1. INTRODUCTION, AIMS

Introduction
Cerebral palsy is a movement disorder most commonly seen in association with perinatal brain hypoxia or damage. Due to developing intensive care unit possibilities the number of individuals who present spasticity is increasing. A complete neurological evaluation is necessary, including evaluation of the lower extremities, before a diagnosis of cerebral palsy can be made. The complexity that the central nervous system injury occurs, and the effect of spasticity on the immature skeleton and muscles needs further investigations.

1.1. Spastic upper limb
The upper extremity surgery has become firmly established as an aid in the management of patients with cerebral palsy. Although surgery cannot be expected to make a functionally poor limb perfect, a marked and measurable improvement in comparison with the preoperative condition can usually be demonstrated. Upper extremity function in cerebral palsy is most commonly classified by an international grading system, moreover some modifications can provide more detailed assessment.

1.2. Iliopsoas muscle
Symptomatic hip flexion deformity secondary to iliopsoas spasticity may interfere with gait, impair sitting balance, or contribute to hip subluxation or dislocation. A noninvasive, functional technique to ameliorate iliopsoas spasticity is presented. This protocol describes an approach to the ultrasound examination of patients with normal or spastic iliopsoas. Location, structure of the muscle and complications after distal tenotomy can be imaged.

1.3. DSC analysis of the skeletal muscles
The standard calorimetric properties of the healthy human skeletal muscle are presented and compared to the same of human skeletal muscles in primary peripheral leg deformities (congenital clubfoot) and secondary deformities caused by the malfunction of the central nervous system (cerebral palsy). To our best knowledge no calorimetric analysis of either the healthy or pathologic human skeletal muscles has been reported previously.
II. RESULTS OF SPASTIC UPPER LIMB OPERATIONS

II.1. Evaluation form in our department
An evaluation form for spastic upper limb has been set up. Outcomes described included rate of success; functional improvement; active and passive range of motion, grasp and release; pinch grip; complications and side effects; and quality of life. Scoring range was 0-5(function absent - near normal function) (Chart 1).

<table>
<thead>
<tr>
<th>Subjective evaluation</th>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drawing figures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turning over pages of book</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turning off door-handle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picking up small objects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picking up big objects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opening up bottles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combing, tooth-brushing, shaving</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buttoning, knitting shoelaces, unzipping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chart 1

II.2. Children's Hospital Boston form
This is the most widely used functional evaluation form (Chart 2).

<table>
<thead>
<tr>
<th>Level</th>
<th>Use</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>No effective use</td>
</tr>
<tr>
<td>1</td>
<td>Poor, passive assist</td>
<td>Paperweight, absent grasp and release</td>
</tr>
<tr>
<td>2</td>
<td>Fair, passive assist</td>
<td>Keep placed object</td>
</tr>
<tr>
<td>3</td>
<td>Good, passive assist</td>
<td>Stabilize other hand usage</td>
</tr>
<tr>
<td>4</td>
<td>Poor, active assist</td>
<td>Active grasp, poor keep</td>
</tr>
<tr>
<td>5</td>
<td>Fair, active assist</td>
<td>Active grasp, good keep</td>
</tr>
<tr>
<td>6</td>
<td>Good, active assist</td>
<td>Active grasp, help other hand manipulations</td>
</tr>
<tr>
<td>7</td>
<td>Partial spontaneous</td>
<td>Poor bimanual activity</td>
</tr>
<tr>
<td>8</td>
<td>Complete spontaneous</td>
<td>Excellent use of hand, voluntary control</td>
</tr>
</tbody>
</table>

Chart 2
II.3. Material and methods

Objective hand functions were scored preoperatively and post rehabilitation according to Children’s Hospital Boston form. Subjective scoring was used according to evaluation form set up by our department. The patients and their parents were asked about their expectations before surgeries and results after the operations. The Children’s Hospital Boston form is the most widely used objective functional evaluation form. We used our own subjective evaluation form because there is no widespread subjective form available for spastic upper limb.

