Chronic Exertional Compartment Syndrome
of the lower leg
A novel diagnosis in diabetes mellitus
A clinical and morphological study of diabetic and non-diabetic patients

David Edmundsson

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From the Department of Surgery and Perioperative Science, Division of Orthopaedics, Umeå University Hospital and Department of Integrative Medical Biology, Section for Anatomy, Umeå University, Umeå, SWEDEN
To my wife Thorey and my son Jonathan
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>AGE</td>
<td>Advanced glycosylated end products</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphatase</td>
</tr>
<tr>
<td>CAFA</td>
<td>Capillaries around fibers related to its cross sectional area</td>
</tr>
<tr>
<td>CAF</td>
<td>Capillaries around fibers</td>
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<td>CECS</td>
<td>Chronic exertional compartment syndrome</td>
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<tr>
<td>CD</td>
<td>Capillary density</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
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<tr>
<td>IMP</td>
<td>Intramuscular pressure</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MyHC</td>
<td>Myosin heavy chain</td>
</tr>
<tr>
<td>NADH-TR</td>
<td>Nicotinamide adenine dinucleotide-tetrazolinium reductase</td>
</tr>
<tr>
<td>NIRS</td>
<td>Near-infrared spectroscopy</td>
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<td>SD</td>
<td>Standard deviation</td>
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SAMMANFATTNING

Kroniskt kompartmentsyndrom (KKS) i underbenen, ett tillstånd med ansträngningsutlösning av smärta orsakad av högt muskeltryck, har tidigare huvudsakligen beskrivits hos idrottare. Orsakerna till KKS är till stor del okända. KKS har inte associerats med andra sjukdomar, och förändringar i muskulatur är inte beskrivna.


36 av 63 undersökta patienter hade KKS i underbenets främre muskelfack: 18 friska, 10 med tidigare skada på underbenet, 4 med diabetes och 4 övriga. Endast 5 av 36 KKS patienter var idrottare. Resultaten 1 år efter operation var utmärkta eller goda i 41 av 57 ben. 16 av 17 undersökta diabetiker hade KKS varav 11 hade typ 1 och 5 typ 2 diabetes. Diabetikerna skiljde sig från övriga i form av längre tid med besvär, kort gångsträcka innan underbenssmärta, fast och öm underbensmuskulatur, underbenssmärta efter 20 tåhävningar och högt muskeltryck. Muskelprover tagna vid operation visade avancerade sjukliga förändringar med extremt små och stora fiber, fiberförtvivning, interna kärnor, kluvna fiber, bindvävsomvandling samt nedsatt mitokondrie-aktivitet jämfört med friska fysiskt aktiva. Diabetikerna hade generellt mer muskelförändringar medan friska med KKS hade en betydligt lägre kapillärtäthet. Operationsresultatet var utmärkt eller gott i 15 av 18 opererade ben. 1 år efter behandling med fasciotomi hade de flesta återgått till obegränsad fysisk aktivitet. Musklerna visade tydliga tecken på regenerationsprocess och läkning. Kvarvarande sjukliga muskelförändringar fanns framför allt hos diabetikerna.

ABSTRACT

**Background:** Chronic exertional compartment syndrome (CECS) of the lower leg, defined as a condition with exercise-induced pain due to increased intramuscular pressure (IMP), has previously mainly been described in running athletes, and etiologic factors are poorly described. CECS has not been reported to occur together with other diseases and information about consequences on muscles morphology after treatment with fasciotomy is largely unknown.

**Patients and methods:** We investigated etiologic and pathophysiologic aspects to CECS in a consecutive series of 63 patients with exercise-related leg pain and in 17 diabetic patients with symptoms of intermittent claudication but no circulatory insufficiency. Clinical examination, radiography, scintigraphy and IMP measurements at rest and after reproduction of symptoms were performed. Patients with CECS were recommended treatment with fasciotomy. Biopsies were taken from the tibialis anterior muscle at time of fasciotomy and at follow-up 1 year later. For comparison muscle samples were taken from normal controls. Enzyme- and immunohistochemical and morphometric methods were used for analysis of muscle fiber morphology/pathology, fiber phenotype composition, mitochondrial oxidative capacity and capillary supply.

**Results:** Thirty-six of the 63 patients fulfilled the criteria for diagnosis of CECS in the anterior tibial compartment. The CECS patients could be divided into different etiologic groups: 18 healthy, 10 with history of trauma against the lower leg, 4 diabetic patients and 4 others. Only 5 of 36 CECS patients were athletes. The results after fasciotomy were good or excellent in 41 of 57 treated legs. Sixteen of the 17 diabetic patients were diagnosed with CECS, 11 with diabetes type 1 and 5 with type 2. The diabetic patients differed from the other groups with longer symptom-duration, short pain-free walking distance, firm and tender lower leg muscles, lower leg pain after 20 heel-raising and high IMP. The postoperative outcome was good or excellent in 15 of 18 treated legs. The muscle biopsies taken at fasciotomy showed frequent histopathological changes including small and large sized fibers, fiber atrophy, internal myonuclei, split fibers, fibrosis, disorganization of mitochondria. In contrast, the main finding in healthy CECS subjects was low muscle capillarization. After 1 year, the majority of CECS patients could return to unrestricted physical activity and the histopathological muscle changes were clearly reduced. The muscle fiber size was larger and the muscles contained signs of regeneration and repair. Remaining muscle abnormalities were present mainly in diabetic patients.

**Conclusion:** CECS is a new differential-diagnosis in diabetic patients with symptoms of claudication without signs of vascular disease. The diagnosis CECS should be confirmed with IMP measurements before treatment. A low ability for physical activity, reflected by the signs of both myopathy and neuropathy, indicates that high IMP and circulatory impairment has deleterious effects for the involved muscles. Increased physical activity and normalization of muscle morphology 1 year after treatment shows the benefit of fasciotomy. The more severe clinical and morphological findings in diabetic compared to healthy subjects with CECS indicate differences in the pathogenesis. Unrestricted physical ability after treatment is very important for diabetic patients, since physical activity is an essential part of the therapy of the disease.
ORIGINAL PAPERS


INTRODUCTION

COMPARTMENT SYNDROMES

Compartment syndrome is a condition caused by an increased intramuscular pressure (IMP) within a closed myofascial compartment compromising blood circulation within the affected space. The result is ischemia, pain and decreased muscle function, and sometimes damage to muscle and nerve tissue. Compartment syndrome may be acute or chronic. Acute compartment syndrome is the comprehensive term of syndromes with high IMP usually caused by trauma or infection. Symptoms worsen acutely and muscle necrosis and nerve injury occur within hours. This syndrome is an emergency condition that usually requires immediate surgical treatment to allow the pressure to decrease (Styf, 2003). Chronic exertional compartment syndrome (CECS) is a slowly progressing disorder that is usually not a medical emergency. The chronic form is most often caused by physical activity and the compartments of the lower leg are particular prone to be affected although other sites such as the forearm may be engaged. This thesis deals only with the chronic form of compartment syndrome of the lower leg.

CHRONIC EXERTIONAL COMPARTMENT SYNDROME

CECS is characterized by exercise-related, recurrent lower leg pain preventing further strenuous exercise. The clinical symptoms occur often bilaterally and also include muscle stiffness along with muscle weakness and sometimes sensory disturbances (Styf, 2003). The anterior and lateral compartments of the lower legs are the most commonly involved although other compartments such as the deep posterior may also be affected. The diagnosis CECS is usually associated with healthy physical active and alternative etiologic factors have been poorly described. Further, CECS has not been reported to occur together with other diseases.

History

Mavor (1956) published the first report of CECS in a professional soccer player. CECS was previously thought to be an atypical form of shin splint. Mavor reported bilateral anterior leg pain during exercise and noted a hernia in the anterior tibial muscle fascia as an indication of high IMP. After fasciotomy the pain was relieved. Later on, CECS has mainly been described
in running athletes and only few have reported CECS in non-athletic patients (Detmer et al., 1985; Styf and Körner, 1986). Most of the patients have no history of predisposing factors, although foot pronation, cavus-foot, venous insufficiency and trauma with a long interval between injury and symptoms have been associated with CECS (Tubb and Vermillion, 2001; Styf, 2003).

