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DSC analysis of the abnormalities of human leg skeletal muscles A preliminary study

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Abstract

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 clubfeet) 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 have been reported previously. Eleven muscle samples from the three groups of patients were analyzed and compared. We hope to add to the understanding of the primary and consecutive functional behavioral changes of the human skeletal muscle due to different etiologic reasons. It has been found that the human muscles have different DSC scans than the rabbit skeletal ones and they are very characteristic for the actual functional and structural state, they behave as a fingerprint. To find the precise biochemical and structural explanation of these alterations there is need for further detailed examinations.

Keywords: Congenital clubfeet; Cerebral palsy; DSC

1. Introduction

Different pathologic conditions of the human locomotion system often go along with consecutive changes of the corresponding skeletal muscles. The reasons and pathomechanical pathways, which lead to the secondary muscular changes have been analyzed by numerous means, however, the exact nature of functional and behavioral characteristics of the muscle itself remain obscured in many aspects [1–3].

It is well known that the human skeletal muscle consists of red and white muscle fibers, however, based on genetic studies of the heavy and light myosin chains it is supposed that there are more types. The main muscle fibers are not congenitally present. Their properties are created and determined by their innervation. Muscle contraction is the classic functional consequence of the nerve supply, however, another significant result of it is the effect on the growth potential of the muscle. This latter is performed through the innervation-controlled enzyme concentration, which leads to the differentiation of more types of muscle fibers [4–7].

In the cases of congenital structural clubfeet the deformity results from peripheral neuromuscular reasons causing deformity and malfunction of the affected leg and foot [8]. Others believe that the deformity itself acts as primary reason, which later leads to secondary muscular changes [9–15].

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In the cases of infantile cerebral palsy the origin of the functional impairment of the leg is caused by the malfunction of the central nervous system. The etiology and the pathomechanism of the condition are well known, and no primary muscle changes play a role in the course of the disease.

Children suffering from both types of problems often need surgical correction. In many cases tendon transfers, tendon elongation are indicated, which allow access to the muscle bodies of the affected leg. We have used this opportunity to remove tissue samples of different muscles from patients suffering from both types of diseases. The healthy muscle samples were removed from cases undergoing surgeries for reasons with no effect on the condition of the skeletal muscles. All these procedures were done according to the prescription of the local ethics committee and to the general rules of any surgical intervention. The samples were analyzed by calorimetric means and compared to each other and to healthy controls, too.

The standard of calorimetric behavior of the human skeletal muscle is also determined by direct analysis and comparing to the results of previous similar studies in different animal models. We know about no reports regarding the calorimetric characteristics of the human skeletal muscle tissue. The normal human standard is used as a reference study. The properties of the normal standard are compared to those of the human skeletal muscles in the two different types of locomotion system diseases. We hope to add to the understanding of the properties of both the healthy and the pathologic human skeletal muscle.

2. Materials and methods

2.1. Sample preparation

Altogether 20 tissue samples were removed from 11 patients. The distribution of the patients and muscles is shown in Table 1. In the cerebral palsy group the

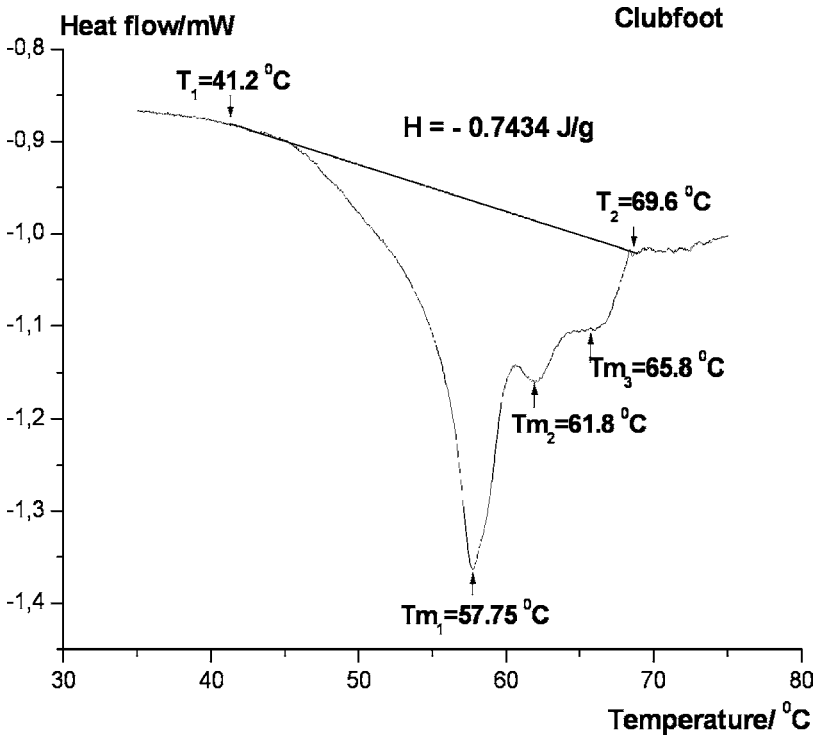

Fig. 1. DSC curve of a clubfoot (the parameters are the transition temperature range [T_1 and T_2], the melting points of the possible structural subdomains [T_{mi}] and the calorimetric enthalpy).