II.3.1. Participants

Thirty-four children with mild or moderate cerebral palsy participated in this study. 39 surgeries were performed on patients aged from 2-18 years (median approximately 6,7 years). 5 participants had bilateral deformity. The pattern of musculoskeletal spasticity were quadriplegia (2 patients), hemiplegia (32 patients, 18 right sided, 14 left sided). Male-female ratio was 19-15. Informed consent was obtained from all children and their parents. Post surgical program was followed for two weeks to three months depending upon type of surgery. Hand function assessment was carried out after 3,7 years post-surgically using the same scoring systems.

II.3.2. Surgical procedures

Surgical options include myotomy, tenotomy, tendon transfers, tenodesis, capsulotomy (Chart 3):

<table>
<thead>
<tr>
<th>Type of surgeries</th>
<th>Number of surgeries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biceps tendon lengthening</td>
<td>11</td>
</tr>
<tr>
<td>Brachioradialis release</td>
<td>2</td>
</tr>
<tr>
<td>Flexor carpi ulnaris tenotomy, Green transfer</td>
<td>13</td>
</tr>
<tr>
<td>Flexor pollicis longus lengthening</td>
<td>7</td>
</tr>
<tr>
<td>Pronator teres slide</td>
<td>6</td>
</tr>
</tbody>
</table>

Chart 3
II.4. Results

II.4.1. Subjective assessment
Overall subjective upper extremity function in cerebral palsy was classified by a five-level grading system. Patients experienced 3,2 level improved hand functions subjectively.

The scale used in this study showed increments according to zero as start level (Chart 1). The better was the function, the higher was the score.

II.4.2. Objective assessment
According to Children’s Hospital Boston objective scale (Chart 2) the average functional score was 3,4 before interventions. The spasticity score for the operated arm could attain an average of 6,1 so the average improvement was 2,7.

One of the functionally improved wrists is presented in the picture below. Pre-surgical score 3 increased to score 6 (Figure 1).

![Figure 1: Active wrist extension before and after surgery](image)

Average stereognosis level was 1,7 before surgery which improved to 3,3 postoperatively.
II.5. Discussion

II.5.1. Improvement in stereognosis and hand function after surgery

Stereognosis is the tactile modality most often evaluated and reported to be impaired in children with CP. Adding the pick-up test, performed with and without looking, the influence on hand function from motor and sensory components respectively is clearly demonstrated. The information regarding whether the child has sufficient sensibility to perform manual activities without visual information is most valuable for treatment planning. Consensus among therapists concerning the choice of tests is very much needed, as is a highly standardized methodology; this would enable therapists to generate important knowledge about somatosensory influences on performance and the outcome of treatment.

II.5.2. Subjective assessment of the results

Although surgery cannot be expected to make a functionally poor limb perfect, a marked and measurable improvement in comparison with the preoperative condition can usually be demonstrated. The major reasons for selecting surgical treatment are to effect improvement in the performance of daily living activities, to increase the speed of hand and forearm movement, and to produce cosmetic improvement. The goals are limited, but a reasonable degree of success can be guaranteed, provided that patient selection is made in a precise way. Surgery of the upper extremity will be helpful to certain patients with neuromuscular dysfunction due to cerebral pathology provided that the preoperative assessment is thorough and accurate, operative procedure are carried out with care and caution and operative care is well planned and executed. However, it needs to mention here that such surgically corrected spastic hand can never be a dominant hand.