**Symptoms and signs**

The typical CECS patient is a young athlete with high, demanding muscle activity; usually a runner, soccer player or recreational runner with bilateral, recurrent lower leg pain that hampers exercise but permits ordinary activity of daily life. The recurrence of leg pain after reproducible work and time span is a characteristic symptom for CECS. The pain is dull or cramping and so severe it ultimately stops activity. The pain usually disappears after 10-30 minutes rest (Blackman, 2000; Tzortziou et al., 2006). Clinically palpable muscle hernias in the tibialis anterior fascia, sometimes painful, are found in about half of the patients with CECS (Blackman, 2000; Bong et al., 2005). Muscle weakness, swelling and stiffness occur frequently and sometimes a peroneal nerve paresis is present with drop foot immediately after exercise. The superficial peroneal nerve may also be affected with numbness and decreased skin sensation antero-laterally over the lower leg down to the dorsal first web space (Styf, 2003; Bong et al., 2005). Dysesthesia over the medial arch of the foot, sometimes with cramping of the intrinsic foot muscles, a sign of tibial nerve affection, indicates involvement of the deep posterior compartment (Blackman, 2000). After exercise about half of the patients have muscle tenderness over the antero-lateral aspect of the lower leg with decreased muscle strength and pain on passive dorsal extension of the ankle joint (Blackman 2000; Styf 2003). Muscle swelling and hypertrophy are inconsistent signs. Arterial circulation is always normal (Rowdon and Abdelkarim, 2008; Bong et al., 2005) and about half of the patients with CECS lack clinical signs (Englund, 2005).

**Muscle morphology**

Information on morphological muscle changes in CECS and the effects of treatment are still largely unknown. Muscle alterations in patients with CECS are only described in a few studies where they reported a high frequency of slow-twitch muscle fibers, alterations in the mitochondria and increased levels of water and lactate that decreased after fasciotomy (Quarford et al., 1983; Wallensten and Karlsson, 1984).
Pathophysiology
The generally accepted pathomechanism for CECS is an abnormal increase in the IMP during exercise resulting in compression of small vessels leading to ischemia and pain (Blackman 2000, Styf, 2003). The pattern of relative ischemia in CECS has been investigated with Near Infrared Spectroscopy (NIRS) and showed rapid, high deoxygenation at the onset of exercise and prolonged reoxygenation post-exercise compared with normal controls (Mohler et al., 1997; van den Brand et al., 2005). After fasciotomy the muscle deoxygenation in CECS patients return to normal levels as seen in healthy volunteers after exercise (van den Brand et al., 2004). Conversely, Magnetic Resonance Imaging (MRI) and thallium-201 single-Photon emission tomography did not show any ischemic muscle changes in patients with CECS (Amendola et al., 1990; Trease et al., 2001; Oturai et al., 2006). Normal muscle compartments are compliant and increase the volume up to 20% at strenuous exercise as a result of increased blood flow (Fraipont and Adamson, 2003). The amount of capillary circulation and interstitial filtration depends on the load of the exercise and normally the compartment can expand to accumulate the oedema seen in muscles during exercise. In CECS, this reserve volume may be reduced by muscle hypertrophy secondary to athletic activity or to an inextensible fascia. A non-compliant compartment may give abnormally high IMP at rest and especially after strenuous activity as well as a long pressure recovery time after exercise. The increased muscle weakness during exercise is probably mostly due to impairment in torque generation and pain in the involved muscle (Varelas et al., 1993). However, although there are a number of hypotheses to the abnormal increase in IMP in patients with CECS, the underlying mechanism and consequences on muscles is still unclear.

Diagnosis
Compartment syndrome is mainly a clinical diagnosis based on a typical history with exercise-related lower leg pain together with increased IMP measured before and after exercise. History, however, is rather unspecific and plain radiography and scintigraphy is recommended early in order to exclude joint and skeletal disorders (Englund, 2005). Also, ankle-brachial index or toe blood pressure measurement should be performed to exclude circulatory disturbances especially in non-athletic patients with these symptoms (Sahli et al. 2005; Englund, 2005). The golden standard for CECS diagnosis is the increase in IMP at rest and after exercise (French and Price, 1962). As the main symptom in CECS is lower leg pain during physical activity, reproduction of pain similar to the clinical situation can be provoked
by treadmill exercise with controlled velocity and slope. The velocity, time and type of exercise are important for the IMP levels (Styf, 2003). Marching 10 minutes on a treadmill with a speed of 6.5 km/h will usually give the typical symptoms at the end of the test in 95% of physical active patients with involvement of the anterior compartment (van den Brand et al., 2004; Bong et al., 2005).

**Intramuscular pressure measurements**

The IMP can be tested by insertion of a catheter within the muscle compartments and gauging the pressure. The IMP measurement is usually done with the patient supine, or prone for posterior compartment, with the ankle joint in 90 degrees and relaxed lower leg muscles. For measurement of the anterior compartment a catheter is inserted in the anterior tibial muscle. For measurements of the deep posterior compartment, a dorso-medial approach behind the medial tibia at the distal third of the leg can be used (Schepsis et al., 1993; van Zoest et al., 2008) (Fig 1).

Fig 1. Cross-sectional image of the lower leg. Location of intramuscular catheters inserted in the anterior and deep posterior compartments. Interosseus membrane is marked green.
A wide range of recording methods, different catheter types and variable transducer systems have been used and often with no specific extremity positions during the measurement (Willy et al., 1999; Blackman, 2000; Verleisdonk, 2002). IMP measurement during exercise is unreliable (Styf, 2003; Edvards et al., 2005) which is why it is commonly done at rest, before exercise and at intervals after exercise (Pedowitz et al., 1990; Verleisdonk, 2002). Alternative diagnostic investigations such as NIRS and MRI have been introduced. NIRS seems to have equal diagnostic accuracy as IMP measurements while MRI is considered less favorable (Mohler et al., 1997; Verleisdonk et al., 2001; Styf 2003; van den Brand et al., 2005).

**Pressure levels indicating CECS**

An IMP above 15 mm Hg at rest, more than 30 mm Hg immediately after exercise and above 20 mm Hg 5 minutes after exercise have been proposed to be sufficient diagnosing CECS (Pedowitz et al., 1990) while others have used other criteria (Styf, 2003; van den Brand et al., 2005). For the deep posterior compartment both higher and lower IMP levels than for anterior compartment have been used (Allen and Barnes, 1986; van Zoest et al., 2008).

**Treatment, prognosis and complications**

Non-operative treatment of CECS, e.g. modification of training activity, physical therapy, massage and shoe adjustments are ineffective (Verleisdonk, 2002; Fronek et al., 1987). The only successful non-operative treatment seems to be decreased activity (Bong et al., 2005). Operative treatment of CECS in the anterior tibial and lateral peroneal compartments includes fasciotomy via one or two incisions (Rorabeck et al., 1983; Fronek et al., 1987; Shepsis et al 1999; Slimmon et al., 2002; Englund, 2005) (Fig 2). An advantage of two short incisions may be an easier approach to both anterior while lateral compartments ensuring an adequate total release of the compartments and also avoiding damage to the superficial peroneal nerve (Shepsis et al., 1993). Endoscope-assisted fasciotomy have recently been introduced (Hutchinson et al., 2003; Lohrer and Nauck, 2007). Fasciectomy has been proposed for cases with recurrence of CECS symptoms (Schepsis et al., 2005). Deep posterior compartment fasciotomy is usually done by a medial incision enabling decompression also of the soleus muscle (Blackman, 2000; Bong et al., 2005; van Zoest et al., 2008) (Fig 3). After fasciotomy of the anterior compartment the result has been reported to be good or excellent in 70-90% of cases, while surgical treatment of posterior compartment syndrome is less favorable with only 50% success rate, which is about the same as reported for placebo (Styf, 2003; Fraipont and Adamson, 2003; Brennan and Kane, 2003). The lower outcome after surgery for posterior
compartment syndrome may be due to problems with the diagnosis, operative technique and complications (Davey et al., 1984; Biedert, 1997; Fraipont and Adamson, 2003; van Zoest et al., 2008).

Fig 2. The site of the skin incision and the two fascia cuts for antero-lateral fasciotomy (A, B). The fascia in each compartment is cut in its whole length (dotted lines, A).
Fig 3. The site of the skin incision and the two fascia cuts for postero-medial fasciotomy (A, B). The superficial and the deep fascia in each compartment is cut in its whole length along the postero-medial border of tibia (dotted line, A).

The general recurrence rate of CECS after anterior fasciotomy varies between 3-20% (Schepsis et al., 2005). This is often due to postoperative bleeding, hematomas and formation of constricting scar tissue in the fascia defect. Therefore, a suction drainage is recommended and is usually removed after 24 hours. Other complications include nerve and vessel injuries.
and wound infections. The overall complication rate is between 5 to 13% in otherwise healthy patients (Fronek et al., 1987; Fraipont and Adamson, 2003).

**Differential diagnosis**

Since the symptoms and signs in CECS are related to unspecific pain and about half of the patients lack clinical signs it is important to consider other diagnoses. When examining the patients it is especially important to analyze the type and localization of pain and when it occurs (Styf, 2003). If CECS is not confirmed with IMP measurements additional investigations, e.g. neurophysiological tests, MRI, CT-scan, ultrasound-led Doppler and angiography may be necessary (Toulipolous and Hershman 1999; Verleisdonk, 2002; Bong et al., 2005).

