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Differential scanning calorimetric examination of the human skeletal muscle in a compartment syndrome of the lower extremities

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Abstract The compartment syndrome—conditions of elevated intramuscular pressure—is one of the most serious complications of the injuries of the lower extremities. Early diagnosis is important, as delayed treatment leads to significant complications. The diagnosis of compartment syndrome is most commonly made by clinical examination and direct measurement of the intra-compartmental pressure. Our hypothesis was that in different stages of compartment syndrome there is a clear pathological abnormality in the tissue elements of the affected muscles, which is responsible for seriousness of the disease, and could be monitored besides the classical histological methods by differential scanning calorimetry. The thermal denaturation of different parts of human samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 K/min. DSC scans clearly demonstrated significant differences between the different types and conditions of samples (control: $T_m = 55.5; 59.9$ °C and $\Delta H_{cal} = 0.52$ J/g, Gr. I.: $T_m = 58.1$; 62.2 °C and $\Delta H_{cal} = 0.28$ J/g, Gr. II.: $T_m = 57.45$; 61.5 °C and $\Delta H_{cal} = 0.24$ J/g, Volkmann's ischemic contracture $T_m = 57.75$; 61.8; 65.8 °C and $\Delta H_{cal} = 0.74$ J/g). These observations could be explained with the structural alterations caused by the biochemical processes. The heat capacity change between native and denatured states of muscle samples was significant, indicating significant water loosing during denaturation, but independent from the structural alterations.

Keywords Compartment syndrome · Ischemic contractures · DSC

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Introduction

Definition

Compartment syndrome is an elevation of interstitial pressure in closed fascial compartment that results in micro-vascular compromise. Compartment syndrome occurs when pressure in a muscle compartment is higher than the pressure in the capillaries, which leads to progressive muscle ischemia and oedema and left untreated can result in infarction of the compartment contents (Figs. 1, 2). As duration and magnitude of interstitial pressure increase, monaural function is impaired and necrosis of soft tissues eventually develops [1]. Whiteside suggests that the perfusion of the compartment depends not only on intra-compartment pressure but also depends on the difference between the diastolic blood pressure and the intra-compartmental pressure [2]. Necrosis of tissue may begin at interstitial pressure as low as 30 mmHg. Ischemia and necrosis of the muscles occur even though the arterial pressure is still high enough to produce pulses. Muscle and nerves can survive for up to 4 h of ischemia, whereas after 4 h, they will show irreversible damage [3].

Fibrotic contracture of skeletal muscle can follow weeks or months after the severe ischemic insult of compartment

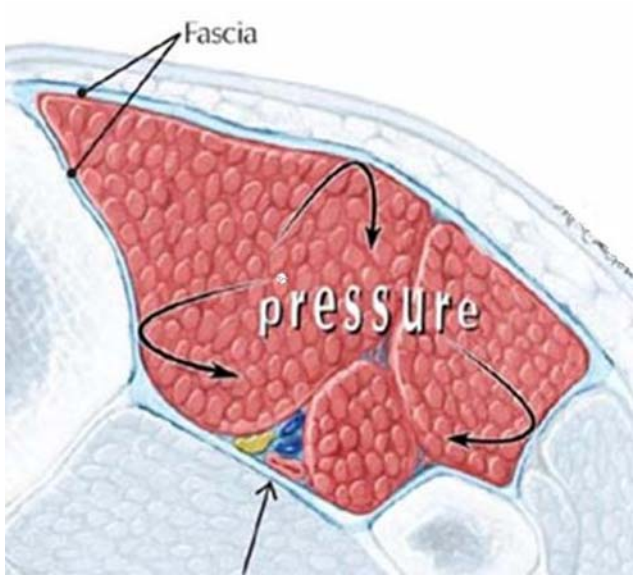

Fig. 1 Compartment syndrome: muscles and blood-vessels are compressed due to increased pressure, capillaries are no longer functional

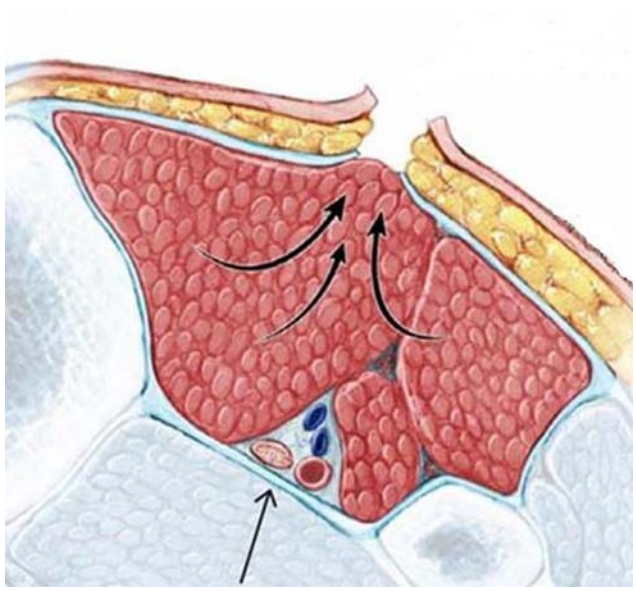

Fig. 2 Fasciotomy procedure: incision in skin and fascia to release pressure. Muscles are no longer compressed, capillaries are functional

