissue, weighed, immediately frozen in liquid nitrogen, and then stored for 6 mo at -80°C until analysis. It has been reported that the range of fiber distribution in the *obliquus internus abdominis* muscle is 55–58% type I, 15–23% type IIA, and 21–28% type IIB in humans (aged 24–54 y) (26). Muscle specimens then were minced and homogenized in a glass-Teflon Porter-Elvehjem homogenizer in 1:25 (wt/vol) dilution of buffer that contained 50 mM of potassium phosphate, 1 mM of EDTA, 1 mM of DTT, and 0.05% Triton X-100 at pH 7.4. The homogenates then were centrifuged at $600 \times g$ at 4°C for 10 min. The resulting supernatant produced an enzyme sample that was decanted and assayed for CK, AK, LDH, OGDH, and CPT activity. The maximum rates of the enzyme activities were measured spectrophotometrically using the Cecil 9200 Super Aquarius spectrophotometer (Cambridge, England) with thermostatic holder at 30°C . All of the assays were performed in duplicate.

The activity of CK (EC 2.7.3.2) was assessed using Test Kit 45 (Sigma Chemical Co., St. Louis, MO; CK, AK, glucose-6 phosphate dehydrogenase coupled assay). The reaction was started with 10 μL of diluted supernatant in potassium phosphate buffer 1:5 at pH 7.4. The increase in absorbance at 340 nm was followed for 3 min. The intra-assay coefficient of variation (CV) was 6.6%.

The maximal activity of AK (EC 2.7.4.3) was measured according to Russell *et al.* (27). The assay medium contained 50 mM of Tris-HCl at pH 7.6, 13 mM of MgSO_4 , 43 mM of KCl, 1 mM of DTT, 0.3 mM of Phospho (enol) pyruvic acid (PEP) in the presence of 0.27 U of pyruvate kinase, 1.5 U of LDH, 230 μM of NADH, 1.3 mM of ATP, 1.3 mM of AMP, and 10 μL of supernatant. The substrates NADH, ATP, and AMP were added immediately before the measurement of the enzyme activity, and the reaction was started. The final volume in the cuvette was 1000 μL . The decrease in absorbance at 340 nm was followed for 15 min. The intra-assay CV was 4.5%.

The activity of LDH (EC 1.1.1.27) was measured according to Leger and Taylor (28). The assay medium contained 50 mM of potassium phosphate at pH 7.2, 1 mM of EDTA, 100 μM of NADH, 2.1 mM of pyruvate, and 10 μL of supernatant. The substrates NADH and pyruvate were added immediately before the measurement of the enzyme activity, and the reaction was started. The final volume in the cuvette was 1000 μL . The decrease in absorbance at 340 nm was followed for 3 min. The intra-assay CV was 5.2%.

OGDH (EC 1.2.4.2) activity was determined according to Cooney *et al.* (29) by measuring the production of NADH when 2-oxoglutarate is converted to succinyl-CoA. The reaction mixture was composed of 100 mM of Tris-HCl at pH 7.4, 250 mM of mannitol, 10 mM of KCl, 5 mM of MgCl_2 , 1 mM of DTT, 10 mM of potassium phosphate with 0.05% Triton X-100, 2 mM of NAD^+ , 0.63 mM of CoASH, and 10 mM of 2-oxoglutarate. A total of 80 μL of supernatant and the substrates NAD^+ and CoASH were added immediately before the measurement of the enzyme activity, and the reaction was started with 2-oxoglutarate. The final volume in the cuvette was 1000 μL . The increase in absorbance at 340 nm was followed for 5 min. The intra-assay CV was 3.1%.

The CPT (total CPT I and CPT II; EC 2.3.1.21.) activity was measured in the supernatant, using methods described by Biber *et al.* (30) and Zammit and Newsholm (31). The reaction mixture was composed of 60 mM of Tris HCl at pH 8.0, 1.5 mM of EDTA with 0.05% Triton X-100 and 0.25 mM 5,5'-Dithiobis (2-nitrobenzoic acid), and 1.67 mM of carnitine. A total of 100 μL of supernatant and the substrates 5,5'-Dithiobis (2-nitrobenzoic acid) and carnitine were added immediately before the measurement of the enzyme activity. The reaction was started by the addition of 0.025 mM of palmitoyl-CoA. The final volume in the cuvette was 1000 μL . The increase in absorbance at 412 nm was followed for 5 min. The intra-assay CV was 3.9%. The analysis of protein content was performed in the supernatant according to Lowry *et al.* (32).

Statistical analysis. Statistical analysis was performed using a software package (Statistica, V. 5.0; Tulsa, OK) in which differences between the means were tested using an unpaired *t* test. The results are expressed as mean $\pm$ SD, and the statistical significance was established at $p < 0.05$.