<table>
<thead>
<tr>
<th>Differential diagnosis to CECS in the lower leg (Styf, 2003; Bong et al, 2005).</th>
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<tbody>
<tr>
<td><strong>Anterior leg pain</strong></td>
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<tr>
<td>Tibia periostitis</td>
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<tr>
<td>Compression of the common peroneal nerve</td>
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<tr>
<td>Peroneal tunnel syndrome</td>
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<tr>
<td>Stress fractures, tibia and fibula</td>
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<tr>
<td>Fascial hernia</td>
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<tr>
<td><strong>Other diagnosis:</strong></td>
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<td>Bone tumors, osteoid osteoma, vascular claudication</td>
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**DIABETES MELLITUS**

Diabetes mellitus (DM) is a major public health problem and one of the most rapidly increasing diseases globally; the number of diabetic patients will increase from 150 millions in 2000 to 366 millions by the year 2030 (Huysman and Mathieu, 2009). DM is characterized by hyperglycaemia resulting from absolute or relative insulin deficiency. There are two main types of DM. Type 1 is caused by an autoimmune reaction with destruction of insulin-producing pancreatic cells leading to insulin deficiency, mostly affecting children or young people. Type 2 is associated with sedentary life style, high daily glucose intake and overweight involving peripheral insulin resistance and a relative insulin deficiency. Type 1 is
treated with life-long endogenous insulin substitution and Type 2 with diet, medication or insulin. Exercise is considered to be one of the cornerstones for optimal diabetes treatment.

**Diabetic complications**

The complications associated with DM are mainly related to vasculopathy and are commonly grouped into macro- and micro-vascular complications. The macro-vascular disease is the most common cause of mortality and morbidity and is responsible for high incidences of stroke, myocardial infarction and peripheral vascular disease (Huysman and Mathieu, 2009). Prolonged hypertension, hyperglycaemia and hyperlipidemia increase cardiovascular risks and the severity and progression of arteriosclerosis, which explains the high frequency of cardiovascular diseases (Girach and Vignati, 2006). The diabetic specific microvascular complications are mainly retinopathy, nephropathy, and arguably, neuropathy (Nathan, 1993; Marshall and Flyvbjerg, 2006). In microangiopathy the capillary walls and arterioles are thickened (Roy et al., 2010) and the glyocalyx contributing to the barrier function on the luminal side is attenuated (Nieuwdorp et al., 2006a; 2006b). Microvascular endothelial injury and hyperpermeability occur when excessive glucose is metabolized to sorbitol forming advanced glycation end-products (AGE) deposited in the endothelial wall (Yuan et al., 2007).

The wide spectrum of vascular abnormalities may cause permeability disturbances in DM, including leakage and local tissue oedema. Moreover, the pathophysiology of diabetic neuropathy is considered to have vascular and metabolic components (Cameron and Cotter, 1997; Yasuda et al., 2003). Although the cause of diabetic neuropathy may be multifactorial, one proposed pathophysiological mechanism is the double-crush syndrome i.e. nerve compression at narrow anatomical spaces together with swelling of the nerve itself by local edema. Occlusion within the neural microcirculation, the vasa vasorum, is regarded as an important contributor to diabetic polyneuropathy (Cameron and Cotter, 1997) and especially acute mononeuropathies (Vinik, 1999). To relieve local nerve pressure surgical decompression of the distal portions of the nerves has been performed with reduction of pain and restoration of sensibility (Dahlin, 1991; Wood and Wood, 2003; Dellon, 2004). Moreover, stiffening of connective tissue in skin, ligaments, tendons and joint capsules due to non-enzymatic glycosylation and cross-linking of collagen is common in DM (Smith et al., 2003; Dellon, 2004). The typical clinical manifestations are stiff joint syndrome, carpal tunnel syndrome and Dupuytrens’ contracture (Smith et al., 2003).
**Leg disorders in DM**

Lower-leg complaints are frequent in DM and often disabling. In fact, these are one of the most serious and expensive diabetes complications and therefore it is very important for health workers to always examine the patients’ feet and lower legs (Kim et al., 2001). Approximately one-third of the diabetic patients get reduced cutaneous foot sensibility with numbness and tingling sensations. Sometimes there is continuous neuropathic pain that is usually not worsened by physical activity. Motor nerves may be affected with paralysis of intrinsic muscles resulting in the typical foot deformity (Kim et al., 2001; Smith et al., 2003). Even progression to complete drop-foot can occur. The senso-motor disturbances and angiopathy increase risks for ulcers, osteopathy and Charcot-joints, e.g. joint destruction. Autonomic neuropathy may result in leg pain in cold or warm environments (Urbancic-Rovan et al., 2004; Devigili et al., 2008). Also spontaneous diabetic muscle infarction in thigh and calf muscles does occur with acute onset of pain and swelling. This condition usually affects female diabetic patients with long-lasting hyperglycemia, multiorgan damage including neuropathy, nephropathy and gastroenteropathy. MRI shows edema and inflammation in the muscle and microscopy reveals necrosis, edema and fibrosis (Yildirim and Feldman, 2008).

Conservative treatment is recommended and the symptoms revert within weeks to months. Another complication to DM is stiffening of arterial walls together with plaque formation obstructing blood flow to the legs (Mackey et al., 2007; Yamagishi, 2009). This gives symptoms of leg pain during walking that is relieved at rest. The incidence of this disorder, termed intermittent claudication, is increased about 3 times in diabetic patients compared to normal population (Sahli et al., 2005) and can progress to gangrene necessitating amputation (Pecoraro et al., 1969; Icks et al., 2009). Others reasons to claudication can be spinal stenosis due to degenerative disease or inflammatory and bone disorders. Some patients lacking pathological clinical signs have been termed as claudication due to neuropathy (Papanas et al., 2005). Thus, in a proportion of diabetic patients with claudication there is no obvious explanation to the symptoms, and the disease itself, per se, is considered as an independent risk factor for exercise-induced leg pain (Wang et al., 2005).

**THE ANATOMY OF THE LOWER LEG**

The anterior compartment contains the tibialis anterior, extensor hallucis longus and extensor digitorum longus and peroneus tertius muscles. The anterior compartment is one of the most inextensible musculofascial compartments surrounded by fascia and located between the tibia,
the fibula and in front of the interosseus membrane (Fig 1). This is probably one of the reasons that it is the compartment most prone to develop compartment syndromes in general. Neurovascular supply contains the deep branch of the common peroneal nerve and anterior tibial artery and vein coursing anterior to the strong and inextensible interosseous membrane. The muscles and nerves involved are therefore vulnerable for circulation disturbances or swelling with raised IMP caused by trauma, due to fact that its main arterial supply is an end-artery crossing the stiff interosseus membrane (Styf, 2003). The lateral peroneal compartment includes the peroneus longus and brevis muscle and the superficial branch of the peroneal nerve. The superficial peroneal nerve passes along the peroneus longus muscle between the longus and brevis muscle to a level of 10-15 cm proximal to the lateral malleolus where it pierces the deep fascia and becomes subcutaneous (Blackman, 2000; Styf, 2003; Bong et al., 2005). Hernias in the muscle fascia appear often in this area, sometimes resulting in nerve entrapments. The superficial posterior compartment contains the medial and lateral gastrocnemis, soleus and plantaris muscles and the sural nerve. A dense superficial fascia surrounds the compartment dorsally and the deep transverse fascia divides it from the deep posterior compartment. The deep posterior compartment contains the flexor digitorum longus, flexor hallucis longus, tibialis posterior muscles and proximally the popliteus muscle. Boundaries for the compartment anteriorly are the tibia, interosseus membrane and fibula and posteriorly the deep transverse fascia. Neurovascular structures in the deep posterior compartment include the tibial nerve and the posterior tibial artery and vein. The tibial nerve and vessels enter the lower leg beneath the soleus muscle further on the posterior surface of tibialis anterior muscle and distally on the posterior tibia (Davey et al., 1984; Bong et al., 2005).

MUSCLE STRUCTURE

Muscle fibers
Human limb skeletal muscles consist of a number of densely packed longish, cylindrical or polygonal shaped fibers specialized for force production and movements. The myofibril and mitochondria are two main components of the muscle fiber, where the myofibril is the actual force generator and the mitochondria is engaged in the energy supply of the fibers. The fiber length varies in different muscles and each fiber has multiple nuclei, normally situated at the periphery of the fibers. A thin layer of connective tissue, the endomysium, surrounds each fiber. Thousands of fibers are then wrapped into the perimysium forming muscle bundles into
groups joining a tendon at each end. All bundles are connected into entire muscles and are enclosed by a surrounding muscle fascia. Each myofibril contains repetitive contractile units along the length of the fiber called sarcomeres. The sarcomere is the functional unit of muscle contraction. It consists of thick filaments, which are mainly composed of myosin, and thin filaments, which are composed of actin, troponin and tropomyosin. Interaction between these two filaments constitutes the basic mechanism for the sliding filament theory of muscle contraction (see Fig. 4). Myosin is the major contractile protein in muscles. Each myosin consists of two myosin heavy chains (MyHC) and four light chains. Myosin is the molecular motor that converts free energy derived from the hydrolysis of ATP into mechanical work. The speed at which ATP can be hydrolyzed determines the speed of contraction. Consequently, the maximum velocity of unloaded shortening of skeletal muscle is related to the ATPase activity.

