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# Higher amount of MyHC IIX in a wrist flexor in tetraplegic compared to hemiplegic cerebral palsy

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**Abstract**

Spastic cerebral palsy can be divided into diagnostic groups by the relative severity of the arm impairment. This study investigates if hemiplegic, tetraplegic or diplegic cerebral palsy (CP) results in different patterns of myosin heavy chain (MyHC) expression in the flexor carpi ulnaris muscle from 17 young patients with CP. Using enzyme-immunohistochemistry and gel electrophoresis techniques we found a higher percentage of fibers expressing fast MyHC IIx (52%) in tetraplegic CP compared to hemiplegic patients (32%), ($p<0.05$). Tetraplegic CP also resulted in a lower amount of fibers expressing slow MyHC I (18%) compared to hemiplegic CP (40%), ($p<0.005$). The proportion of muscle fibers containing fetal MyHC was higher in tetraplegic CP compared to other groups, ($p<0.005$). Taken together theses results indicate that tetraplegic CP is associated with a shift from slow to fast myosins and that regenerative events are more prominent in tetraplegic CP compared with milder brain damage.


*Keywords:* Cerebral palsy; Spasticity; Upper limb; Muscle; Fiber type

## 1. Introduction

Cerebral palsy (CP) is a motor impairment due to a non-progressive lesion in the brain before the age of two [1]. Since more than 30 years the patients with spasticity have been sub-diagnosed according to the limb impairments. Depending on the impairment of the arms relative to the legs, cerebral palsy can be categorized into hemiplegic CP with unilateral limb disorder, diplegic CP with greater impairment of the legs than of the arms, and tetraplegic CP with all four limbs equally affected. Children with tetraplegic CP also have a mental retardation, while the intellect often is spared in diplegic and hemiplegic CP.

The impairment of the hand may be the major disability for children with cerebral palsy. Spasticity is accompanied by weakness, loss of dexterity and often contractures. The decreased fine motor control and neglect of the paralyzed arm play a great role for the handicap. Musculotendinous surgery of the upper limb may improve function and hygiene, and also the mobility and reach of the arm. In planning surgery or occupational therapy, concern about the length, strength and volitional control of the muscles should be taken. The endurance is to a large extent centrally driven, but there are also inherent factors in the muscle affecting endurance. Myopathic changes might influence contractility, but the most important factor is the composition of different myosin heavy chains (MyHCs).

Traditionally muscle fibers have been divided into different types by staining for mATPase activity [2]. The

*Abbreviations:* mATPase, myofibrillar adenosine triphosphatase; CP, cerebral palsy; FCU, flexor carpi ulnaris muscle; mAb, monoclonal antibody; MyHC, myosin heavy chain; NADH-TR, nicotineamide adenine dinucleotide-tetrazolium reductase; PAP, peroxidase–antiperoxidase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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different staining patterns of the different fiber types are correlated to the content of MyHCs, which have different contractile properties. Human limb muscle fibers are thus divided into three main pure fiber types: type I fibers containing MyHC I and two subtypes of fast fibers, type IIA containing MyHC IIa and type IIX containing MyHC IIx.. Fibers containing MyHC IIx have the fastest contraction velocity (for review see [3,4]). The majority of recent studies use the term IIX and not IIB to describe the fastest myosin isoform in human limb muscles [5–7]. Human skeletal muscle also contains two types of hybrid muscle fibers: type I/IIA containing both MyHC I and MyHC IIa and type IIA/X containing both MyHC IIa and IIx [4]. Humans have to a certain extent a genetically predetermined fiber type composition [8]. Training long distance running e.g. will thus result in a higher amount of MyHC I in some individual's leg muscles compared to others [8]. Different brain damages might predispose muscles to express different MyHCs, which could influence the functional efficacy of different training regimes for patients with spasticity.

An alteration in fiber type composition has been reported in adult patients with upper motor syndromes, but the findings are not uniform. An increased proportion of type II fibers (known to contain MyHCs IIA and IIX) has been reported in one study [9], while an increase in type I fibers (containing MyHC I) were reported in two studies [10,11]. To our knowledge there are no reports on the influence of cerebral palsy severity on the contractile properties of skeletal muscle in children.

