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Skeletal muscle fiber-type specific succinate dehydrogenase activity in cerebral palsy

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Abstract

Introduction—Children with cerebral palsy (CP) exhibit increased energy expenditure during movement, but whether this is due in part to decrements in skeletal muscle mitochondrial oxidative capacity is unknown. Accordingly, we compared fiber-type specific succinate dehydrogenase (SDH) activity in children with CP to typically developing (TD) children.

Methods—SDH activity and myofiber areas of type 1 and 2A fibers were measured in semitendinosus biopsies of both groups (n=5/group)

Results—SDH activity was ~35% higher in type 1 compared to type 2A fibers, but there were no differences between groups. Average myofiber area was 45% smaller in CP vs. TD ($P<0.05$), and type 2A fibers were 32% larger than type 1 fibers ($P<0.05$) only in TD children.

Discussion—Fiber-type specific SDH activity is similar between TD children and children with CP. This suggests that increased energy expenditure in children with CP is not related to impaired mitochondrial oxidative capacity.

Keywords

cerebral palsy; oxidative capacity; skeletal muscle; fiber type; energy expenditure; succinate dehydrogenase

Introduction

Children with cerebral palsy (CP)¹ display skeletal muscle weakness, increased energy expenditure with movement^{2–5}, and decreased physical activity⁶ relative to typically

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developing (TD) children. While increased energy expenditure has traditionally been attributed to inefficient movement and cardiorespiratory factors³, it is not known whether impaired skeletal muscle oxidative metabolism may also contribute to these deficits. The activity of succinate dehydrogenase (SDH, complex II of the mitochondrial electron transport chain)⁷, is a marker of skeletal muscle oxidative capacity^{7,8}. Since skeletal muscle oxidative capacity varies by fiber-type (type 1>type 2A>type 2X), categorizing SDH activity by fiber type⁹ is important, particularly if there are differences in fiber type between groups. While expression of metabolism-related genes, including SDH, is reduced in skeletal muscle from children with CP^{10, 11}, no direct measurement of mitochondrial oxidative metabolism has been reported^{12, 10, 11}. To address this gap, we measured fiber type-specific SDH activity and fiber size in skeletal muscle from children with CP and TD children.

Materials and Methods

Participants and Setting

Ten children [5 spastic diplegic CP: 12.2 ± 5.1 years (3 boys/2 girls), 5 TD: 15 ± 0.7 years (4 boys/1girl)] participated. Age-appropriate consent/assent was obtained under a protocol approved by the Institutional Review Board of UC San Diego. Muscle biopsies were obtained from affected spastic semitendinosus of children with CP undergoing hamstring lengthening and normal semitendinosus of TD children undergoing ACL reconstruction.

Muscle tissue preparation

Biopsies were flash frozen intraoperatively in liquid nitrogen cooled isopentane and stored at -80°C. Subsequently, 10µm serial transverse sections were cut at -20°C using a cryostat (Leica CM3050S, Buffalo Grove, IL, USA), mounted on glass slides and stored at -80°C until experimental assays were performed.

SDH assay

SDH activity was measured histochemically as a marker of muscle fiber oxidative capacity, as described by others^{7, 8, 13}. Briefly, tissue sections were incubated in an SDH reaction mixture [1.5mM nitroblue tetrazolium (NBT), 5mM EDTA, 48mM succinic acid, 0.75mM sodium azide, 30mM methyl-phenylmethyl sulfate, phosphate buffered to pH 7.6^{7, 8, 13}]. During electron transfer from succinic acid to NBT by SDH a color change occurs, the rate of which represents SDH activity [change in optical density (OD)/minute]; endogenous SDH activity was determined by incubating a serial section in a medium lacking succinic acid. Images were captured on a microscope (Leica CTR 6500, Buffalo Grove, IL) using a 10X objective every 60 seconds for 10 minutes. Sensitivity and linearity of the assay were ensured by testing different substrate (succinic acid) and electron acceptor (NBT) concentrations as well as within-biopsy and between-day variation in SDH activity. For CP vs. TD comparisons, all assays were performed on the same day at 22°C (room temperature) with identical camera acquisition parameters.

A mask in Adobe Photoshop was applied to 10 randomly selected interspersed type 1 and type 2A fibers per section to determine mean pixel intensity normalized to area (pixel intensity/mm²) for 10 minutes. In total we analyzed 200 fibers (100 per group, 10 per fiber

per subject). SDH activity was expressed as the rate of change in OD/minute after subtracting background activity (Fig. 1B). Only those fibers that clearly expressed laminin and type 1 or type 2A MHCs were chosen (Fig. 1A). Fibers with a lower expression of green fluorescence and no expression of red fluorescence were assumed to be either hybrids or 2X fibers⁹ and were not selected in analysis of 2A fibers. Experimenters were blinded to experimental groups during image and data analysis.