syndrome. Commonly known as Volkmann's ischemic contracture, the affected limb often becomes dysfunctional and painful, and may lose sensibility [4]. The pathogenesis of the muscle contracture includes prolonged ischemia, myonecrosis, fibroblastic proliferation, contraction of the cicatrix, and myotendinous adhesion formation. The resultant deformity is thus a combination of varying degrees of contracture and weakness depending on which muscles and nerves are affected. Therefore, a variety of clinical presentations can be encountered following compartment syndrome of the leg and foot [5].

Causes

The most common reason of compartment syndrome is the fracture; which could be open or closed. The typical localisation of the disease is the lower leg, the four muscle compartments around the tibia [6]. The compartment syndrome could be developed during the surgical treatment, especially reamed intra-medullar nailing, when the procedure itself increase the pressure in the soft tissues of the extremities [7–9]. The second reason is the improper casting of the fractures when the bandage is too strong and increases the compartmental pressure too. The other causes of the syndrome could be burns, infiltration of IV medications (chemotherapy), intra compartment haemorrhage and tumors.

Diagnosis

Early diagnosis of the compartment syndrome is important, as delayed treatment leads to significant complications. In a conscious, alert patient, acute compartment syndromes usually are easy to diagnose clinically; however, in the unconscious patient, a diagnostic aid such as the intra-compartmental pressure monitor is useful (Fig. 3) [10, 11]. In our practice the Whiteside's injection method was used (using hospital available materials) for the measurement of the intra-compartmental pressure.

Therapy

When the compartmental pressures excess of 30–35 mmHg in a normally perfused patient, it is suggested to apply a

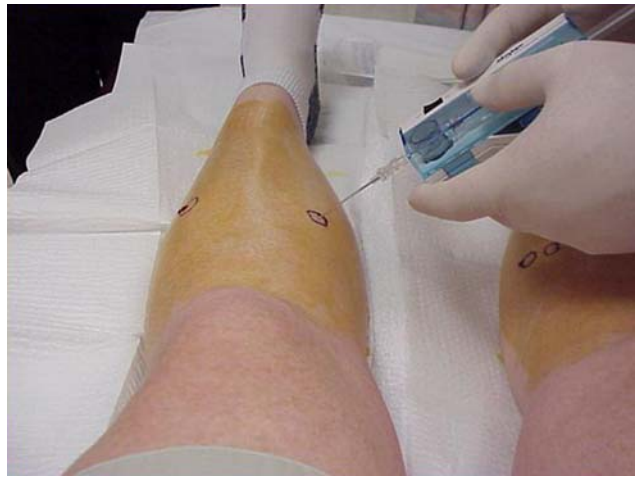

Fig. 3 Measurement of compartmental pressure in the lower leg compartments

surgical decompression, for open compartment fasciotomy. According to Whiteside's suggestion when the difference between the diastolic blood pressure and the intra-compartmental pressure, known as the DeltaP, is less than or equal 30 mmHg fasciotomy is necessary [12, 13]. All of affected compartments of the injured extremities need complete decompression.

The treatment of the Volkmann's contracture is based on an appreciation of the pathoanatomy of the deformity. Non-operative therapy is aimed at obtaining or preserving joint mobility. Operative management is usually reserved for treatment of the contractures with infarct excision, myotendinous lengthening and tenotomy [4].

Hypothesis

Our hypothesis was that in different stages of compartment syndrome there is a clear pathological abnormality in the tissue elements of the affected muscles, which is responsible for seriousness of the disease, and could be monitored besides the classical histological methods by differential scanning calorimetry. With differential scanning calorimetry (DSC) we planned to carry out investigations of muscle destruction caused by the disease. The thermograms may prove and follow the changes in the structure of affected muscles in different stages of compartment syndrome. Earlier examinations have demonstrated that differential scanning calorimetry (DSC) is a useful and well-applicable method for demonstration of thermal consequences of local and global conformational changes in the organs of the musculoskeletal system. Different authors have demonstrated thermal effects of degenerative processes in various human tissue samples [14–24].

