

Alterations of hamstring muscle properties in patients with varying severity of spastic cerebral palsy

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Background:

Cerebral palsy (CP) is a disorder in which children experience a non-progressive brain lesion that results in permanent and progressive secondary postural and movement disorders [1]. CP has an incidence of 2.0-2.5 occurrences per 1000 live births in developed nations, making it the most common cause of physical disability in children [2]. There is a spectrum of disease states in CP that affect upper and lower limbs to varying degrees. The commonly accepted standard for severity measure is the Gross Motor Classification System (GMFCS) [3].

Since the secondary effects of CP disrupt posture and movement and most surgical treatments such as muscle lengthenings, address the musculoskeletal system [4]. The most common drugs used in CP, Baclofen and Botulinum Toxin Type A, are designed to induce muscle relaxation. These therapies can address some of the muscular problems associated with CP (hypertonicity and spasticity), but often exacerbate others (weakness and fatigue) and ultimately contractures still form [5]. Although the neurological insult is primary to CP, it is also irreversible and thus the secondary effects on CP muscle represent a target for new therapeutics.

Skeletal muscle from children with CP has been characterized at a variety of levels, with most studies reporting muscle tissue and muscle fiber atrophy, decreased muscle cross-sectional area, muscle shortening, and decreased specific tension [6, 7]. Additionally, muscle fiber stiffness was significantly increased and the muscle tissue itself contained a hypertrophic extracellular matrix [7, 8]. However these experiments are limited by comparing different muscle groups and further research is required to more completely characterize spastic muscle.

Open question:

How does the altered neuronal input of muscle contraction associated with spastic cerebral palsy progress into a pathological state with weakness, fatigability, hypertonicity, and contractures? Specifically:

- How are the mechanical properties of spastic muscle altered
- How are the signaling properties of spastic muscle altered?
- How do these muscular alterations correlate with disease severity?

Hypothesis:

Spastic muscle in cerebral palsy is in a pathologic state that has fundamentally different mechanical properties induced by the pathologic neuronal stimulus creating altered muscle signaling. These muscle alterations are represent important disease parameters and are correlated with disease severity.

Experimental approaches:

One of the significant difficulties in CP research is that no commonly accepted animal model exists and collecting biopsies from children is very difficult. The previous research on mechanical properties of CP muscle compared different muscle groups between CP patients and control patients. We will take advantage of the fact that Dr. Chambers frequently performs operations on patients with CP and in sports medicine. Patients with CP often require hamstring lengthening procedures that exposes the gracilis and semitendinosus muscles. Patients undergoing an anterior cruciate ligament replacement from hamstring autograft require

the removal of muscle from gracilis and semitendinosus tendons. After informed consent, the muscle sample used for study purposes will be obtained during the surgical procedure through the procedural incision. The muscle biopsy obtained for this study will be a 60 milligram sample and will be removed from the hamstrings (gracilis and semitendinosus). The sample will only be taken from a muscle that is already exposed in the procedure. A sample size calculation done based on preliminary results suggest a sample size of 10 patients per group. This will allow us to collect muscle biopsies from children with CP and also a control group.

Upon collection of the biopsy one half of the muscle tissue will be placed in a glycerol relaxing solution in order to test the passive mechanical properties. Spastic muscle is thought to be more stiff than control muscle and this has been attributed to increases in the passive mechanics of the muscle. Since the level of this increased stiffness is unknown we will measure the mechanical properties at two levels, the single fiber and the fiber bundle. The comparison of the material properties at the fiber and bundle level will allow us to investigate the role of the extracellular matrix in the increased stiffness.

In order to make the measurements the fiber or bundle will be attached to a force transducer on one side and a motor arm on the other with suture. The force will be recorded at increasing lengths dictated by the motor arm. The basic unit of skeletal muscle is the sarcomere, which will be measured to normalize the length of the muscle using laser diffraction. The stress-relaxation will also be measured during the stretch. This provides data on the visco-elastic properties of the muscle and velocities role in muscle stiffness.

How spastic muscles become stiff, weak, and small is an important question. Our experiments will include a microarray study to investigate this question. Microarrays allow the investigator to explore the entire genome for alterations in transcription that relate to the disease. Many muscle pathways related to muscle can be explored simultaneously as well as providing the possibility of novel genes important to spastic muscle pathology.

RNA will be isolated from the muscle biopsy and run on Affymetrix HG-133 microarrays. The results will be confirmed for select genes using quantitative PCR. Microarrays are limited to looking at transcriptional activity of genes and thus protein level analysis will be conducted on genes of interest. Protein quantification methods to consider will be dependent on the protein of interest, but could include ELISA, Western blotting, or SDS-PAGE gels.