Immunohistochemistry

MHC isoforms were labeled using primary antibodies for type 1 (BA-D5, 1:50) and type 2A (SC-71, 1:50) (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) combined with fluorescent secondary antibodies (type 1, Alexa Fluor 647 IgG_{2b}, 1:200; type 2A, 488 IgG₁, 1:200, Life Technologies, Carlsbad, CA), basal lamina using a primary antibody for laminin (Laminin 9393, 1:1000, Sigma-Aldrich, St. Louis, MO) and secondary fluorescent antibody (Alexa Fluor 594 IgG, 1:200, Life Technologies, Carlsbad, CA) after SDH staining.

Statistics

A significance level (α) of 0.05 was used. Statistical tests are detailed in Results. *A priori* power analysis indicated that, for a power of 0.80, our sampling was sufficient to demonstrate a medium effect size, i.e. 30% difference in SDH activity between groups.

Results

SDH activity

SDH activity was significantly higher (~35%) in type 1 vs. type 2A fibers in both groups ($P < 0.01$) (Fig. 1), but was not different between groups (type 1: CP 4.1 ± 0.8 vs. TD 4.5 ± 1.3 OD/min, $P > 0.20$, type 2A: CP 2.7 ± 0.4 vs. TD 3.0 ± 0.5 OD/min, $P > 0.20$) using a 2-way ANOVA.

Fiber area

Average myofiber area (type 1 and type 2A fibers combined) was 45% smaller in children with CP vs. TD (CP 2713 ± 2567 vs. TD 4975 ± 1579 μm^2 ; $P < 0.05$, Fig. 1) on a *t*-test and Wilcoxon-Mann-Whitney test. Only in the TD group, type 2A average fiber size was 32% larger than type 1 fibers (5666 ± 1626 vs. 4284 ± 1330 μm^2 ; $P < 0.05$); within the CP group, type 2A and type 1 fiber areas were not significantly different (2887 ± 3070 vs. 2540 ± 2308 μm^2 ; $P = 0.37$, Fig. 1D) using a pairwise *t*-test. Additionally, the coefficient of variation of myofiber area was significantly higher in CP (CP $25 \pm 5\%$ vs. TD $18 \pm 2\%$, $P < 0.05$); *t*-test.

Discussion

We compared fiber-type specific oxidative properties of muscle in children with CP and TD by measuring SDH activity. In both groups, SDH activity in type 1 fibers was higher than type 2A, which is consistent with other adult human studies⁹. Importantly, SDH activity per unit area was not reduced in children with CP in type 1 or type 2A fibers, suggesting that oxidative capacity, at least as represented by SDH, is preserved. Consistent with previous

reports of impaired muscle development^{12, 14, 15}, we found that children with CP had significantly reduced myofiber areas and increased coefficient of variation, and only children with TD had significantly increased myofiber area of type 2A compared to type 1 fibers. This might be due to inadequate and uneven myoblast fusion during postnatal development, related to a reduced muscle stem cell number in children with CP^{16, 17}. The reduced average fiber size in children with CP is likely associated with peripheral weakness and may be also related to increased energy expenditure during ambulation due to lower force generating capabilities of the muscle.

A limitation of this study was the small number of fibers analyzed as well as small sample sizes; however, *a priori* power analysis indicated that our sampling could detect differences in SDH activity between groups. Also, SDH activity is not informative of other complexes in, or flux through the electron transport chain. While the children with CP are slightly younger (12.5 yrs) than the children with TD (15 yrs), based on our previous meta-analysis of muscle fiber size and age¹⁶, this 2.5 year difference would only be expected to decrease fiber area by about 400 μm^2 , far less than the 2,200 μm^2 observed. Lastly, this study only involved fairly ambulatory children with CP (GMFCS I & II)¹⁸.

In conclusion, fiber-type specific SDH activity per unit area is maintained in children with CP, suggesting that increased energy expenditure during movement in these children is not accounted for by decrements in skeletal muscle oxidative metabolism.

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Abbreviations

CP	cerebral palsy
GMFCS	Gross Motor Function Classification System
MHC	myosin heavy chain
NBT	nitroblue tetrazolium
SDH	succinate dehydrogenase
TD	typically developing

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Figure 1.

A) Representative images of laminin, SDH staining, type 2A fibers (bright green), and type 1 fibers (bright red). Type 1 fibers correspond with darker SDH staining (higher SDH activity) indicated by asterisk. Scale bar = 100 μm , **B)** Optical density over time of type 1 and type 2A fibers and background, **C)** SDH activity in type 1 and type 2A fibers (optical density/min) in children with TD and CP, **D)** Pairwise change in myofiber area between type 1 and type 2A fibers and boxplots of average myofiber areas across both type 1 and type 2A fibers for the TD and CP groups. Asterisks in plots, significant differences ($P<0.05$), ns, no significant differences.