Institute for Experimental Medical Research
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Fatigue mechanisms and skeletal muscle function in experimental and human heart failure

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1 Abbreviations

ACEi – angiotensin converting enzyme inhibitor
AMPK – AMP activated kinase
AP – action potential
ATII – angiotensin II
CaMKII – calmodulin dependent protein kinase II
cAMP – cyclic AMP
CHF – chronic heart failure
CICR – calcium induced calcium release
CK – creatine kinase
CO – cardiac output
CRP – C-reactive protein
CS – citrate synthase
EC-coupling – excitation contraction coupling
ECM – extra cellular matrix
EF – left ventricular ejection fraction
HADH – 3-hydroxy acyl-CoA-dehydrogenase
HF – heart failure
IL – interleukin
LD – lactate dehydrogenase
EDP – left ventricular end diastolic pressure
MDH – malate dehydrogenase
MI – myocardial infarction
MLC – myosin light chain
MLCK – myosin light chain kinase
MMP – matrix metalloproteinases
MyBP-C – myosin binding protein C
NKA – Na⁺-K⁺-ATPase
NCX – Na⁺/Ca²⁺-exchanger
NF-κB – nuclear factor-kappa B
NO – nitric oxide
PGC-1α – proliferator activated receptor gamma co-activator 1α
Pi – inorganic phosphate
PKA – protein kinase A
PKC – protein kinase C
PLB – phospholamban
PPARδ – peroxisome proliferator-activated receptor δ
RAAS – renin angiotensin aldosterone system
RBC – red blood cell
RyR – ryanodin receptor
Ser – serine
SERCA – sarcoplasmic reticulum Ca²⁺ ATPase
SOL – soleus muscle
SR – sarcoplasmic reticulum
Thr – threonine
TnC – troponin C
TnI – troponin I
TnT – troponin T
VO₂max – maximal oxygen uptake
2 List of papers

Paper 1 (accepted)

Causes of fatigue in slow twitch rat skeletal muscle during dynamic activity


Paper 2 (submitted)

Attenuated fatigue in slow twitch skeletal muscle during isotonic exercise in rats with chronic heart failure

Morten Munkvik, PK Lunde, M Aronsen, JAK Birkeland, I Sjaastad, OM Sejersted

Paper 3 (in manus)

Skeletal muscle fatigue and trainability in heart failure; Background and design of the TRUST Study

TA Rehn, Morten Munkvik, G Slettaløkken, B Rud, PK Lunde, OM Sejersted, J Hallén, I Sjaastad

Paper 4 (in revision)

Training effects on skeletal muscle calcium handling in human chronic heart failure

Morten Munkvik, TA Rehn, G Slettaløkken, A Hasic, J Hallén, I Sjaastad, OM Sejersted, PK Lunde
3 Introduction

3.1 Muscle structure and function

3.1.1 Muscle structure

There are three types of muscle tissue in the human body: smooth, cardiac and skeletal muscle. Cardiac and skeletal muscle are commonly known as types of striated muscle because the strict organization of myofilaments in these muscles results in a characteristic striated pattern easily detectable through a standard light microscope. Smooth muscle lacks this striation and is found in several internal organs. Cardiac muscle is only found in the heart. Skeletal muscles move joints - giving rise to movement- and are voluntarily controlled. The remainder of this chapter will deal exclusively with skeletal muscle.

During the prenatal stage, primitive myoblasts merge together, forming muscle cells that are distinguished from most other cells by having several nuclei per cell. In skeletal muscle, the nuclei are peripherally located within the cells. There are 600-650 skeletal muscles in the human body, each consisting of bundles of roughly cylindrical muscle cells. Transverse tubules (t-tubules) are deep invaginations of the plasma membrane. These were described in a scientific paper as early as 1902, but the paper was not translated from Italian to English until 1961 (390). On either side of the t-tubules are the terminal sacs of the sarcoplasmic reticulum (SR), called terminal cisternae. A t-tubule and two flanking terminal cisternae (about 12 nm from the tubules) constitute the triad.