We will also investigate the effect of any significant differences on the severity of the disease. There are potentially many alterations in spastic muscle that do not have a role in the disability. To address this we will look for significant differences from above that also correlate with severity in cerebral palsy. Severity of hamstring contracture is traditionally quantified by measuring the passive range of knee extension. However, this does not always correlate with functional disabilities and we will also use the GMFCS level of the patient to correlate our data to the functional limitations of the patient.

Alternatives:

Our study may be neglecting important differences in several areas. If the passive mechanical properties are not altered active mechanical properties of spastic muscle could be more prominent. Additionally, since the microarray only has transcriptional differences, post transcriptional alterations may be playing a role and require looking at additional protein level changes. There are other severity measures we may need to look into for our analysis, such as Ashworth or House, which look at muscle tonicity or spasticity respectively.

Schematic:

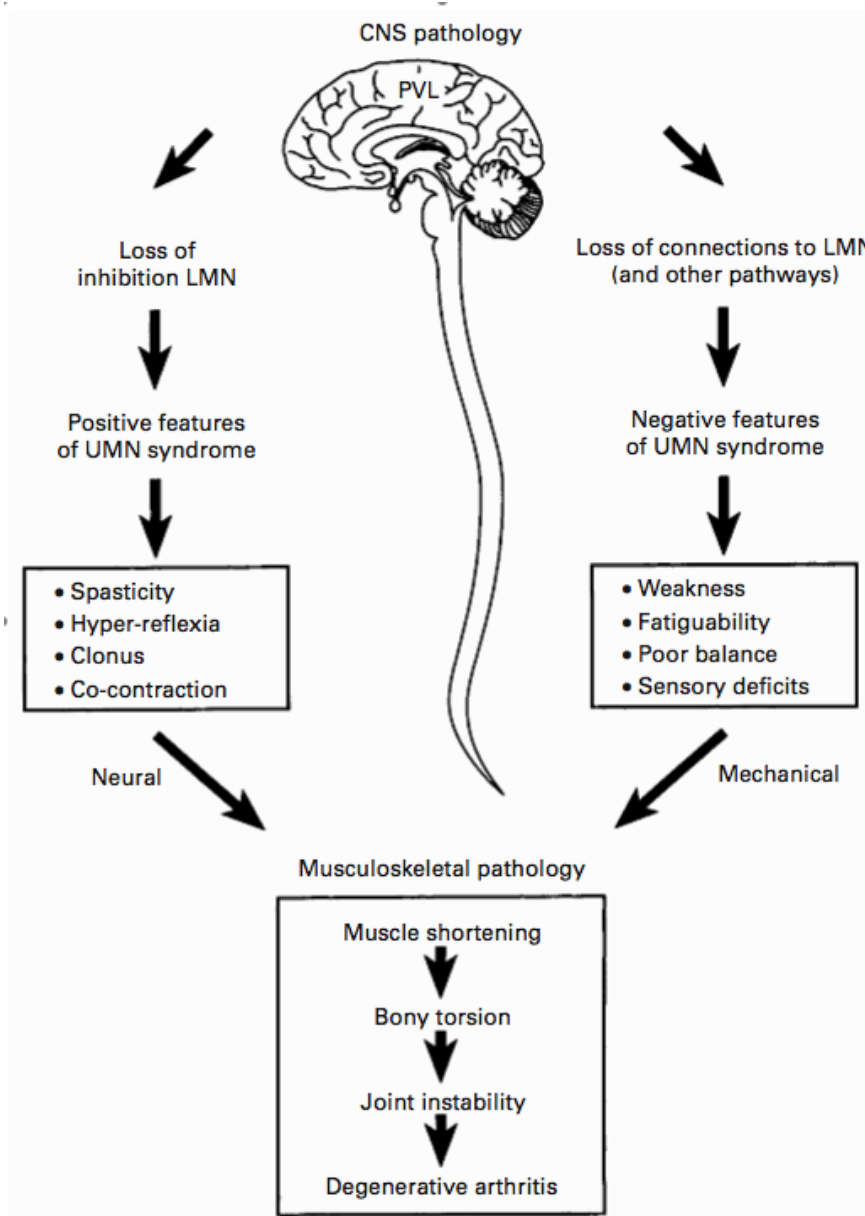


Diagram showing the neuromusculoskeletal pathology in cerebral palsy. The pathology of the central nervous system in cerebral palsy is defined as a static encephalopathy. Given the infinite variability of the location and severity of the lesions the clinical syndromes are in turn extremely variable. In motor terms, cerebral palsy results in an upper motor neurone lesion which in this diagram is considered to have a series of positive and negative features that interact to produce the familiar musculoskeletal pathology. Historically, clinicians have concentrated on the positive features and the negative features have been relatively ignored. It is probable that the optimum management of children with cerebral palsy will require integrated management of both the positive features and the negative features. From [4]

References:

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Additional Figure