Aim

The aim of current study was to set up thermal characteristic of healthy human muscle, to investigate muscles in case of acute compartment syndrome and Volkmann's ischemic contracture with DSC and to prove with the examinations that there is a definitive difference in the structure of the healthy and pathological muscles, which can be reproduced. The calorimetric examination of this kind, we hoped could give answer to the following questions:

- is it possible to detect differences between thermal features of the intact muscles, muscles from compartment syndrome and muscles from ischemic contractures.
- is there any correlation between the thermal effect of the injured muscle and the detected pressure changes in the affected compartment.

Material and method

Sample preparation

The control samples—healthy human skeletal muscle—2 × 4 cm muscle pieces were taken from three different compartments of the lower leg: gastrocnemius muscle, tibialis anterior muscle and peroneus brevis muscle. The donors taken into our study were all under age of 65 at their death, we considered these persons to be free any degenerative changes in their muscles. We took samples only from leg muscles where any other kind of ischemic, septic or post traumatic changes could not be verified macroscopically. All the medical interventions were made according to the ethic regulations of the University of Pécs.

The pathologic muscles were derived during operations of different seriousness of compartment syndromes after the fracture of the tibia. There were three different group according to the different stages of the compartment syndrome. Group I.: acute tibia fracture borderline compartment syndrome, intra-compartmental pressure was between 30 and 35 mmHg, group II: acute tibia fracture, definitive compartment syndrome, pressure was over 35 mmHg and in Volkmann's group: healed tibia fracture, muscle ischemic contractures as the sign of complication of untreated compartment syndrome.

In acute cases after the stabilization of the tibia fracture we made fasciotomy and decompression of the muscles from three different incisions over the different compartments. From these approaches we excised pieces of 2 × 1 cm muscles from the macroscopically most affected ischemic part. All of the patients were operated within 12 h after the accident and the pressure injury were detected minimally 3 h.

In chronic cases (Volkmann ischemic contracture) during the operative corrections of the foot deformities we made a small incision over the affected compartments and excised a same size sample from the muscles.

We measured 4 samples from control group, 11 pathologic muscles, 4 from group I, 4 from group II and 3 from chronic cases. In the pathologic group there were three females and five males being in average 48 years (28–57) of age.

After removing the muscle pieces we cut them into two parts. One part has been sent to histological examinations the other underwent DSC investigation.

Histological examination

The samples subject for histological examination were fixed in 4% formaldehyde, longitudinal and cross section slides have been made and stained with haematoxylin and

eosin. Light microscopic control (Nikon Eclipse E400) has been performed.

DSC investigation

The pieces of different samples have been prepared and measured within 6 h of removal. The thermal denaturation of different parts of human samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (samples plus buffer) in average. Typical sample wet masses for calorimetric experiments varied in the range of 100–200 mg. RPMI-1640 solution was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration. The data treatment after ASCII conversion was done by Origin 6.0.

Result and discussion

With our histological examination we could demonstrate that cadaveric muscle tissues showed no sign of degeneration, regular structure could be seen. The pathologic samples from compartment syndrome group I showed moderate and the samples from group II showed marked signs of myonecrosis. The samples from Volkmann's ischemic contracture muscles showed degeneration and myofibroblastic proliferation microscopically.

The pressure level of the affected muscle compartment is the most important parameter for the clinical outcome of the disease. There is a well known significance between the pressure value and the hypoxemic degeneration and structural changes of the muscle tissue. With our investigation we proved strong significances between the pressure values and the thermal parameters of the affected muscles too (see Table 1).

According to our knowledge this study is the first in the line of compartment syndrome research that used thermal analytical method. In Fig. 4 one can see the thermal denaturation of control as well as samples of group I and II. The heat capacity change between native and denatured states of different muscle samples was practically the same. In case of control we obtained an endothermic transition with two different thermal domains with T_ms 55.5 and 59.9 °C, that could be the melting of myosin and actin [25, 26]. The separation of myosin and actin contributions is well definite with greater myosin enthalpy contribution, and with high cooperativity in case of both muscle proteins (the half width of its thermal transition is smaller). In group I (mild stage) we have observed a significant change in the shape and melting temperatures (T_ms 58.1 and 62.2 °C) of denaturation. The separation of two meltings is more pronounced with a bigger myosin structural change. It could be the sign of severer damage in the actomyosin system. In group II (severe stage) a further transition temperature change can be observed (T_ms 57.45 and 61.5 °C) with increased actin damage (smaller ΔH_{cal}) as a consequence of marked myonecrosis. In case of Volkmann's ischemic contracture a well definite third thermal compound ($T_m = 65.8$ °C) could be identified (Fig. 5) but the shape of DSC scan is similar to the normal stage. The DSC scans clearly demonstrate the significant differences between the different stages of compartment syndrome (see Table 1) and the calorimetric enthalpy values support this too: in the control $\Delta H_{cal} = 0.52$ J/g, in group I (mild) 0.28 J/g, in group II (severe) 0.24 J/g and in Volkmann's ischemic contracture are 0.74 J/g respectively. These observations could be explained with the structural alterations caused by the biochemical processes and by the effect of