II.5.3. Outcomes of successful treatment

The goals of operative treatment in the child with cerebral palsy should be very specific and should be aimed at providing useful grasp and release and acceptable hygiene. The ideal candidate for surgery of a spastic hand is the one who is cooperative, intelligent and well motivated with pattern of grasp and release, with gross function so that the hand is already useful to some extent. The hand that has serve contractures, surgery is indicated only for the purpose of appearance. Surgical options include myotomy, tenotomy, tendon transfers, tenodesis, capsulotomy,
excision arthroplasty and arthrodesis. Surgical interventions followed by proper post surgical therapeutic regime in the form of hand splints and activity training improve functions in cerebral palsy spastic hands, but the goals need to be decided properly. It is difficult to assess outcome as a measure of surgical results by use of functional measures such as range of motion, grip strength, or standardized testing because patients with spastic hand deformities have such varying levels of functional use, varying degrees of central nervous system involvement, and varying pictures of spasticity. By the Children’s Hospital Boston upper extremity functional use classification we reported at least one functional grade of improvement with surgical treatment of upper limb deformities. In another view of all upper extremity surgical procedures we reported an average improvement of 2.7 functional levels for individuals with an average preoperative use of fair passive assist (level 3,4). Patients with fair to good voluntary control had the greatest functional improvement.
III. ULTRASOUND STUDY OF THE ILIOPSOAS MUSCLE

III.1. Material and methods

III.1.1. Participants
Between 2002-2005 forty-one children aged from 1.7-12.5 years (median approximately 6.7 years) participated in this study. 16 normal and 25 mild or moderate iliopsoas spasticity have been found on patients with 8 unilateral and 17 bilateral deformity. All individuals who presented spasticity in the iliopsas were seen in association with cerebral palsy. Informed consent was obtained from all children and their parents. Post surgical program was followed for six weeks, three and six months.

III.1.2. High frequency ultrasound transducers and techniques
A high frequency linear array transducer is preferred even in large patients. The patient lies in the supine position with the hips adducted. Standard disinfectant fluid is used as contact medium. The iliopsoas muscle is examined in the groin by a 5 or 7.5MHz linear transducer in the longitudinal plane. Sonographically, the femoral head serves as a bony landmark and is identified in the groin in the standard longitudinal sectional plane. The relation between the iliopsoas muscle and the femoral vessels is demonstrated in the transverse sectional plane. The iliopsoas muscle lies in direct contact to the capsule over the femoral head. For optimum scanning the transducer is moved cranially, following the iliopsoas muscle. The size, mobility, echogenity, vascularity and surroundings can be easily estimated. Intramuscular botulinum toxin A injections are beneficial for the treatment of functional shortening of the iliopsoas muscle, but it is difficult to achieve precise needle positioning and injection. A possible solution can be an ultrasound guided technique for the iliopsoas muscle using an anterior approach from the groin. Postoperative adverse events or complications can be observed as well.
III.2. Results

III.2.1. Healthy iliopsoas muscle

Transverse and sagittal view allowed clear identification of the iliopsoas muscles because of their known fixed position relative to the vertebrae, bony pelvis, and hip joints. On ultrasound imaging the muscles demonstrated low echogenity. Sharply marginated, irregular zones of high echogenity could be seen within the muscles because of the presence of fascia and tendon. In most people the muscles are bilaterally symmetric in size, contour, and echogenity. However, asymmetry of the psoas muscles can be simulated in healthy subjects by malpositioning tilting the pelvis at the time of scanning (figure 2-3: A-acetabulum, C-cartilage, F-femoral head).

![Figure 2: Iliopsoas (arrow = tendon) cross section](image)

![Figure 3: Iliopsoas (arrows = fascia and tendon) longitudinal section](image)
III.2.2. Spastic iliopsoas muscle

On ultrasound imaging the spastic muscles demonstrated same size but the structure of connective tissue demonstrated higher echogenicity. The ratio of low echogenic areas decreased according to connective tissues. In the opposite, sharply margined widened irregular zones of highly echogenic tendons could be seen within the muscles. Connective tissue accumulated and bundled. In most people synovitis, and poor vascularity can be observed. In standard transverse sectional plane femoral neck and partially head are identified as semi-curved echogenic structures. Joint capsule follows contour of bony structures. Iliopsoas muscle lies directly above joint capsule. Femoral artery can be identified in real-time mode anteromedially of iliopsoas muscle as a pulsating low-echogenic structure. Vein lies medially, femoral nerve laterally, sartorius and rectus muscle can be seen as well (figure 4-5).