Table 1
The distribution and age of the monitored patients (n_s = number of muscle samples)

Groups	No. of cases
Healthy controls	3 (10 ± 2 years, $n_s = 5$)
Cerebral palsy group	4 (1.4 ± 0.6 years, $n_s = 7$)
Clubfoot group	4 (1.5 ± 0.5 years, $n_s = 8$)

muscle samples ($n = 7$) were removed from the gastrocnemius muscle during surgeries performed for the correction of spastic equinus deformities. In the cases of congenital clubfeet the samples ($n = 8$) were taken from the abductor of the hallux. Five of the healthy samples were also removed from the m. abductor hallucis during surgical correction of the hallux valgus deformities.

2.2. DSC measurements

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. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μl sample volume in average. 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.

3. Results and discussion

The thermal denaturation of the investigated human muscles can be seen in Figs. 1–3. It is obvious at a

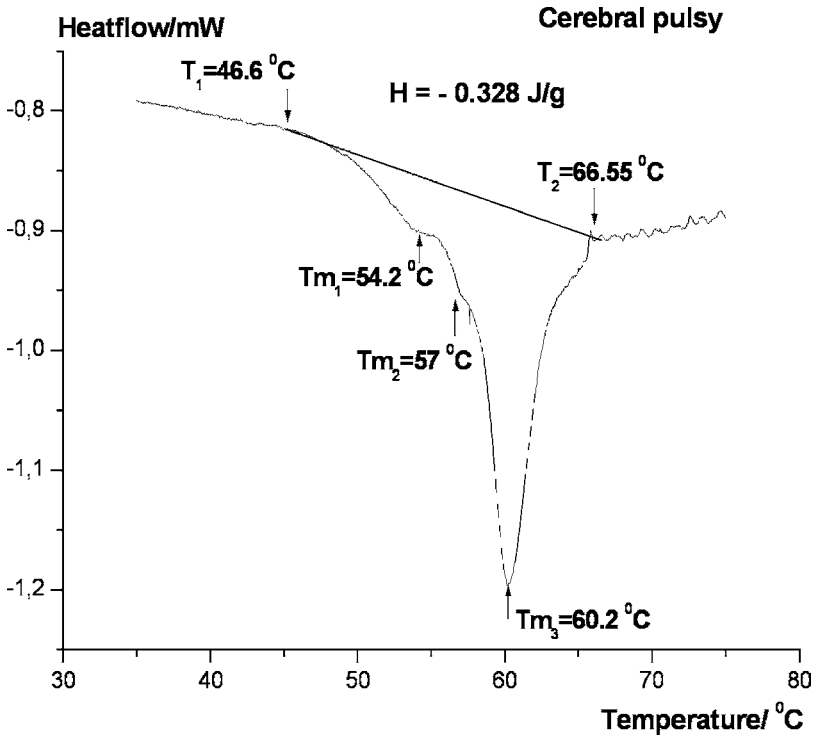

Fig. 2. DSC curve of a cerebral pulsy muscle (the parameters are the same as in Fig. 1).