Most of the cell volume consists of myofibrils, which are cylindrical protein networks with a repeating pattern of dark and light bands. Dark bands are called A bands and the light bands are named I bands. The smallest repeating unit in the myofibril is the sarcomere. Although a complex framework of proteins makes up the sarcomere, the principle elements are the two myofilaments actin, a thin filament, and myosin, a thick filament (Figure 1).
**Thin filament associated proteins**

Actin (43 kDa) is a spherical molecule spun helically around the 6-900 kDa heavy protein nebulin. Neighboring actin monomers interact via subdomain 3 and 4, and one helical turn compromises 14 actin monomers repeating every 36 nm (16, 63). This thin filament starts at the Z-disc (Figure 1) and runs toward the M-line. The length of this filament is about 1 μm. Nebulin and other proteins such as tropomodulin and CapZ (63) are important in defining thin filament length.

**Figure 1. Selected proteins of the sarcomere.**

See text for details

Tropomyosin (TM) is a 66 kDa polypeptide located in the groove between the actin filaments, and was previously considered a backbone of the thin filament (182). TM is
associated with three interacting subunits: the troponins (Tn). Troponin C (TnC) is an 18 kDa Ca\(^{2+}\) binding subunit, while Troponin I (TnI) is a 21 kDa polypeptide that inhibits the actomyosin ATPase in a Ca\(^{2+}\)-insensitive manner. The last subunit (Troponin T, TnT) has a molecular weight of 33 kDa and binds the other subunits to TM. The stoichiometry of TM, TnC, TnI and TnT is 1:1:1:1 (147, 148) and the complex repeats at every seventh actin monomer (1) along the thin filament. Neighboring TM overlaps from head-to-tail along the filament. Given that TM covers the binding site for myosin on actin, it became clear early on that TM/troponin could exert some control over contractile function (157). Interactions between TM, Tn subunits and actin are Ca\(^{2+}\) sensitive. As Ca\(^{2+}\) binds to TnC, the other subunits bind tighter to TnC and weaken the TnI-actin interaction (164). Additionally, TM moves azimuthally by about 25° along the actin surface. The precise amount of movement varies in different publications, ranging from 0° of movement to 60°. For illustrative reconstructions, see Xu et al. (412).

**Thick filament associated molecules**

Myosin is composed of two heavy chains (MHC) and four light chains (MLC). The two heavy chains (220 kDa each) are identical, and make up the head (subfragment 1, S1) and tail (S2) of the molecule. The C-terminal end of the tail is referred to as light meromyosin (LMM), and is essential for myosin polymerization. S2 connects the myosin head to the thick filament backbone, while S1 has ATPase activity that is activated upon binding to actin. Herein lays the basis for the sliding filament theory of muscle contraction, which will be described in more detail in section 3.1.3. There are two essential (ELC, “alkali” or MLC1) and two regulatory (RLC or MLC2) light chains, each about 20 kDa. These are located near the neck of the MHC molecule (Figure 1). A giant protein, titin (3-4 MDa), is thought to be a ruler for sarcomere architecture, as it runs through the whole length of a half sarcomere, from Z-disc to M-line (63). Myosin binds to titin in a helical fashion so that two of these molecules are organized at an angle of 120 degrees to each other with three myosin molecules every 14.3 nm (261). Consequently, there will be 43 nm between myosin heads projecting in the
same direction. With antibody labeling, seven to nine stripes, also with 43 nm spacing, are visible across the A band. These were named C-protein by Offer et al. (281), but later became known as myosin binding protein – C (MyBP-C, 140 kDa). Separate genes encode three isoforms of MyBP-C in adult muscle (387): slow (previously known as MyBP-X), fast skeletal, and cardiac isoforms. There are most likely three MyBP-C molecules per stripe in skeletal muscle (266). These are not always the same isoform (94). The C-terminal part of MyBP-C has both an LMM and a titin binding site and could thus be important in linking myosin to titin. Together with another myosin binding protein (MyBP-H), MyBP-C seems to be important in regulating thick filament assembly and length (35). In vertebrae, this length is set, rather precisely, to 1.6 μm. The cardiac isoform of MyBP-C can regulate cardiac muscle contraction (406), but the role of skeletal muscle MyBP-C remains unknown.