The purpose of the present study was to examine if MyHC composition of the flexor carpi ulnaris muscle differs in children with different types of CP. Occurrence of myopathic findings, fibrosis and alterations in the cytoskeleton (desmin) was also investigated. Analysis included SDS-PAGE (Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis), enzyme and immunohistochemistry.

## 2. Materials and methods

### 2.1. Muscle sampling

Muscle biopsies were taken during tendon transfer surgery from the flexor carpi ulnaris muscle (FCU) of 17 young patients with CP. Nine had hemiplegic CP (mean age 9 years, range 4–19 years), 3 had diplegic CP (mean age 9 years, range 5–14 years) and 5 had tetraplegic CP (mean age 14 years, range 3–24 years). Four of the patients with tetraplegia had no function of the hand and one used it as a passive helper hand [12]. One of the diplegic patients used the operated hand as an active helper hand, while two used it as a passive assist. In the hemiplegic patients three used the affected hand as an active assist, and 6 as a passive helper hand.

The muscle specimens (15 mm long and 3–8 mm in diameter) were mounted in embedding medium (Tissue Tek, Miles laboratories, Naperville, Illinois, USA), snap frozen in propane chilled with liquid nitrogen, and stored at −80 °C until analyzed.

### 2.2. Enzyme- and immunohistochemistry

Frozen specimens were stained for the demonstration of the mitochondrial enzyme NADH-TR and for myofibrillar adenosine triphosphatase (mATPase) after preincubation at pHs 10, 4, 4.6, and 4.3 [13]. Muscle cross-sections (5 μm) were also stained with monoclonal antibodies against specific myosin heavy chain isoforms (MyHCs). The following primary monoclonal antibodies (mAb) were used: mAb A4.840 and mAb N2.261 [14] (Developmental Studies Hybridoma Bank). Five fiber types were delineated (type I, I/IIA, IIA, IIA/X, IIX). Type I fibers were strongly stained with mAb A4.840, weakly stained with mAb N2.261 and were stable in the acid ranges but labile at pH 10.4. Type IIA fibers were strongly stained with mAb N2.261, unstained with mAb A4.840 and were labile in the acid ranges but stable at pH 10.4. Type IIX fibers were unstained with both mAb N2.261 and mAb A4.840 and were stable at pH 10.4 and 4.6 but labile at pH 4.3. Type IIA/X were weakly stained with mAb N2.261, unstained with mAb A4.840 and stained intermediately between types IIA and IIX at pH 4.6. Type I/IIA fibers were strongly stained with both mAb N2.261 and mAb A4.840 and were stable at all pHs.

Identification of fibers expressing developmental MyHC isoforms was assessed using the following antibodies: mAb F1.652 against embryonic MyHC (Developmental Studies Hybridoma Bank), and mAb NCL-MHCn against fetal MyHC (Novocastra Laboratories Ltd) [15]. Delineation of muscle fiber borders was made using a mAb against extracellular matrix laminin chain α5 [16]. The cytoskeletal protein desmin was identified using the mAb D33 (Dako Corp, Carpenteria, CA).

Four randomly selected areas of the specimens were photographed using a light microscope interfaced with a computerized image analysis system to determine fiber area and fiber type composition (IBAS, Kontron electronic GMBH, Eching, Germany). The percentage of the specimen area occupied by a given fiber type was calculated. From each specimen a mean of 429 (range 163–848) fibers were studied.

### 2.3. Biochemistry

Frozen cross-sections (10–20 μm) were extracted with Laemmli sample buffer (Bio-Rad). MyHC isoforms were separated on 8% polyacrylamide gels in the presence of sodium dodecyl sulphate [17] using the Bio-Rad mini PROTEAN 3 Cell system. The upper running buffer contained 10 mM 2-mercaptoethanol [18].

The gels were run at constant voltage (70 V) and temperature (7 °C) for 24 h and finally silver stained. Three bands were identified corresponding to MyHC I, MyHC IIa/ fetal and MyHC IIx/ embryonic. In this gel embryonic MyHC cannot be separated from MyHC IIx and fetal MyHC cannot be separated from MyHC IIa.