Fig. 4. Schematic illustration of skeletal muscle structure. Muscle, muscle fibers with capillaries, myofibrils, myofilaments and contractile molecules are shown.

**Muscle fiber composition**

The human skeletal muscle is composed of several different fiber types that can be distinguished on the basis for differences in the ATPase activity or by the dominant MyHC isoform. Based on the myofibrillar ATPase reaction at different pH, muscle fibers can be divided in slow contracting type I fibers and fast contracting type II fibers. Slow type I fibers are fatigue resistant and have high mitochondrial oxidative capacity. Fast type II fibers can be subdivided into IIA, IIB and IIC fibers, where IIA are more fatigue resistant and have higher
mitochondrial oxidative capacity than type IIB. Type IIC fibers have characteristics in between type I and II fibers and are normally rare in human muscles.

Myosin contains at least eight genes for MyHC (Schiaffino and Salvati, 1997; Weiss et al., 1999) of which two are code for developmental MyHC isoforms, MyHC fetal and MyHC embryonic. These two isoforms are expressed during early muscle development and as muscle differentiates and matures, the developmental MyHCs are down-regulated and replaced by adult isoforms in human limb muscles (Butler-Browne et al., 1990; Barbet et al., 1991). The predominant contractile MyHC isoform in human limb muscles are slow twitch MyHCI, fast twitch MyHCIIa and fast twitch MyHCIIx. ATPase type I fibers express MyHCI, type IIA fibers express MyHCIIa, and type IIB fibers express MyHCIIx. Type IIC fibers co-express MyHCI and MyHCIIa.

Muscle capillarization
A network of parallel and cross-anastomosing capillaries, with some turtuosity and branching, surrounds all muscle fibers. The dimension of this network of micro-vessels is the major determinant for oxygen delivery to the muscle cell and is therefore important for muscle performance and endurance. However, the oxygen supply depends also on an adequate vascular function and intact autoregulation. Microcirculation varies widely between rest and work, partly due the autonomic change of the diameter of pre-capillary arterioles. Blood flow disturbances in the circulation result in an energy crisis, ischemia and accumulation of metabolic by-products, which may lead to muscle pain, fatigue and deprived function. The extent of the capillary network is normally related to fiber phenotype composition and fiber size (Hudlicka et al., 1987; Ponten and Stål, 2007). Thus, large muscle fibers are surrounded by more capillaries than small fibers and slow contracting fibers containing MyHCI have generally higher oxidative mitochondrial capacity and are supplied by more capillaries than fast contracting fibers containing MyHCII.

Muscle plasticity
Muscle fibers are dynamic structures capable to change their size and phenotype under various conditions. Physical training usually results in fiber hypertrophy and alteration of fiber phenotypes as well as increased mitochondrial oxidative capacity and extension of the capillary network (Wang et al., 1993; Hudlicka et al., 1992; Eggington et al., 1998) whereas inactivity and denervation often gives the opposite (Lu et al., 1997; Borisov et al., 2000; Dedkov et al., 2002). The adaptive reaction of the muscle to physical activity is not only
influenced by the neuronal signal intensity and mechanical load on the muscle but also by hormones and growth factors (Wang et al., 1993; Fitts and Widrick, 1996; Andersson et al., 2005). Strength training results in increased myofibrillar protein synthesis, activation of precursor cells and satellite cells. Satellite cells fuse with existing myofibrils and contribute to increased number of myonuclei and hypertrophy of muscle fibers (Eriksson et al., 2006).

AIMS OF THE STUDY

The overall aims of this thesis were to study etiologic aspects of CECS in lower legs and to learn more about possible muscle alterations after treatment with fasciotomy.

The specific aims were:

1. To study etiologic factors resulting in CECS in unselected patients with exercise-related lower leg pain independent of age, gender and activity levels.

2. To analyze possible morphological alterations in the anterior tibial muscle in otherwise healthy physically active individuals with CECS.

3. To describe history, clinical findings and treatment of CECS in DM.

4. To describe morphological alterations in the anterior tibial muscle in diabetic patients with CECS.

5. To analyse the effect of treatment with fasciotomy on muscle morphology 1 year after treatment in diabetic and healthy non-diabetic patients with CECS.

6. To compare the morphological results between diabetic and healthy non-diabetic patients with CECS.
PATIENTS AND METHODS

PATIENTS

Patients included in each study, patient demographics and clinical data for all subjects are summarized in Table 1.

Subjects in study 1

Seventy-three patients were referred to the division of Orthopaedics, University Hospital, Umeå, from 1996 through 2000 because of a suspicion of CECS due to a history of pain in the lower leg on exertion. None of the subjects had clinical signs of arterial circulatory disturbances in the legs. Seven patients were excluded since they refused to participate in the study and 3 had been treated earlier for a similar disorder. Thus, 63 patients (27 males and 36 females, mean age 39y, range 16-73y) were included in the study. Mean duration of symptoms was 2.6y (0.5–15y).

Subjects in study 2

In the clinical study of patients suspected for CECS, 4 patients were unexpectedly found to have DM. This prompted us to ask the diabetic clinic at Umeå University Hospital to send us all diabetic patients with activity-related leg pain without clinical signs of circulatory insufficiency in order to explore our finding. During a 2-year period, we got 13 additional diabetes patients referred for suspicion of CECS. Thus, 17 patients were included in study 2 (3 male and 14 females, mean age 39y, range 18-72y).

Abbreviations

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<th>No= No trauma against lower legs</th>
<th>Preop symptoms</th>
<th>Preop signs</th>
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<tr>
<td>CB= Chronic back pain</td>
<td>1. Pain</td>
<td>1. Tenderness over anterior compartment</td>
</tr>
<tr>
<td>DM= Diabetes mellitus</td>
<td>2. Sensory deficit</td>
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<tr>
<td>FF= Fibula fracture</td>
<td>3. Edema</td>
<td>3. Fascial hemia</td>
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<td>MC= Muscle contusion</td>
<td>5. Muscle stiffness</td>
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<td>PN= Polyneuropathy</td>
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<td>6. Muscle rupture</td>
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<td>RA= Rheumatoid arthritis</td>
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24
Table 1. Demographic data of patients with CECS included in studies 1-4. R = right leg, L= left leg, B= both legs  A = athletes, R =Recreational runners, W = walkers.

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**Subjects in study 3**

Fourteen of the physically active and otherwise healthy patients in study 1 who were recommended surgical treatment after diagnosis of CECS agreed to a muscle biopsy at fasciotomy. Nine of them agreed to a second muscle biopsy at follow-up 1 year later (3 males and 6 females, mean age 32 y, range 18-51y). The duration of symptoms was 3 years (1-10y).

**Subjects in study 4**

Seven of the diabetic patients who participated in study 2 agreed to a muscle biopsy at fasciotomy (5 females, 2 males, mean age 37y, range 18-53y). Five had diabetes type 1 and 2 had diabetes type 2. One year later, five of these patients agreed to a second muscle biopsy. The mean duration of exercise-induced leg pain was 6.8y (0.5-15y) and the mean duration of diabetes was 23y (11-30). All were on insulin treatment.

**Controls**

For comparison of morphological muscle findings, biopsies from the tibialis anterior muscle were collected from a control group of nine healthy and physically active individuals (5 males and 4 females, mean age 34y, range 19-51y). None of the subjects had leg pain or clinical signs of neurological or circulatory disturbances.

**CLINICAL EVALUATION**

History, symptoms and clinical signs were noted, with special attention being paid to neurological and circulatory disturbances. Conventional plain radiography and scintigraphy were performed to exclude other causes of lower leg pain.

**Criteria for diagnosis of CECS**

For diagnosis of CECS the following should be fulfilled: (1) history of exercise-related lower leg pain, but normal pedal pulse, normal radiograph and bone scans (2) reproducible pain during exercise test, (3) IMP values at rest of > 15 mm Hg and/or IMP of > 30 mm Hg 1-2
minutes after the end of exercise and / or IMP of > 20 mm Hg 5 minutes after exercise together with the reproduced leg pain (Pedowitz et al., 1990).

Reproduction of symptoms

A treadmill test was used to reproduce the symptoms. The duration of the test was 10-15 min and during this period the velocity and slope of the treadmill was adjusted in an attempt to reproduce the lower leg pain. The patients with CECS reported increasing pain in the lower legs, usually rating 5 or 7 on the 10-point Borg scale, and/or rated exertion as 17 (very heavy) on the 20-point Borg scale at the end of the test (Borg, 1973).