**Skeletal muscle fiber type**

In 1960, researchers histochemically identified (103) two different types of muscle fiber that displayed reciprocal activities of oxidative and glycolytic enzymes. The oxidative fibers were called type I fibers. They had a low level of myosin ATPase activity, while type II fibers (glycolytic) had high myosin ATPase activity. The type I fibers are recruited at lower exercise intensities, but at about 30% of VO$_{2\text{max}}$ the type II fibers are also activated (139). There are subgroups of type II fibers. Importantly, the different fiber types constitute important differences regarding other parameters such as fatigability (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the different fiber types</th>
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<tr>
<td><strong>Type</strong></td>
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<tr>
<td>Contraction time</td>
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<tr>
<td>Force production</td>
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<tr>
<td>Mitochondrial density</td>
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<tr>
<td>Capillary density</td>
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<td>Myoglobin density</td>
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<td>Oxidative capacity</td>
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<td>Glycolytic capacity</td>
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<td>Major storage fuel</td>
</tr>
<tr>
<td>Type of activity</td>
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<td>Fatigue resistance</td>
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* Fast twitch fibers contain 15-20% more CrP than slow twitch fibers (345)
3.1.2 Calcium handling

The sarcoplasmic reticulum (SR) is the most important calcium store in skeletal muscle. The \([\text{Ca}^{2+}]_{\text{SR}}\) has been measured to 1.5 mM (60), while the cytosolic concentration (\([\text{Ca}^{2+}]_i\)) in a resting muscle fiber ranges from 100 to 250 nM (27). The concentration gradient over the SR membrane is therefore four orders of magnitude. Since the mid-1900s, it has been known that intracellular \(\text{Ca}^{2+}\) initiate skeletal muscle contractions (165, 170). It is therefore an important condition for the normal function of the muscle fibers that the cytosolic \(\text{Ca}^{2+}\) levels are tightly regulated. In resting conditions there are three main active transport mechanisms that keep \([\text{Ca}^{2+}]_i\) at low levels:

1) The SR ATP-dependent \(\text{Ca}^{2+}\) pump (SERCA). This single 110 kDa polypeptide spans the SR membrane 10 times (Reviewed by Inesi and Kirtley (186)), and is coded for by three different genes giving three isoforms (411). SERCA1 is exclusively found in fast twitch skeletal muscle, SERCA1a in adult and SERCA1b in neonatal cells. SERCA2 is present in all tissues, SERCA2a only in muscle (slow twitch fibers) and SERCA2b in smooth muscle and all non-muscular tissue. SERCA3 is present in many tissues but not in muscle tissue, except transiently and together with SERCA2a in early heart development (18). Two \(\text{Ca}^{2+}\) ions are transported over the membrane per ATP hydrolyzed (140). It has a high affinity for \(\text{Ca}^{2+}\) (\(K_D \approx 0.5 \mu\text{M}\)). In cardiac and slow twitch skeletal muscle (not in rat cases (84)), SERCA2 is regulated by phospholamban (PLB), which inhibits the pump by reducing the \(\text{Ca}^{2+}\) affinity (337). PLB can be phosphorylated by protein kinase A or CaM kinase at Ser16 and Thr17 respectively. Phosphorylation will cause phospholamban to dissociate from SERCA2, thereby eliminating the inhibition. SERCA function is also regulated by Sarcolipin (280), but since this protein is mainly active in cardiac atrial cells (288) it will not be discussed further.

2) The plasmalemmal \(\text{Ca}^{2+}\) pump (PMCA). This pump comes in four different isoforms. PMCA1, 3 and 4 have been demonstrated in mammalian skeletal muscle (287). It pumps one \(\text{Ca}^{2+}\) over the membrane per ATP hydrolyzed, and is believed to regulate \(\text{Ca}^{2+}\) in the gap between the T-tubule and terminal cisternae of SR where the concentration of \(\text{Ca}^{2+}\) can be
large (mM range) (36). Compared to SERCA, PMCA has a much lower affinity to Ca$^{2+}$
($K_D \approx 0.1$ mM), but in the presence of Ca$^{2+}$:calmodulin, the affinity to Ca$^{2+}$ is increased toward
SERCA levels, implying boosted activity at elevated [Ca$^{2+}$] (140).

3) The Na$^+/Ca^{2+}$ exchanger (NCX). There are three isoforms of the exchanger, NCX1, 2
and 3, with NCX2 and 3 restricted to the brain and skeletal muscle (301). NCX exchanges 1
Ca$^{2+}$ per 3 Na$^+$ ions and hence is electrogenic. Depending on the membrane voltage, NCX
can move Ca$^{2+}$ either out of or into (“reverse mode”) the cell. The physiological role for NCX
is different in the different skeletal muscle fiber types (214), and it has been speculated that
the exchanger is involved in Ca$^{2+}$ extrusion in slow twitch fiber, but not in fast twitch fibers
(41).