Table 1 Pressure and thermal parameters of human skeletal samples [T_m melting temperature, ΔH_{cal} calorimetric enthalpy (− stands for endothermic), average ± standard errors]

	Number of samples	Compartmental pressure	T_m (^{0}C)	ΔH_{cal} (J/g)
Healthy	4	<30 mmHg	55.5 ± 0.2	−0.52 ± 0.04
			59.9 ± 0.3	
Compartment syndrome Gr. I. (mild)	4	30–35 mmHg	58.1 ± 0.2	−0.28 ± 0.02
			62.2 ± 0.3	
Compartment syndrome Gr. II. (severe)	4	>35 mmHg	57.45 ± 0.2	−0.24 ± 0.02
			61.5 ± 0.3	
Volkmann's ischemic contracture	3		57.75 ± 0.2	−0.74 ± 0.06
			61.8 ± 0.3	
			65.8 ± 0.3	

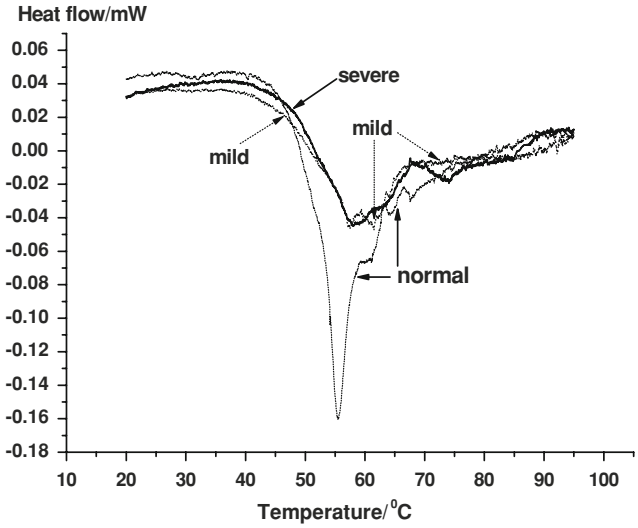

Fig. 4 Thermal denaturation scans of normal, mild and severe clinical stages

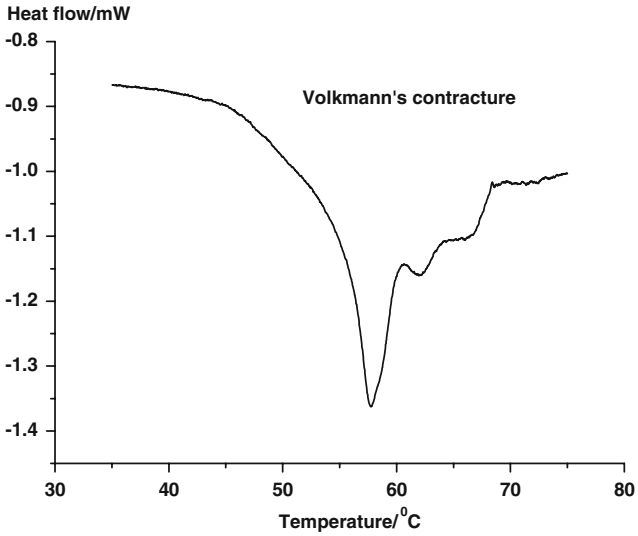

Fig. 5 DSC scan of Volkmann's ischemic contracture

compartmental pressure change. The thermal parameters of the healthy and pathologic muscles were absolutely different. There was a significant structural alteration between the control and group I. (mild pressure injury in compartment syndrome) samples, and the change in the melting temperatures seems to be a good monitor of it. In a group II (severe pressure injury) samples a complete disorganisation of the muscle tissue were proven, that could be seen from the smaller melting temperatures and calorimetric enthalpy than in case of control. The thermal results of Volkmann's ischemic contracture group could be the manifestation of a further destroying of the muscle structure with locally more densely packed subunits.

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of the pathologic human muscles. With differential scanning calorimetry it is possible to detect differences between thermal features of the intact muscles, muscles from compartment syndrome and muscles from ischemic contractures. There is a strong correlation between the thermal effect of the injured muscle and the detected pressure changes in the affected compartment. Our results may be of clinical relevance in the future i.e. to follow the pathologic changes in the muscle tissues in the cases of compartment syndrome and Volkmann's ischemic contracture, and to choose the optimal time of surgical therapy.

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