Measurements of IMP

IMP measurement was monitored using a micro-capillary technique with infusion of a low volume of isotonic saline (0.1-0.3 ml/h) via a catheter (Myopress; Athos Medical, Höör, Sweden). The catheter has an outer diameter of 1.05 mm and the tip has four side holes, which gives a surface tissue contact area of 1.5 mm². A cannula with the catheter filled with saline was inserted into anterior tibial muscle and connected to a pressure transducer (PMSET 2DT-XO 2TBG; Becton Dickinson, Singapore). During the procedure the patients were supine, and relaxed with the ankle joint in 90 degree. For posterior compartment measurements we performed the dorso-medial approach (Schepsis et al., 1993). The location of the catheter tip was checked by palpation and gentle compression with an amplitude reaction on the pressure curve. Measurements were performed in both legs. The use of a myopress catheter is considered as an accurate method for IMP measurements (Styf, 2003). The advantage of this method is less volume load to the interstitial tissue at rest and in exercise avoiding false high values. It also enables a rapid detection of pressure oscillations during dynamic measurements.

Treatment with fasciotomy

All patients with diagnosis of CECS were recommended treatment with fasciotomy of the anterior tibial and peroneal compartment. The surgical procedure of the anterior compartment
included a 5 cm skin incision halfway between the fibular shaft and the tibia crest in the mid portion of the leg (Fronek, 1987). After an extended subcutaneous dissection, the fascia of both compartments was decompressed with fasciotomy. A 1 cm wide strip of the fascia was removed and an over-night suction drainage was used. Posterior compartments were treated with fasciotomy of the superficial soleus and gastrocnemius muscles and the deep posterior compartments according to Styf (2003).

MUSCLE BIOPSIES

At fasciotomy, a muscle sample (approximately 8 x 5 mm) was obtained under general anesthesia from the anterior tibial muscle, 15 cm distal to the knee joint and 1 cm deep in the muscle. A second biopsy was obtained under local anesthesia 1 year after fasciotomy at the same level and area, but in order to avoid scar tissue, not at the identical site as the first biopsy. Muscle samples from the corresponding region were obtained from the control subjects. The muscle samples were mounted for serial sectioning in OCT compound (Tissue Tek®, Miles laboratories, Naperville, IL, USA) and frozen in liquid propane chilled with liquid nitrogen.

METHODS FOR ANALYSIS OF MUSCLES

Immunohistochemistry

Serial transverse muscle cross-sections (5 μm thick) were cut in a cryostat microtome at -20°C, mounted on glass slides, and processed for immunohistochemistry with well characterized monoclonal antibodies (mAbs) against different human myosin heavy chain (MyHC) and laminin isoforms. Laminin is a major component of the basement membrane. Data on used mAbs are shown in Table 2. Visualization of cell borders (i.e., basal lamina) of the muscle fibers and capillaries was performed by using mAb 4C7 which labels the basement membrane of capillaries strongly and the basement membrane of muscle fibers weakly, and mAb 5H2 which labels only the basement membrane of muscle fibers strongly (Ponten and Stål, 2007). An antibody against desmin (D33) was used for visualization of fiber regeneration and abnormalities in fiber structure. Immunohistochemical visualization of
bound antibodies was performed using the indirect unconjugated immunoperoxidase technique. For details of the laboratory procedures see Stål and Lindman (2000).

Enzyme-histochemistry.

Eight µm thick cross-sections, serial to those used for immunohistochemistry, were stained for the demonstration of myofibrillar ATPase activity (EC 3.6.1.3) after preincubations at pH 10.3, 4.6 and 4.3 (Dubowitz, 1985). Hematoxylin & Eosin and Gomori trichrome staining were used to visualize general morphology and muscle pathology. To demonstrate oxidative capacity of fibers, a mitochondrial enzyme, NADH-TR (EC 1.6.99.3), was assayed. Muscle fibers characterized by focal or multifocal zones without mitochondrial NADH-TR activity were characterized as moth-eaten fibers (Banker and Engel, 1994).

Fiber classification.

Based on the staining pattern for the different MyHC mAbs, the fibers were classified as fibers containing only MyHCII, MyHCIIa, or MyHCIIx or as hybrid fibers coexpressing MyHCII and MyHCIIa or MyHCIIa and MyHC IIx. The basis for classification is shown in Fig 5. For control and comparison, the muscle fibers were enzyme-histochemically typed according to their staining intensities for myofibrillar ATPase (mATPase) after alkaline and acid preincubations (Dubowitz, 1985).
Morphometric analysis.

Randomly chosen areas of the immunohistochemical and enzyme-histochemical stained muscle cross-sections were scanned and analyzed in a light microscope connected to an image analysis system (IBAS, Kontron elektronik GMBH, Eching, Germany). The fibers were classified in fiber phenotypes based on their MyHC isoform composition and the proportion of different types was estimated. The fiber area was measured by tracing the circumferences of each fiber along the periphery of the basement membrane and the numbers of capillaries were counted on whole muscle cross-section and around each individual fiber. Small atrophic or regenerative/degenerative muscle fibers (<20μm²) were excluded as they highly bias the calculation of capillary variables. The calculation of fiber area and capillary variables included 10,745 muscle fibers and 22,626 capillaries. A single investigator, who was blinded regarding the clinical data of the subjects, determined all morphological analyses.

Capillary variables.

Capillary density (CD) was calculated as the total number of capillaries per mm² muscle cross-section. The number of capillaries around fibers (CAF) included all capillaries within a distance of 5 μm from each individual muscle fiber. Capillaries related to each fiber relative to its cross-sectional area (CAFA) were calculated according to the formula: CAFA / fiber cross-sectional area x 10³. This variable relates CAF parameter to fiber size and is a hypothetical measure of the cell volume each capillary supplies.

Statistical analysis.

In study 1 a non-parametric test (Kruskal-wallis) was used for analysis of differences between groups. A Mann-Whitney test (Holm’s correction of the Bonferroni method) was used in study 2 (Statistical package Statistic, version 6.0). In studies 3 and 4 an Anderson-Darling test was used to analyze the normality in distribution of the samples. Since no indication of skewed distribution was observed within each group, an unpaired t-test was used to test possible differences between patients and controls and a paired t-test for differences between baseline and follow-up. A Chi-square test was performed to analyze differences in fiber type proportion. The variability in muscle fiber diameter was expressed as the coefficient of
variation (CV) according to the formula CV = SD/fiber area x 100 (%). The statistical software Statview 4.5 (SAS Institute Inc., Cary, NC, USA) was used to generate measurements and Minitab (Minitab Inc, State College Pennsylvania, USA) to calculate the statistics. The null hypothesis was rejected on $p$-values $\leq 0.05$ in all used statistical tests.

RESULTS

CECS IN UNSELECTED PATIENTS WITH EXERTIONAL LOWER LEG PAIN

Of the 63 patients with exercise-induced lower leg pain, 36 patients (mean age 36 (16-65) y, 22 females, 14 men) had CECS with engagement of 66 anterior, 2 lateral and 7 posterior compartments. Clinically they were tender over anterior lower leg and muscle hernias were found in 4 patients. All had normal findings on radiography and bone-scan. Only 5 patients were athletes and 5 recreational runners while the majority were walkers (n=26).

The age, proportion of walkers and outcome differed in the four different groups of patients as shown below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Age</th>
<th>Proportion walkers/athletes, runners</th>
<th>Sex female/male</th>
<th>Treated with fasciotomy/1y follow-up</th>
<th>Outcome of treated legs at 1y follow-up (mean rating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overuse</td>
<td>18</td>
<td>29</td>
<td>8/18</td>
<td>10/8</td>
<td>17/16</td>
<td>2</td>
</tr>
<tr>
<td>Previous trauma</td>
<td>10</td>
<td>40</td>
<td>10/0</td>
<td>6/4</td>
<td>9/9</td>
<td>3</td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>4</td>
<td>38</td>
<td>4/0</td>
<td>4/0</td>
<td>3/3</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
<td>51</td>
<td>4/0</td>
<td>2/2</td>
<td>3/3</td>
<td>2</td>
</tr>
</tbody>
</table>

The rating according to Abramovitz et al (1994).

<table>
<thead>
<tr>
<th>1. Excellent</th>
<th>No pain during or after exercise</th>
<th>Patient considers herself/himself cured</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Fair</td>
<td>Pain on running/ exercise or afterwards</td>
<td>Still has a limitation Recurrent symptoms Only slightly improved</td>
</tr>
<tr>
<td>2. Good</td>
<td>Minimal discomfort or soreness during/after exercise</td>
<td>Significantly improved Glad to have had surgery</td>
</tr>
<tr>
<td>4. Poor</td>
<td>Unchanged or worse complications</td>
<td></td>
</tr>
</tbody>
</table>
According to the clinical history the patients were divided into four different etiologic groups: 18 with overuse (otherwise healthy), 10 with earlier trauma, 4 insulin-treated diabetic patients and 4 others. Diabetic patients and the 4 others had higher IMPs than the overuse group (Fig. 6). Fifty-seven legs in 32 patients were treated with fasciotomy. The surgical results were graded according to Abramovitz et al (1994) and were excellent or good in 41 of 57 treated legs.