![Figure 2. Selected proteins involved in Ca$^{2+}$ handling in skeletal muscle.](image)

Some proteins important in setting membrane potential are also outlined. See text for details.
Also worth mentioning here is the 12 kDa protein parvalbumin (PA). PA is found only in cytosol of fast twitch skeletal muscle (285) and binds two Ca\(^{2+}\) with high affinity. There is a positive correlation between PA and relaxation rate (175), indicating that increased concentration of the protein could increase the rate of relaxation in contracting fast twitch muscle fibers.

The mitochondria (274) and the cell nucleus (201) can take up and release Ca\(^{2+}\), but it is not known whether these stores contribute to the cycling of calcium during muscle contraction and relaxation.

**Calcium in the sarcoplasmic reticulum**

Calsequestrin (Csq) is located inside the terminal cisternae (283), probably positioned here by either triadin, junctin, or both (57, 188). These are anchoring systems that could link Csq to important Ca\(^{2+}\) handling proteins (DHPR and RyR, discussed later). Csq has a high capacity for low affinity Ca\(^{2+}\) binding (36). Most of the Ca\(^{2+}\) buffering capacity of SR is due to Csq (234). Of the total calcium in SR (43.75 mM) only about 3.5% (1.5 mM) is in free form (140). Recent publications suggest that Csq can be phosphorylated. This both enhances Ca\(^{2+}\) binding capacity and promotes the association with junctin (28). The level of Ca\(^{2+}\) in SR is higher in fast twitch than in slow twitch fibers (230). This could explain some of the differences we see between the two main types of skeletal muscle (Table 1).

**Release of Ca\(^{2+}\) from SR**

At excitation of the muscle cell membrane, sodium flows into the cell through specific channels. In turn, sodium influx alters membrane potential, which is sensed by voltage sensors in the t-tubule (325). These sensors are specialized L-type Ca\(^{2+}\) channels called dihydropyridine receptors (DHPR). It is believed that they are mechanically coupled with the ryanodine receptor (RyR) in a strictly ordered fashion (36, 135), distinguishing the skeletal from cardiac muscle, where there is no such connection (140). At first this mechanical link
was hypothesized only on a functional basis (325) but later also confirmed experimentally (267).

There are three RyR isoforms, where RyR1 is the dominant in skeletal muscle. A low amount of RyR3 is also detected in slow twitch skeletal muscle (73). The RyR density is higher in fast fibers (83). Each RyR consists of four 565 kDa subunits and 50% of these homotetramers are located in close proximity to the DHPR (134, 300). This arrangement was nicely illustrated by Block et al as early as in 1988 (43). Ca²⁺ itself affects RyR in a dose-dependent fashion, so that low concentrations activate the channel and higher concentrations inhibit it. Ca²⁺ free calmodulin (CAM) activates and Ca:CAM inhibits RyR (311). Calstabin1 (FKBP12) binds to RyR in a stoichiometry of four to one (382) and stabilizes the closed state of the channel (50), while PKA phosphorylation of RyR dissociates calstabin1 from the channel and increases activity (309). PKA is anchored to RyR via mAKAP (309), together with the phosphodiesterase PDE4D3 that degrades cAMP locally and thereby regulates RyR activity (212). Another anchoring protein, spinophilin, links PP1 to the release channel (33). PP1 dephosphorylates RyR (314).

3.1.3 Cross-bridge theory and force generation

History

Before the sliding filament hypothesis was put forward independently by two groups in the 1950s (156, 179, 181), it was believed that folding or coiling of long protein filaments formed the basis for muscle contraction. Both lactic acid and CrP were thought to bring about these conformational changes. The idea that myosin crosses bridges to actin, and undergoes a conformational change before it “rows” on actin, was hypothesized in 1969 (183). Although modified several times, this hypothesis is now more or less uniformly accepted as the model for skeletal muscle force production, even though several problems with the theory exist (178). It is apparent from the literature that the multiple steps and different configurations of myosin and actin involved in the force-producing process are hypothetical, but several researchers have tried to create a simplified scheme of what are thought to be the most
important steps in the cycle. The original model was proposed in 1971 (232) and has been modified several times (55, 74, 75, 305). A simplified version of the different steps that make up the cross-bridge cycle theory will be discussed in the next section. See Figure 3, or reviews (74, 75, 143).

Figure 3. Simplified view of the cross-bridge cycle.
M – Myosin, A- Actin. Pi – Inorganic phosphate. Blue and red colored circle denotes low and high force generation configurations of the myofilaments, respectively. See text for details.

