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# The role of muscle imbalance in the pathogenesis of shoulder contracture after neonatal brachial plexus palsy: a study in a rat model

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**Background:** An internal rotation contracture of the shoulder is common after neonatal brachial plexus injuries due to subscapularis shortening and atrophy. It has been explained by 2 theories: muscle denervation and muscle imbalance between the internal and external rotators of the shoulder. The goal of this study was to test the hypothesis that muscle imbalance alone could cause subscapularis changes and shoulder contracture.

**Materials and methods:** We performed selective neurectomy of the suprascapular nerve in 15 newborn rats to denervate only the supraspinatus and the infraspinatus muscles, leaving the subscapularis muscle intact. After 4 weeks, passive shoulder external rotation was measured and a 7.2-T magnetic resonance imaging scan of the shoulders was used to determine changes in the infraspinatus and subscapularis muscles. The subscapularis muscle was weighed to determine the degree of mass loss. An additional group of 10 newborn rats was evaluated to determine the sectional muscle fiber size and muscle area of fibrosis by use of images from type I collagen immunostaining.

**Results:** There was a significant decrease in passive shoulder external rotation, with a mean loss of 66°; in the thickness of the denervated infraspinatus, with a mean loss of 40%; and in the thickness and weight of the non-denervated subscapularis, with mean losses of 28% and 25%, respectively. No differences were found in subscapularis muscle fiber size and area of fibrosis between shoulders after suprascapular nerve injury.

**Conclusions:** Our study supports the theory that shoulder muscle imbalance is a cause of shoulder contracture in patients with neonatal brachial plexus palsy.

This study was approved by the Ethic Commission of Vall Hebron Research Institute (Act No. 24, Extraordinary Session, November 20, 2008).

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**Level of evidence:** Basic Science Study, Animal Model.

**Keywords:** Neonatal brachial plexus palsy; glenohumeral dysplasia; shoulder contracture; subscapularis muscle; muscle imbalance; muscle growth

Shoulder impairment is the most common long-term complication and the major cause of morbidity after upper-trunk neonatal brachial plexus palsy (NBPP).[7] A shoulder internal rotation contracture can develop in patients with incomplete recovery of the upper trunk.[1,2,10] The constant position of internal rotation leads to early glenohumeral joint deformity, which is characterized by increasing glenoid retroversion and posterior humeral head subluxation (also known as glenohumeral dysplasia).[4,9,12] The changes in bone and articular alignment have been extensively studied, whereas the pathogenesis of muscular changes has received little attention.[1,2,8] The mechanism leading to subscapularis contracture has been postulated by 2 differing hypotheses: (1) denervation and (2) muscle imbalance. The denervation hypothesis states that denervation of the subscapularis muscle causes progressive muscle fibrosis, shortening, and contracture.[8] This hypothesis has recently been supported by an experimental study in a mouse model.[6]

Biopsy specimens from the subscapularis from children with NBPP do not show muscle fiber atrophy or fibrosis as predicted by the denervation hypothesis,[1,3] despite loss of thickness in the muscle.[8-10] Furthermore, several clinical and radiographic studies support the muscle imbalance hypothesis over the denervation hypothesis.[3,5] The imbalance theory suggests that the subscapularis changes are due to incomplete or delayed external rotator muscle reinnervation.[12] The weakened external rotators become overpowered by the internal rotators, leading to a persistent internal rotation posture. The lack of a passive stretch of the subscapularis leads to progressive shortening and muscle growth impairment.[1,3,10]

The purpose of this study was to develop an animal model of muscle imbalance to test the hypothesis that muscle imbalance could lead to the internal rotation contracture seen in NBPP.

## Materials and methods

This study was carried out following the National Institutes of Health guidelines for the use of laboratory animals and with the approval of the local ethics committee for experimental animal use. We used 30 newborns rats from 2 pregnant Sprague-Dawley Oncins France Strain A rats, 15 rats from each, in this study. The rat pups were randomly divided into 2 groups of 15: one group underwent an isolated neurectomy of the suprascapular nerve (suprascapular injury group), whereas the other group underwent an identical approach to the nerve without neurectomy (sham group). Only 1 upper limb from each pup was used, leaving the uninjured contralateral limbs as the native control group. Functional evaluation, magnetic resonance imaging (MRI), and subscapularis muscle weighing were performed 4 weeks after surgery, as described later.

An additional group of 10 newborn rats undergoing a neurectomy of the suprascapular nerve was evaluated at weekly intervals to measure passive shoulder external rotation and to determine the sectional muscle fiber size and muscle area of fibrosis by use of images from type I collagen immunostaining (histology group).

### Newborn rat surgery

Five-day-old rat pups from the suprascapular injury group and the histology group underwent right brachial plexus surgery under general anesthesia with isoflurane. A surgical microscope was used for dissection. A transverse incision inferior to the clavicle was made with splitting of the pectoralis major and minor muscles to expose the brachial plexus. The suprascapular nerve was identified, and a neurectomy was performed with micro-scissors just distal to the branch point of the superior subscapular nerve. The skin incision was closed with running No. 7-0 polypropylene suture. Rat pups from the sham group underwent the same incision, exposure, and closure, but no neurectomy was performed.

### Functional evaluation

Any gross gait abnormality was recorded just before the rats were killed. The animals were then killed with pentobarbital sodium injected intraperitoneally after sedation. Passive glenohumeral joint external rotation was measured immediately after the animals were killed. We used a modification of the method described by Nikolaou et al.[6] Unlike the previously described method that measured external rotation in 90° of forward elevation of the shoulder, we performed our measurements with the rats positioned in full adduction with neutral flexion and extension of the shoulder. Our method replicates the way in which we measure external rotation clinically in children. The neutral position of the shoulder was defined as the shoulder in 0° of abduction and the elbow in 90° of flexion with the front limbs up ventrally perpendicular to the examination table. After scapular stabilization with the thumb, each shoulder was placed in maximal external glenohumeral joint rotation and photographed with a 12-megapixel digital reflex camera (Canon EOS 1100D; Canon, Tokyo, Japan) stabilized on a tripod perpendicular to the rat (Fig. 1).

The angle formed by the forearm and the animal's midline was defined as glenohumeral external rotation. Measurements were performed digitally with Osirix software (Apple, Cupertino, CA, USA). The nonoperative side was measured and used as a control. A pilot study of 10 rats tested the reliability of this measurement method. Both limbs were positioned and photographed 3 times each, and each photograph was measured twice. The intraclass