![Fig. 6. Preoperative IMP (mean values in mmHg) in the anterior tibialis muscle at rest, 1, 5, 10 and 15 min after exercise. The diabetic group had higher mean IMP values than the trauma and overuse group with significant differences to the overuse group at rest and 15 min after exercise (p<0.05).](image)

**CECS IN PATIENTS WITH DIABETES MELLITUS**

Seventeen patients with DM and lower leg pain were investigated. Their mean age was 39 (18-72) y, 14 were females and the mean duration of diabetes was 22 (1-21) y. The duration of claudication was 6 (0.2-15) y. Twelve had type 1 and 5 had type 2 diabetes. Twelve had other diabetic complications as well. Clinical examination revealed firm muscles of the lower leg, with and leg pain was provoked by 20 heel-raisings. Muscle hernias were present in 4 patients and impaired cutaneous sensibility was found in 9 patients. None had signs of circulatory insufficiency. Sixteen patients were confirmed to have CECS. IMP was
significantly higher (p<0.05) in diabetic patients compared with a group of healthy physically active patients treated for CECS (Fig. 7).

![Graph showing IMP values](image)

Fig. 7. Preoperative IMP (mean values in mmHg) in the anterior tibialis muscle in overuse (blue) and diabetic (purple) groups at rest, 1, 5, 10 and 15 min after exercise. Bar represents SD. The diabetic group (n=16) had higher IMP than the overuse group (n=18). The differences in IMP between the two groups were statistically significant at all time intervals (p<0.05), except at 5 min.

Fifteen of the 16 patients were treated with fasciotomy of the antero-lateral compartment and one with a posterior compartment release. At surgery the fascia seemed thickened and whitish in some patients. Nine patients were followed more than 1 year and rated their outcome as excellent in 4, good in 11 and fair in 3 legs. The walking time before leg pain increased to unlimited in 8 patients. Postoperatively, 1 had superficial peroneal nerve injury and 2 had infections.

<table>
<thead>
<tr>
<th>Number</th>
<th>Female/male</th>
<th>Age (mean y)</th>
<th>DM type 1/2</th>
<th>Disease duration (mean y)</th>
<th>Number with diabetic complication</th>
<th>Years of leg pain (mean y)</th>
<th>Outcome of treated legs 1y follow-up (mean rating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>13/3</td>
<td>39</td>
<td>11/5</td>
<td>21</td>
<td>13</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

Basic data and outcome for the entire diabetic group diagnosed with CECS.
BASELINE MUSCLE MORPHOLOGY

Muscle pathology

Histopathological muscle changes were common in CECS especially in diabetic patients having more frequent, severe and widespread alterations than healthy non-diabetic patients. The most common abnormalities were presence of extremely small-sized fibers partly expressing developmental MyHC. Most cases showed signs of fibrosis and focal fascicular atrophy were observed in some subjects. A number of fibers, or clusters of fibers, had low or lacked NADH-TR activity in the centre and others had mitochondrial disorganization fulfilling the criteria for moth-eaten fibers in the CECS. This pattern was especially prominent in diabetic patients. More diabetic specific findings were fiber hypertrophy, fiber type grouping, group atrophy, fiber necrosis, infiltration of inflammatory cells, abnormally formed fibers including angulated fibers, fiber split, increased number of internal nuclei (>3%) and fat infiltration. Ring-fibers, pyknotic nuclear clumps and fibers with a tendency to lobulated form and an irregular trabecular and coarse granular appearance in NADH-TR were also found in diabetic patients (Fig. 8).

Fiber types and their mitochondrial oxidative capacity

Fibers expressing MyHC I, MyHC IIa, MyHCIIa+IIa and MyHCIIa+IIx were distinguished in all tibialis anterior muscles in both patients with CECS and in normal controls. No muscle fibers expressed only MyHCIIx in any cases. Fibers containing MyHC I had usually a higher mitochondria oxidative capacity than fibers containing MyHCII. The NADH-TR staining intensity was generally lower in the CECS samples than in controls, especially in fibers containing MyHCII isoforms.

Relative frequency of fiber phenotypes, fiber area and variability in fiber size

The proportion of different fiber phenotypes or fiber area did not differ between diabetic and healthy non-diabetic patients with CECS and controls. When small atrophic or degenerative/regenerative fibers were excluded, no statistical differences in fiber area and CV values were observed between patients with CECS and normal controls.
Fig 8. Muscle pathology in the anterior tibialis muscle in diabetic (A-H) and physical active patients with CECS (I-L). Figs A-D are stained for Hematoxylin & Eosin, fig. E for mAb A4.840 (MyHCl), figs F, G for mAb D33 (desmin), figs. H, I for NADH-TR, figs. J, K for mAb N2.261 (MyHClIIa stained strongly and MyHCl weakly) and fig. L for mAb NCL-MHCn (developmental MyHCl). Note the large variability in fiber size and fiber form (A, C, D), increase of internal myonuclei (A), a necrotic fiber (B), increased infiltration of fat and connective tissue (C, D), small angulated fibers (D, K), fiber type grouping (E), a multi split fiber (F), ring and split fibers (G, H), abnormal mitochondria distribution (I), fascicular atrophy (J) and presence of developmental MyHCl (L).

**Muscle capillarization**

All capillary parameters differed significantly between healthy non-diabetic patients with CECS and controls. The capillary density (CD) tended to be 28% lower (p=0.06), the number of capillaries around fibers (CAF) was 21% lower (p=0.004), and the number of capillaries around fibers relative to its cross-sectional area (CAFA) was 27% lower (p=0.01) than in controls. For fiber phenotypes, significantly lower CAF and CAFA values were observed for MyHCl and MyHClIIa fibers (p<0.04, respectively). In contrast, no significant difference were
found between diabetic patients with CECS and controls, except that those with DM showed significantly lower CAF for MyHC IIa fibers (p=0.02). However, there was trend to lower CD (p=0.06) and lower CAF (p=0.07) (Figs. 9 and 10).

Fig. 9. Serial crossections of tibialis anterior muscle in a diabetic CECS patient (A-D) and control subject (D-F) stained for mAb N2.261 (MyHCIIa strongly stained and MyHCI weakly stained) (A,D), mAb A4.951 (MyHCI) (B,F) and mAb 4C7 (C,F).

Fig. 10. Muscle cross-sections of the tibialis anterior muscle of a diabetic patient and a healthy active subject with CECS and normal control stained for mAb C47. Note the lower capillary density in a healthy active subject (B) compared with a diabetic patient with CECS (A) and a normal control (C).
MORPHOLOGY AT FOLLOW-UP 1 Y AFTER FASCIOTOMY

Muscle pathology

Histopathological muscle alterations were less common 1 year after treatment, especially in diabetic patients with dramatically reduced changes (Fig10). Although morphological abnormalities still were present in most diabetic, and in some healthy non-diabetic patients, the frequency of the remaining alterations was low. A general finding was decreased frequency of fibers with mitochondrial disruptions and the NADH-TR staining activity of muscle fibers was slightly increased in some cases, especially for fibers containing fast MyHCII. Connective tissue alterations within the muscle was general reduced. In healthy non-diabetic patients, the number of small-sized fibers was decreased contrary to an increase of small fibers expressing developmental MyHC (Fig. 11).

Fig 11. Muscle cross-sections of the anterior tibial muscle of a diabetic patient with CECS at fasciotomy (A) and at 1 year follow up (B) and a normal control subject stained for Hematoxylin & Eosin. Note the normalization of the muscle 1 year after fasciotomy (B).

In DM, the most pronounced alteration was a reduction of small-sized fibers, necrotic fibers, split fibers, inflammatory cells and fibers containing developmental MyHC. The remaining histopathological alterations 1 year after fasciotomy consisted in the healthy non-diabetic group mainly of small fibers containing developmental MyHC and in those with DM the most common findings were atrophy, fiber hypertrophy, fiber type grouping and internal nuclei.
Fig. 12. Muscle cross-sections of the anterior tibial muscle of a healthy active patient with CECS at fasciotomy (A) and at 1 year follow-up (B, C) stained for mAb N2.261 (MyHCIIa strongly stained, MyHCI weakly stained) and for mAb NCL-MHCn (developmental MyHC) (C). Note the presence of developmental MyHC 1 year after fasciotomy indicating on fiber regeneration (C).

**Fiber types and their mitochondrial oxidative capacity**

The expression of MyHC isoforms in fibers was similar 1 year after treatment, with exception that no fibers contained MyHCIIa+IIx in diabetic patients. The mitochondrial NADH-TR activity of muscle fibers was slightly increased in some cases, especially for fibers containing fast MyHCII (Fig 13).