Hydrolysis of ATP (1, Figure 3)
A premise for myosin and actin interaction is the presence of Ca^{2+}. When this ion binds to TnC, TM uncovers the myosin binding site on actin. Hydrolysis of ATP bound to myosin to form M·ADP·Pi is favored by a factor of 10 compared to if ATP is not bound to myosin. The complex both before (M·ATP) and after (M·ADP·Pi) hydrolysis forms weak bonds to actin.
(A·M·ATP and A·M·ADP·Pi). Importantly, hydrolysis is associated with a movement of the myosin neck region, so that the configuration returns to the pre-power stroke configuration. The ATP cleavage is 30 times faster when myosin is not bound to actin, compared to if myosin is bound to actin (399), favoring rearrangement of the myosin molecule in the unbound state.

**Release of P<sub>i</sub> (2, Figure 3)**

The rate of P<sub>i</sub> release is 50 times faster if released from A·M·ADP·P<sub>i</sub> compared to M·ADP·P<sub>i</sub>. This is important because the release of P<sub>i</sub> causes a strong cross bridge to form between actin and myosin (168), and favors a high force generation at the same time (173, 284). To complicate matters, there are reports of strong actin binding prior to P<sub>i</sub> release due to isomerization, and that the release of P<sub>i</sub> stabilizes this binding (85, 257). Isomerization is probably regulated by Ca<sup>2+</sup> (233). Various studies appear to agree that after release of P<sub>i</sub> the actin myosin interaction is high force generating (A·M·ADP), and that this step is rate limiting in the myofibrillar ATPase cycle (223). The high force states are thought to be dominant during isometric contraction, while only 5% of cycle time has cross-bridges in this configuration during isotonic shortening (367).

**The power stroke (3, Figure 3)**

The power stroke probably occurs over several steps, with the neck region of myosin operating as a lever arm (227, 381). The neck region changes its angle about 70° (99, 174) during the power stroke. As ADP is cleaved off, the binding between actin and myosin (A·M) generates even higher force, called the rigor state (74). The shortening velocity of the muscle is highly dependent on the ADP release (336).

**ATP binding of myosin (4, Figure 3)**

The affinity of myosin for ATP is even higher than the affinity for actin. Therefore myosin dissociates from actin and binds to this nucleotide (M·ATP). If Ca<sup>2+</sup> is still present, the contractile apparatus can repeat the cycle in Figure 3. If not, TM will block the myosin binding
site on actin, and filament sliding will stop. In addition to Ca\(^{2+}\), also ATP needs to be available. The effect of one cross-bridge cycle is hydrolysis of one ATP molecule and displacement of thick and thin filament relative to each other, about 10 nm (130, 184).

**Elastic components contribute to force production?**

An elastic component of the cross-bridge was hypothesized over 50 years ago by A.F. Huxley (180). Such an elastic component also provides an explanation for some experimental data (130). Following from the previous section, myosin can be regarded as an active force generator. Elastic elements are passive force generators (209). Elasticity could be a property of the myosin molecule itself (131, 185). It could also be part of other sarcomere proteins, like titin (222, 258), but the alternatives are not mutually exclusive. How these elastic, springlike qualities of the thick filament behave during a cross-bridge cycle, and how much they contribute to force production, is not fully understood.

### 3.2 Integrated view of the normal skeletal muscle function

Contraction of skeletal muscle is triggered by activity in somatic neurons, ultimately depolarizing the skeletal muscle cell membrane. This leads to a conformational change in DHPR that induces an opening of RyR. Ca\(^{2+}\) is released from the SR through the open RyR and the cytosolic concentration rises sharply. Ca\(^{2+}\) binds to TnC and cross-bridge formation and force production can occur.

The amount of Ca\(^{2+}\) that enters cytosol upon stimulation is dependent on the stimulation frequency in the motoneuron. A high stimulation frequency will allow more Ca\(^{2+}\) to enter cytosol and consequently more extensively activate the myofilaments, compared to a lower frequency, where only a limited amount of Ca\(^{2+}\) will enter cytosol. As a result, the skeletal muscle force production will be higher at higher stimulation frequencies in comparison to lower.
As the motoneuron activity terminates, the DHPR returns to the resting configuration and RyR closes. Ca\(^{2+}\) dissociates from TnC due to removal of Ca\(^{2+}\) from cytosol, and TM blocks further attachment of myosin to actin. Due to this, the force generation drops.