RESULTS

Enzyme Activities Expressed as $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight

Anaerobic enzymes. The range of maximal LDH activity in children was 14.0–51.3 and in adults was 77.4–146.7 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight (Fig. 1A). The LDH activity was 4-fold higher in adults compared with children ($p < 0.0002$).

The range of maximal activity of AK in children was 59.3–98.1 and in adults was 69.0–117.4 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight. The AK activity was 20% lower in children compared with adults ($p < 0.006$; Fig. 1A).

The range of maximal CK activity in the skeletal muscle of children was 346.7–592.4 and of adults was 496.0–715.6 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight. The CK activity was 28% lower in children compared with adults ($p < 0.0005$; Fig. 1B).

Aerobic enzymes. The range of maximal activity of OGDH in children was 0.48–1.50 and in adults was 0.66–1.60 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight (Fig. 2). OGDH activity was lower in children compared with adults ($p < 0.04$; Fig. 2). The range of

Figure 1. (A) The effect of age on muscle LDH and AK activity. The results are statistically different (* $p < 0.0002$, ** $p < 0.006$) in children ($n = 16$) vs adults ($n = 12$), respectively. (B) The effect of age on the CK activity in both tested groups. *Significantly different ($p < 0.0005$) from children ($n = 20$) to adult ($n = 12$) groups. The LDH, AK, and CK activities are expressed as mean $\pm$ SD $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight.

Figure 2. The effect of age on muscle OGDH and CPT activity. *Significantly different ($p < 0.04$) OGDH activity between children ($n = 19$) and adults ($n = 12$). There was no difference in CPT activity between children ($n = 17$) and adults ($n = 12$). The results are expressed as mean $\pm$ SD $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight.

maximal activity of total CPT in children was 0.15–0.51 and in adults was 0.23–0.61 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet weight (NS; Fig. 2).

The CPT/LDH ratio of enzyme activities in skeletal muscle was significantly higher in children *versus* adults (3-fold; $p < 0.0002$). The ratio of CPT/OGDH had a tendency to be higher (16%) in children compared with adults, but the difference did not reach the level of significance ($p = 0.16$; Table 1).

Table 1. CPT/LDH and CPT/OGDH ratio of enzyme activities in skeletal muscle

Ratio	Children (<i>n</i> = 16)	Adults (<i>n</i> = 12)
CPT/LDH	0.0132 $\pm$ 0.0048	0.0034 $\pm$ 0.0012*
CPT/OGDH	0.46 $\pm$ 0.13	0.39 $\pm$ 0.09

The age-dependent changes on CPT/LDH and CPT/OGDH ratios measured in skeletal muscle of children and adults.

* Children vs adults $p < 0.0002$.

Protein content. Mean total protein content was 21% lower in children (110.6 ± 17.4 mg/g wet weight; $n = 16$) than in adults (133.4 ± 17.0 mg/g wet weight; $n = 11$; $p < 0.005$).

Enzyme Activities Expressed as $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein

When the activities of skeletal muscle CK, AK, OGDH, and CPT were expressed relative to total protein content, there were no longer significant differences between children and adults (Table 2). Only LDH activity remained significantly different between the two groups when expressed relative to protein content, with the children 3.5-fold lower than the adults ($p < 0.0001$; Table 2).

DISCUSSION

To date, there has been a paucity of information concerning the potential differences in human skeletal muscle metabolic enzyme capacity between children and adults. To our knowledge, the current data represent the first report of AK, CPT, and OGDH enzyme activity in children compared with adults.

The most striking age-related changes were seen with LDH activity. Children were shown to have 3.5-fold lower LDH activity compared with adults, which is consistent with the findings of other groups (10,15). However, it should be noted that one study found no differences in LDH activity (16), but the children were 13–15 y and had most likely begun or had finished sexual maturation. A 3-fold higher PFK activity (the rate-limiting enzyme of glycolysis) (33) has been previously reported in adults compared with children (11,14). Together, these findings are consistent with studies showing lower blood (17–19,34,35) and muscle (18) lactate concentration in children after exercise, compared with adults. In addition, it was found that boys (9–12 y) recover faster than men (19–23 y) from high-intensity short-term exercise, and it was suggested that a lower reliance on glycolysis during the Wingate anaerobic test led to decreased acidosis in boys (36). Moreover, it has been reported that venous blood pH was less acidic (7.32) in 10-y-old children compared with 25-y-old adults (7.18) (37). Finally, blood lactate and base excess were significantly lower in boys *versus* young adults and after repeated supramaximal exercise (38).