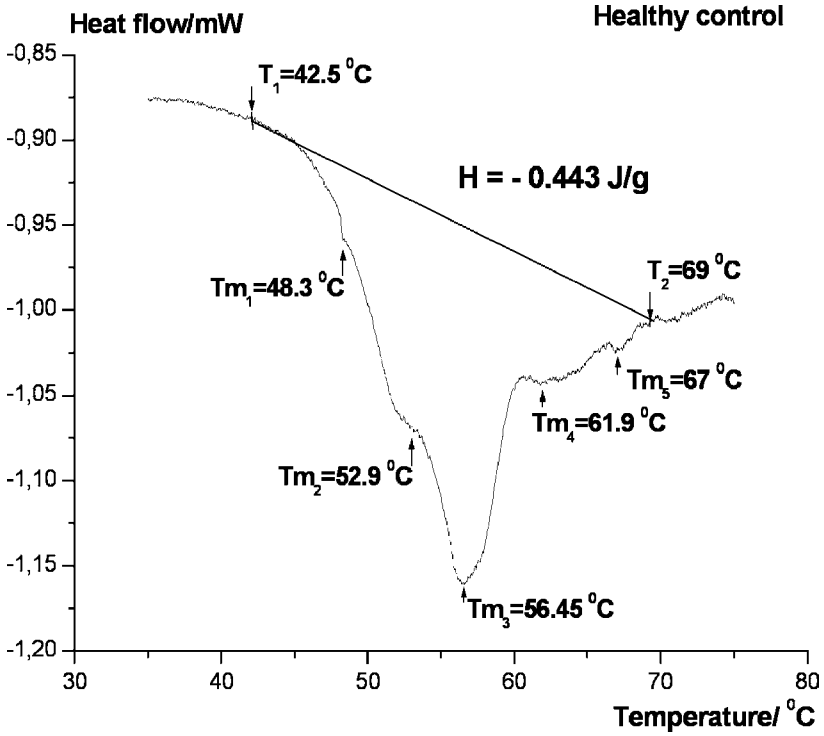

Fig. 3. Denaturation curve of a healthy human muscle as a control (see Fig. 1 for symbols).

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 pulsy (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 pulsy 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 sarcoplasma as a sign of pathologic change [8]. 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 [8].

DSC scans for m. psoas of rabbit in different intermediate states of ATP hydrolysis cycle can be seen in Fig. 4 [16–19] for the sake of comparison as an animal skeletal muscle control (the structural and functional characteristics of this muscle is the same as of the human one). It can be seen that the middle part of DSC scans was not affected by the presence or absence of nucleotides (that is the protein conformation of this

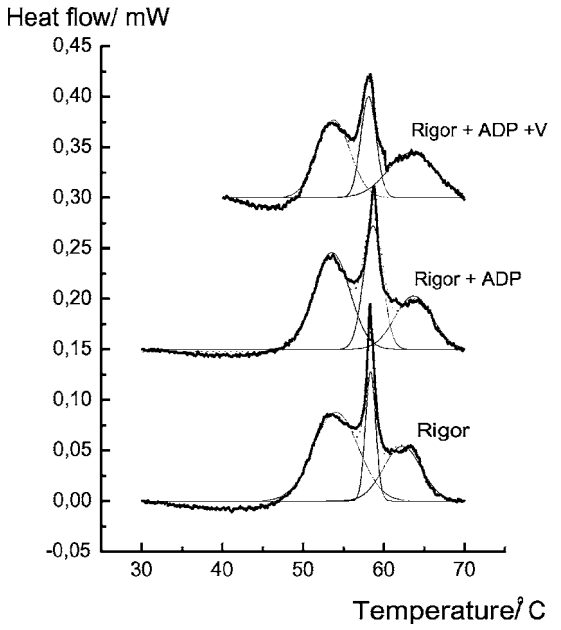

Fig. 4. Thermal denaturation of rabbit psoas muscle fibers. Symbols: RIGOR + ADP + V_i stands for weak binding state and RIGOR+ADP refers to the strong binding state of ATP hydrolysis cycle.

subunit during the different stages of ATP hydrolysis cycle remains practically uneffected), therefore it was used as a reference point in the comparison with the investigated samples. This central part of thermograms in our human muscles varies from 56.45 to 60.2 °C that is it is affected by the different muscle diseases.

On the basis of the shape and melting temperature range of the clubfoot muscle denaturation curve it is similar to the higher temperature part of weak-binding state of rabbit psoas mimicked by ADP + V_i (V_i is orthovanadate, an inorganic phosphate analogue, Fig. 4 upper panel) [17], or AMP PNP (a non-hydrolizable ATP analogue) [20,21]. The transition at around 63 °C could be the sign of the F-actin melting [22], because the weaker interaction between actin and myosin in the simulated states of rabbit muscles separated the strong overlapping melting curve of myosin from actin [16], and a lot of facts support that the structure of a clubfoot muscle differ from the normal healthy one evoked by the abnormal anatomic position [8], which could be manifested as a weaker actin–myosin interaction.

The most surprising finding of our measurements is that both the malfunction of central nervous system

Table 2
The thermal parameters (means ± S.E.M.) of the denaturation of human leg muscles (T_m = melting temperature, ΔH = calorimetric enthalpy change)

Sort of muscle	T_m (°C)	ΔH (J/kg)
Healthy controls	56.5 ± 0.3	−540 ± 60
Cerebral palsy	60.5 ± 0.5	−640 ± 85
Clubfoot	57.7 ± 0.4	−850 ± 110

(cerebral palsy) and the abnormal structural change in the muscle caused by the clubfoot appear as an increased global thermal stability compared to the healthy control (Table 2 and Figs. 1–3). We could observe such a deviation from the normal thermal parameters (but in opposite sign) only under the effect of oxygen free radicals [23], which was the consequence of the damage of SH sidechains in the myosin head caused by the free radical attacks [24]. This may refer to the worse interdomain communication of the different subunits of muscle proteins which can appear as a malfunction of the whole muscle and/or the different "packaging" of the affected parts.

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.

Acknowledgements

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