Fig. 13. Muscle cross-sections of the anterior tibial muscle of a diabetic (A, B) and a healthy active patient (C, D) with CECS stained for NADH-TR at fasciotomy and 1 year follow up. Note the lower mitochondria NADH-TR staining activity and more irregular mitochondria distribution at time for fasciotomy (A, C) compared to 1 year follow-up (B, D).
Relative frequency of fiber phenotypes, fiber area and variability in fiber size

The frequency of different fiber phenotypes was, with exception of the lack of MyHCIIa+IIx fibers in those with DM, unchanged 1 year after treatment. The mean fiber area values were larger in patients with CECS than in controls, although the difference was only significant for MyHCIIa fibers in healthy non-diabetic patients (p=0.04). However, in diabetic patients there was a trend to larger MyHCl fibers (p=0.06). After treatment, small-sized fibers (< 100μm²) were more frequent in non-diabetic patients with CECS, but less common in those with DM, reflecting the higher CV values in non-diabetic and lower CV in diabetic patients.

Muscle capillarization

In healthy non-diabetic patients, CD was decreased by 34% 1 year after treatment (p=0.01), but CAF remained at the same level. However, when compensating for differences in fiber size, the CAFA values were 27% lower (p=0.01). CAFA values were lower for all fiber phenotypes (p<0.01), except hybrid MyHCIIa+IIx fibers. In DM, all capillary parameters were lower 1 year after treatment, but significant difference was only found for CAFA (p=0.04).

DISCUSSION

Main findings

CECS of the lower leg is a disorder mainly reported in running athletes (Abramowitz and Schepsis, 1994; Black and Tailor, 1993). This thesis shows that CECS is also associated with intermittent claudication in non-athletes, and particularly in diabetic patients without signs of distal circulatory impairment. In contrast to young physically active individuals with CECS, diabetic patients had higher IMP and got leg disabling pain already after a short walking distance. Fasciotomy gave good results, which is especially important in diabetic patients, since physical activity is crucial for them. The pronounced alterations in the anterior tibial muscle with signs of ischemia in diabetic patients were mainly normalized one year after fasciotomy supporting that the diagnosis really exists in DM.
CECS in diabetes mellitus

This thesis is the first to report of CECS in DM. The typical diabetic patient with CECS is a female with longstanding disease often with other diabetes complications. The long duration of the disease is probably crucial for the development of CECS. CECS also appears to be more frequent in type 1 than in type 2 diabetic patients and the latter in our series were often on insulin treatment implying that they had a more difficult-to-treat disease. The main symptom of CECS, intermittent claudication, is common in diabetic patients and is mostly caused by arterial disease. The symptoms are similar between vascular disease and CECS but clinical examination reveals no signs of circulatory insufficiency in CECS. One reason why CECS has not previously been detected in DM may be that the focus has been on vascular disease. In contrast to healthy active subjects with CECS, the diabetic patients got disabling leg pain already after a short walking distance, often a few hundred meters, and slowly adapted their activity. Most of these patients had been examined for arterial disease, and as the results of this investigation was normal, they were prescribed pain-relieving medication or physiotherapy but the results were poor. The patients had thus reduced their physical activity in order to avoid pain and adapted to the condition especially since the treatment of training only made it worse. Further, the reason to the female preponderance in diabetics as well as in healthy active subjects with CECS is unclear, but it seems to be some predisposition to gender. The majority of CECS patients came from the diabetes care unit at our hospital which is a selection bias since we had informed the care unit about the disorder and urged them to send claudication patients without clear explanation to the symptoms.

The mean age of diabetic patients with CECS was lower than usually reported in diabetic patients with arterial disease or spinal stenosis (Arinzon et al., 2004; Sahli et al., 2005). The walking-induced pain in CECS was situated in the anterior part of the leg in contrast to those with vascular claudication having primarily calf pain (Stewart et al., 2002). The leg pain was easily provoked by 20 heel-raisings although we have not validated this test. The IMP of the anterior compartment in diabetic patients with CECS was higher than in healthy physically active individuals with CECS both at rest and after exercise. The IMP was so high in diabetic patients during the measuring procedure that we feared a development into acute compartment syndrome (Jose et al., 2004). Even at rest the mean IMP was above the values (25-35 mm Hg) reported to reduce capillary perfusion in muscle (Hargens et al., 1981). Spontaneous diabetic muscle infarction has been diagnosed especially in females with multiple diabetic complications (Yildirim and Feldman, 2008); this is similar to the clinical
characteristics in this thesis. Thus, pre-existing CECS may be the origin to development of muscle necrosis.

At surgery the fascia seemed considerably thicker, whitish and stiff in diabetic patients than in healthy athletes with CECS, but our preliminary results of fascia examination indicate great variability and thus more material is needed to verify our assumption. However, the engagement of the whole leg indicates a general affection with connective tissue thickening of similar type seen in stiff-joint syndrome in DM (Smith et al., 2003). Hyperglycemia increases the capillary permeability resulting in edema, thereby increasing the pressure in the compartment (Perin et al., 2007). The patients’ long history of physical inability perhaps initiates a vicious circle resulting in hyperglycemia with subsequent further pathologic changes in capillaries and fascia. The patients had thus reduced their physical activity in order to avoid pain, theoretically with aggravated DM as a consequence. The rating of outcome at one year after antero-lateral fasciotomy was the same as in healthy physically active patients with CECS. However, the activity level differed between the two groups and the most important result was that the vast majority of the diabetic patients were able to walk without restrictions.

**Clinical implications**

It is important to notice that about 50% of the diabetic patients with CECS treated with antero-lateral fasciotomy relapsed after more than 1y with walking-induced leg pain from the medial side. Additional investigations with IMP measurement revealed CECS in the deep posterior compartment. Posterior CECS is reportedly uncommon and even its existence had been questioned (Styf, 2003). However, after additional posterior fasciotomy the exercise-related pain disappeared in nearly all cases. Consequently, 1 year follow-up time was too short.

CECS is assumed to be rather prevalent in DM as our preliminary data indicate that around 5% of patients from a diabetic clinic have CECS. It is, however, curious why muscle necrosis or spontaneous acute compartment syndrome in DM is so rare if according to our assumption CECS is rather common. This may be due to the chronic situation where the muscles have adapted to a lower oxygen metabolism. Our circulatory assessment consists of ankle/brachial index but in DM this index may sometimes be unreliable why toe blood pressure measurements also are performed (Sahli, 2009). When circulatory tests are normal and symptoms and signs are characteristic for CECS, we continue with IMP measurements. If the
intramuscular pressures are normal we extend the investigations further with circulatory and spinal examinations. Diabetic patients with long disease duration have increased risk for postoperative complications. In order to reduce these risks we always operate on one leg and only one of the anterior or posterior compartments at the time. Further, we recommend preoperative preoperative antibiotics to reduce the risk for infection. Suction drainage is used to avoid hematoma, which according to our experience increases the risk for postoperative scaring with subsequent inferior results (Schepsis et al., 2005).

Why does CECS occur in diabetic patients?
Several specific complications to diabetes may contribute to high IMP and ischemia in diabetic patients with CECS. Firstly, the micro-vascular disease might cause an imbalance in vascular exchange and disturbed regulation of blood flow. Leakage of macromolecules and fluid through the endothelium might result in tissue edema that increases IMP (van den Berg et al., 2006; Simionescu, 2007; Yuan et al., 2007). Evidence of compartment syndrome due to capillary leakage is suggested by the fact that patients with a rare but devastating disease, systemic capillary leak syndrome (SCLS), are complicated by plasma leakage into muscle and compartment syndromes (Matsumura et al., 2007; Sanghavi et al., 2006). Moreover, disturbed blood flow regulation due to the inability of arteries and arterioles to dilate appropriately or diabetic autonomic neuropathy involving the vasomotor nervous system might cause disturbed homeostasis (Bakker et al., 2009; Verrotti et al., 2009). Disturbed vascular regulation could also be a result of a defect function or loss of the vascular pericytes that envelopes capillaries and are proposed to be involved in regulation of blood flow, vascular permeability, angiogenesis and endothelial proliferation (Shepro and Morel, 1993; Allt and Lawrenson, 2001; Hammes et al., 2002). Pericyte loss is a hallmark in diabetic retionopathy (Yafai et al., 2004). Diabetic arteriosclerosis might also contribute to a lower capillary perfusion pressure making the muscle blood flow more sensitive for increased IMP. Secondly, an inextensible compartment due to a thicker and/or stiffer muscle fascia secondary to generalized stiffening of connective tissue in DM might also contribute to high IMP (Sternberg et al., 1985; Avery et al., 2009; Aoki et al., 1993). Although an inextensible fascia might be a part of the pathogenesis, several other factors are probably crucial for the development of CECS in DM (see Fig 14).
Muscle alterations in diabetic patients with CECS