Figure 4: Spastic iliopsoas (3) cross section
(1-femoral head, 2-capsule, 4-sartorius and rectus muscle, 5-femoral artery)

Figure 5: Spastic iliopsoas (4) longitudinal section
(1-femoral head, 2-acetabulum, 3-joint capsule, 5-subcutaneous fat)
III.2.3. Position of the iliopsoas muscle after surgery

Distal tendon is released and allowed to move 2-5 (average 3,4) cm proximally from its insertion on the trochanter minor. End of the tendon is surrounded by proliferated synovial tissue with low echogenity. Vascularity of the tendon is decreased, scared tendon demonstrates high echogenity (Figure 6).

Figure 6: Iliopsoas muscle (2) after surgery, longitudinal section

(1-acetabulum, arrows-scared tendon)

Occasionally the tendon inserted to the medial wall of the pelvic bone (Figure 7).

Figure 7: Iliopsoas (thin arrow) inserted to the pelvic bone (thick arrows)
III.2.4. Complications

Cause of the complications was mostly the connection between hip joint and retroperitoneal space maintained by iliopsoas muscle. Haematomas could be seen in 3 cases, which divided soft tissues (Figure 8).

![Figure 8: Postoperative haematoma](image)

Once a distended iliopsoas bursa, as a fluid collection with internal echoes was seen anterior to the contiguous iliofemoral ligament (Figure 9).

![Figure 9: Postoperative cyst](image)
III.3. Discussion
The ultrasound examination of the iliopsoas muscle from the groin provides real-time visual guidance into the muscle and confirms correct placement of the interventions. Intraoperative or postoperative adverse events or complications can be observed. It is a safe, cost effective, reliable, and time-saving method. It reduces the risk of complications, especially accidental injuries of vessels or intestines. The ability to locate the exact position of the muscle in real-time guarantees accurate placement of therapeutic interventions. The presented technique allows for a reliable and safe diagnostic procedure, but it should only be used by experienced practitioners with appropriate training. As for alternative techniques, some kind of sedation (e.g. midazolam) is necessary, especially for children as the patient needs to lie still. Another possibility for identifying the psoas muscle is MR or computed tomography assistance. Beside the logistical and economic arguments against this methods, this technique has the additional side effect of narcotics or exposure to radiation. Ultrasound, on the other hand, is a readily available real-time imaging modality without any risk for the patient.
IV. DSC ANALYSIS OF SKELETAL MUSCLES

IV.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a well-established method for the demonstration of thermal consequences of local and global conformational changes in biological systems. The device is a SETARAM Micro DSC-II calorimeter is a special block which can be heated and cooled. Two cells are placed inside, one with the investigated material, the other with buffer fluid as a reference. The calorimeter continuously measures the temperature of each cell and keeps the difference zero. If we slowly start heating up the cells, at a certain point some changes happen in the structure of the measured biological system, leading to an exogenous or endogenous reaction. To keep the temperature difference zero, some extra energy has to be given to or taken away from the reference cell. This can be detected and analyzed with computerized systems.

IV.2. Material and methods

IV.2.1. Samples

Altogether 26 tissue samples were removed from 18 patients. In the cerebral palsy group the muscle samples (n=11) were removed from the gastrocnemius muscle during surgeries performed for the correction of spastic equinus deformities. In the cases of congenital clubfeet the samples (n=9) were taken from the abductor of the hallux. Six of the healthy samples were also removed from the m. abductor hallucis during surgical correction of the hallux valgus deformities.