Our findings of lower CK and AK activity in children further support that children have lower anaerobic capacity compared with adults. However, others have found no difference in CK activity in skeletal muscle of children *versus* adults (15,16). The absolute values for AK activity of our adult subjects is consistent with other reports (39,40), and to our knowledge,

Table 2. The effect of age on CK, AK, LDH, OGDH, and CPT enzymes activity in the obliquus internus abdominis muscle measured in children and adults

Group	CK	AK	LDH	OGDH	CPT
Children (n = 16)	4404 ± 820	742 ± 167	247 ± 65	7.6 ± 2.1	3.0 ± 0.8
Adults (n = 11)	4664 ± 565	738 ± 104	886 ± 141*	7.8 ± 1.7	3.0 ± 0.9

Enzyme activities are expressed as nmol·min⁻¹·mg⁻¹ protein.

* Children vs adults $p < 0.0001$.

this is the first report of this enzyme's activity in children compared with adults. Although our data showed that CK and AK values were no longer different from adult values when expressed per milligram of total protein, we believe that power output is likely a function of total muscle mass and not protein content *per se*. Consequently, enzyme activities expressed relative to net muscle weight likely correlate better with indices of muscle function.

The activity of OGDH in skeletal muscle is rate limiting in the tricarboxylic acid cycle. In addition, it seems that the enzyme is fully activated during exercise (41–43). The absolute OGDH activity values measured in skeletal muscle of adults in the present study was similar to those previously reported (42,44). However, to our knowledge, the activity of this enzyme has not yet been reported in children. We found a marginally (25%) lower OGDH activity in children compared with adults. In contrast, studies have found significantly higher NADP-isocitrate dehydrogenase, fumarase, and malate dehydrogenase in skeletal muscle of pubescent children compared with adults, with no differences in citrate synthase activity (16). In general, little or no differences in aerobic enzyme capacity are consistent with other reports that aerobic power is similar in children and adults (10,45).

The oxidation of long-chain fatty acids in the mitochondria plays an important role in aerobic energy production, in skeletal muscle. Long-chain fatty acids are shuttled across the mitochondrial membrane by two CPTs (CPT I and CPT II). There are no published comparative studies of CPT activity in healthy children and adults. In the current study, we did not find any age-related changes in CPT activity in children compared with adults. This finding is consistent with studies showing no differences in enzyme activities of fatty acid metabolism (acetoacetyl-CoA thiolase and 3-hydroxyacyl dehydrogenase) in pubescent children compared with young adults (16). However, it has been shown that children use proportionally more fat and less carbohydrate than adults during exercise performed at the same relative intensity (21,34,46). Recently, higher rates of total fat oxidation were reported before and after exercise in children *versus* adults, even when fed carbohydrate (21). The higher fat oxidation in boys may be a “default” mechanism as a result of an underdeveloped glycogenolysis and/or glycolytic system (21). Moreover, the CPT/OGDH ratio of enzyme activities in skeletal muscle tended to be higher (16%) in children *versus* young adults, but the differences did not reach statistical significance ($p = 0.16$). This finding may suggest that there is higher oxidation of fatty acids than other substrates by mitochondria in children compared with adults. For example, the CPT/LDH ratio was 3-fold higher in children compared with adults in the current study. It is also possible that there are

age-related differences in the ratio of CPT I:CPT II enzyme activities; however, our assay did not distinguish between the two.

Some studies have found a higher proportion of type I muscle fibers in children *versus* adults (11,12), whereas one group did not show these findings (13). Even if there is a greater proportion of type I muscle fibers in children, there does not seem to be a strong correlation between fiber type composition and enzyme activity in skeletal muscle (47). Consequently, differences in fiber type cannot explain the markedly lower LDH activity found in children compared with adults.

The potential mechanism(s) for lower total protein in the skeletal muscle of children *versus* adults could relate to higher total water and fat-free body mass content in children (10,14,48). This is an important observation because it may explain some of the discrepancies in current literature with respect to the effect of age on muscle enzyme activity. After expressing enzyme data relative to total protein, only LDH activity remained different between the two groups (higher in adults *versus* children).

In conclusion, the activities of the anaerobic enzymes CK, AK, and LDH in children were lower than in adults. The lower anaerobic performance in children *versus* adults might be due to their smaller muscle mass, lower protein content, or a lower percentage of fast-twitch muscle fibers. However, children's statistically significant lower LDH activity is likely to be the major factor of their decreased anaerobic performance. The OGDH activity measured in our subjects was slightly lower, and CPT activity was similar for children *versus* adults, but when the data were expressed relative to protein content, there was no significant difference. The ratio of CPT/LDH was much greater in children and the ratio of CPT/OGDH tended to be higher. Together, these results suggest that children have a greater ability to oxidize lipids during exercise. Overall, the mechanisms behind the enzymatic differences reported here in children and adults are not clear.

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