The pronounced and widespread histopathological changes in the lower legs of diabetics with CECS are probably a consequence of local circulatory impairment and to some extent of a general neuropathy. Diabetes micro-vascular dysfunction may cause neuropathy (Nathan, 1993; Marshall and Flyvbjerg, 2006; van den Berg et al., 2006; Bakker et al., 2009) followed by atrophy and progressively reduced muscle strength in lower legs (Andersen et al., 1997; Bus et al., 2002; Andersen et al., 2004a; Greenman et al., 2005). Consequently, neuropathy might explain some of the histopathological muscle changes in diabetic patients with CECS, but the often considerably increased IMP suggests that local ischemia has the main detrimental effect on muscles. The clusters of necrotic fibers and infiltration of inflammatory cells as well as fibers with mitochondrial disorganization is probably a direct consequence of circulatory impairment (Dubowitz, 2007; Heffner and Barron, 1978; Larsson et al., 1990), although chronic low-grade inflammation is also reported to be associated with the disease. A secondary effect of high IMP and ischemia might be local neuropathy due to compression of capillaries supporting blood flow to nerves. The clusters of atrophic fibers, fascicular atrophy and high number of small-sized fibers support motor nerve damage. The high frequency of large sized fibers is probably derived from frequent activation of some motor units to uphold the muscle function in diabetic patients with CECS. However, there were also signs of a parallel process of repair and regeneration. The presence of fiber type grouping indicates that sprouts of adjacent intact motor axons have re-innervated neighboring denervated fibers (Morris and Raybould, 1971). Other signs of regeneration and repair were an increased frequency of fibers stained for developmental MyHCs, a high number of split fibers and fibers with increase number of internal nuclei (Eriksson et al., 2006). The mechanism behind fiber split is unclear, but fusion of activated and multiplying satellite cells is supposed to cause the formation of branched fibers or they may develop secondary to defect regeneration after segmental muscle fiber damage (Eriksson et al., 2006). The internal myonuclei may come from longstanding degeneration and regeneration resulting in enclosure of these within the fibers after fusion of regenerating myocytes and myotubes (Schmalbruch, 1985). In contrast to healthy patients with CECS, capillary supply of diabetic muscle was more similar to normal controls, although there was a trend towards lower microvascularization. The more advanced histopathological changes, but decreased alterations in capillary supply than in healthy active with CECS, suggest differences in pathogenesis and emphasize vascular complication as an important factor in development of CECS in diabetic patients.
The normalization of muscle after fasciotomy was especially prominent in DM, although abnormalities were still present after 1 year. The healing of the muscles after decompression together with clinical improvement supports the diagnosis CECS in DM. Some of the remaining pathological alterations in the tibialis anterior muscle are probably complications to a general diabetic myopathy and neuropathy. Nevertheless, the normalization of muscle morphology together with clinical improvement with unrestricted walking ability in most cases is of vital importance since physical activity, as previously mentioned, is a vital part of the therapy in DM.

**CECS in non-diabetic patients**

Traditionally, CECS of the lower leg has been described in physically active young individuals probably due to the fact that the first reported CECS cases were athletes. In contrast to almost all previous studies (Detmer et al., 1985; Englund 2005), we found that even less physically active middle-aged subjects may get lower leg symptoms diagnosed as CECS. One reason is perhaps that general practitioners and internists rarely meet patients with compartment syndromes. Thus, CECS should be considered as a differential diagnosis to exertional leg pain even in walkers. About half of the patients referred to the orthopaedic clinic suspected for CECS of the lower leg were verified having CECS. Compared with other studies (Quarford et al, 1983; Bong et al., 2005), this is a high proportion probably due to some selection bias since many of the patients were sent from the sports clinic and the general practitioners were informed of the diagnosis before the start of the survey. Even today many patients are treated for CECS without IMP measurement. This is not correct since the unspecific symptoms may be similar to other disorders. We have routinely used the micro-infusion method for muscle pressure measurements for many years and are aware of the sources of error. It should be remembered that the posterior compartment pressure measurement is more difficult to perform and that the values are less reliable (Allen & Barnes, 1986; van Zoest et al., 2008).

CECS has different etiologies. In our series about one third of the cases had a history of trauma long before the symptoms appeared. Trauma has been earlier proposed to promote development of CECS (Tubb and Vermillion, 2001) but we had a large proportion of these cases. It is also important to notice that the results after fasciotomy of those with history of trauma were inferior to others probably due to posttraumatic soft-tissue alterations. Thus, it is important for the surgeon to inform these patients about the prognosis before surgery.
Pathophysiologic theories to CECS in non-diabetic patients

The pathophysiology to CECS in healthy physically active individuals is unclear, but as proposed in DM, an abnormally increased IMP during exercise impedes muscle blood flow causing ischemia and pain (Blackman, 2000; Styf, 1987; 2003). Although the mechanisms are not well-understood, anatomical tight compartments of lower legs, inextensible fascia and muscle hypertrophy can all be hypothesized to be involved in the detrimental increased of IMP during exercise (Turnipseed et al., 1989). A high IMP will compress the capillaries and stop blood flow just above the intra-capillary pressure (25-35 mmHg) in the muscle (Murabak et al., 1978; Hargens et al., 1981). High IMP levels will consequently lead to ischemia and pain. Prolonged periods of ischemia give endothelial swelling, increased vascular permeability, interstitial edema, nerve damage and fiber necrosis (Menger et al., 1997; Blackman, 2000; Blaisdell, 2002). The observed histopathological alterations suggest that IMP and ischemia in some patients reach levels high enough to damage muscle and nerves. Moreover, a low muscle microvascularization might be a part of the etiology to CECS, as indicated by lower capillary supply in healthy active subjects with CECS. The capillary blood flow starts to be blocked in capillaries already at a compartment pressure of 15 mmHg (Hartsock et al., 1998). A muscle with low capillary supply might therefore be susceptible for increased IMP by reaching critical levels of insufficient blood supply of muscle and nerve tissue during exercise. A low structural capacity for blood flow is supported by a higher degree of relative de-oxygenation during as well as delayed re-oxygenation after exercise (Mohler et al., 1997; van den Brand et al., 2004), along with a slower recovery of voluntary force, and slower return of muscle volume towards normal after exercise (Birtles et al., 2003). There are factors pointing towards a constitutional cause to low muscle microvascularization in healthy physical active with CECS. It is well-known that inactivity lowers the number of capillaries, decreases oxidative metabolism and fiber size, and alters fiber type composition (Borisov et al., 2000). However, decreased physical activity seems not to be the major cause to low microvascular supply since all healthy patients in this study reported a relatively normal or even high physical activity before treatment, and the muscles contained no alterations in fiber size or fiber phenotype composition compared to normal controls. Moreover, the capillary network was still low one year after treatment when the physical activity of patients had increased, which was supported by the increased size of fast fibers expressing MyHCIIa. The restricted capillary network together with lack of neovascularization after treatment may reflect constitutional differences or a deficiency in
specific angiogenic factors regulating the interaction between muscle fibers and the vascular bed.

The improvement in physical activity one year after fasciotomy was reflected by the normalization of histopathological abnormalities, increased fiber size and increased proportion of hybrid fibers, i.e. fibers containing multiple MyHC isoforms. The presence of developmental MyHCs implies that decompression triggers muscle regeneration and repair (Schiaffino et al., 1986). In adults, small fibers expressing developmental MyHC have been associated with activated satellite cells involved in the process of fiber repair or formation of new fibers after muscle damage (Kadi et al., 1999; Bischoff, 1994). Although fasciotomy induces a healing process in muscle, the low capillary supply and absence of neovascularization after treatment indicate low micro-vascularization as a pathogenic factor in healthy active subjects with CECS.
SUMMARY
CECS of the lower leg occurs in middle-aged adults with low or moderate physical activity. Previous trauma seems to be an etiologic factor for development of CECS. Importantly, this thesis shows that CECS has to be considered as a novel differential diagnosis in diabetic patients with symptoms of claudication without signs of vascular disease. Diabetic patients with CECS got disabling leg pain after a much shorter walking distance, compared to healthy non-diabetic patients with CECS, and the IMP was higher both at rest and after exercise. The typical patient was a female with longstanding diabetes often with other diabetic complications. Fasciotomy gave good results with unrestricted walking ability in majority of cases. Low ability for physical activity, reflected by the signs of both myopathy and neuropathy, indicates that high IMP and circulatory impairment has deleterious effects for the involved muscles. Healthy physical active patients with CECS of the anterior compartment of the lower leg seem to have a low structural capacity for muscle blood flow suggesting low microvascularization as an integral part of the pathogenesis. The more severe clinical and histopathological findings in diabetic patients than healthy subjects with CECS indicate differences in the pathogenesis and emphasize vascular complication as an important factor in development of CECS in DM. Increased physical activity and normalization of muscle morphology one year after treatment showed the benefit of fasciotomy. The unrestricted physical ability in majority of patients treated for CECS is very important for diabetic patients, since physical activity is an important part of the therapy of the disease. The symptoms and signs are characteristic if searched for, and the treatment is simple. Before treatment, IMP measurements should always be done to verify the diagnosis.

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