IV.2.2. Method

The thermal unfolding of muscle proteins in different samples after fiber preparation was monitored by a SETARAM Micro DSC-II calorimeter. All experiments were conducted between 5 and 80 °C and finally the samples were irreversibly denatured during each cycle. Physiological buffer was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction between the sample and reference vessels. The repeated scan of denatured sample was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration.
IV.3. Results

IV.3.1. Control group

6 measurements showed similar pattern (Figure 10). It is obvious that these DSC scans behave as a very characteristic fingerprint of the physiological state of muscles. Age of the donors was 1.5-8 (average 4.2) years, 2 males and 4 females.

Figure 10: Denaturation curve of a healthy human muscle as a control
IV.3.2. Muscle in clubfoot

9 measurements showed similar pattern (Figure 11). Age of the donors was 1-9 (average 2.9) years, 6 males and 3 females.

Figure 11: DSC curve of a clubfoot
IV.3.3. Muscle in cerebral palsy

11 measurements showed similar pattern (Figure 12). Age of the donors was 0,7-12 (average 3,6) years, 6 males and 5 females.

Figure 12: DSC curve of a spastic muscle
IV.3.4. Comparison

Comparison of DSC curves of healthy control (human standard), clubfoot and spastic muscles (Figure 13).

Figure 13: Comparison of DSC curves of healthy control (human standard), clubfoot and spastic muscles.
IV.4. Discussion

It is obvious at a first glance that these DSC scans behave as a very characteristic fingerprint of the physiological state of muscles. The smallest transition temperature range can be observed in cerebral palsy (it seems to be a more cooperative transition) while the clubfoot is the less cooperative one. If we try to decompose the thermograms into subsets which could be probably assigned to different structural subdomains of the muscle the cerebral palsy shows the most uniform picture that is the less capability for the different kind of interaction between the distinguishable subunits.

In our former structural (light and electronmicroscopic) study we have found the next results. Generally the clubfoot cases are plastered prior to operation. Because of the longer immobilization marked fatty degeneration occur. The most serious changes were observed concerning the contractile elements: discontinuous, ruptured myofibrillar structures, decreased numbers of myofibrillar elements with highly granulated sarcoplasm as a sign of pathologic change. The slightest change was the decreased number of myofibrillar structures by itself, combined with maintained continuity and transverse linearity of them. Other observed characteristic changes were the decreased number and enlargement of the mitochondria together with atrophic cristae, and the number of sarcoplasmic reticular system decreased while they were enlarged with increased glycogen content. These are also typical changes in muscular atrophy caused by inactivity. The follow-up (after surgical treatment) examinations of our earlier cases showed normal-like appearances of the feet, with good or fair functioning and stable statuses. They supported the fact that the analyzed muscles were contractile despite their ultrastructural changes, so normal walking could be achieved by our treatment.

Our data prove that differential scanning calorimetry could be a useful tool in the investigation of muscle dysfunction in the orthopedic surgery, too. We have demonstrated that abnormalities in the muscle function evoked either by the passive structural constrain (clubfoot) or by the functional disorder of central nervous system (cerebral palsy) resulted in the change of global conformation of muscle proteins which appear in different DSC thermograms.
V. NEW RESULTS

1. RESULTS OF SPASTIC UPPER LIMB OPERATIONS
   - Creating an exact and detailed functional evaluation form
   - Creating a subjective evaluation form
   - Establishing new aspects of measured results after surgery

2. ULTRASOUND STUDY OF THE ILIOPSOAS MUSCLE
   - Describing ultrasound characteristics of normal muscle and tendon
   - Describing ultrasound characteristics of spastic muscle and tendon
   - Discovering situations after distal tenotomy
   - Judging possible complications
   - Establishing noninvasive functional examination

3. DSC ANALYSIS OF SKELETAL MUSCLES
   - Defining DSC curve of healthy human muscle
   - Defining DSC curve of spastic human muscle
   - Defining DSC curve of clubfoot human muscle
   - Establishing further molecular biologic researches