Several consequences of muscle activity need immediate attention both during and after muscle stimulation. Firstly, Ca\(^{2+}\) needs to be removed from cytosol. This is done mainly by SERCA. Further, the balance of ions over the cell membrane must be reestablished. This is primarily taken care of by the NKA. Next, the amount of energy that was used (foremost to remove Ca\(^{2+}\) from cytosol and by movement of myosin heads) needs to be rebuilt. Different muscle fiber types differ as to which cellular machinery is preferred in this process (Table 1). Lastly, products from skeletal muscle metabolism need to be removed, and potential alterations in proteins need to be reversed.

During activity, a lag or dysfunction in any of the processes mentioned above can potentially limit skeletal muscle function and cause skeletal muscle fatigue. This will be discussed further in the following sections.

### 3.3 Skeletal muscle fatigue

The transition from rest to exercise increases energy demand more than 100-fold (317). Depending on the intensity, nature and duration of the exercise, the skeletal muscle will have decreased function after exercise. The common perturbations in muscle performance include reduction in maximal force, shortening velocity and power and prolongation of relaxation. This phenomenon is known as fatigue and have been reviewed numerous times (11-14, 75, 125, 126). Skeletal muscle fatigue has been most recently defined as “any decline in muscle performance associated with muscle activity” (13). Muscular fatigue is reversible by rest (2), which distinguishes fatigue from muscle weakness or damage. The causes of these changes can be many, and the relative importance of particular factors remains controversial. The field is in constant flux as old, well-known factors seem to lose significance and other possible candidates enter the field. The picture is additionally obscured by the fact that
different kinds of exercise (i.e. high intensity vs. low intensity, isometric vs. dynamic/isotonic) will influence different types of muscle fiber in varying ways. Thus, there is no clear cut picture which can elucidate all aspects of skeletal muscle fatigue.

**Figure 4. Potential sites of central (1-4) and peripheral (5-8) fatigue.**


Bigland-Ritchie hypothesized 8 potential sites of fatigue (39). These are illustrated in Figure 4. Central fatigue (Figure 4, 1-4) denotes a nervous basis for the muscle fatigue. The highly motivated athlete will be less susceptible to central fatigue compared to a jogger on an exercise run (120). Most experimental studies investigate peripheral fatigue (Figure 4, 5-8) and often subdivide these steps further, as Fitts and Metzger (127) do, for example. Only peripheral fatigue will be dealt with in the following and central fatigue will not be described further. Some of the assumed causes of fatigue will be briefly described in the following.
3.3.1 Alterations in electrolytes and Ca\(^{2+}\) handling

Associated with the action potential (AP) is a Na\(^{+}\) current into the muscle cell and a K\(^{+}\) current out. Repeated activation will lead to a net K\(^{+}\) efflux from the cell (64, 172), increasing the extracellular [K\(^{+}\)] from 4 mM in resting conditions to 9 mM in high intensity working muscles, possibly reaching 10 mM in localized regions (329). The K\(^{+}\) accumulation is probably higher in the t-tubules because the membrane surface is large and combined with a small volume (13). The shift in electrolyte balance over the sarcolemma alters membrane polarization, which could cause the DHPR to become unresponsive (7). During activity magnesium concentration could rise from 1 to about 2 mM (13). This could have implications on Ca\(^{2+}\) release rate from SR (see next section).

Both high H\(^{+}\) and P\(_{i}\) reduce maximal isometric force (76). The effect could have impact on the number of cross-bridges in the high force state or on the force per cross-bridge. It has also been shown that P\(_{i}\) decreases myofilament Ca\(^{2+}\) sensitivity (256). H\(^{+}\) was also a candidate for reducing Ca\(^{2+}\) sensitivity by competing with Ca\(^{2+}\) on binding to TnC (40), but the Ca\(^{2+}\) affinity to SERCA is also reduced at lower pH. In sum, elevated H\(^{+}\) probably increases the amount of Ca\(^{2+}\) available for TnC binding (13). Westerblad et al (13) argues that the modest increase in tetanic [Ca\(^{2+}\)]\(_{i}\) seen at the early phase of fatigue could be due to the effects P\(_{i}\) has on RyR and SERCA. Elevated P\(_{i}\) increases SR Ca\(^{2+}\) release (22) through RyR and inhibits (355) or even reverses Ca\(^{2+}\) pumping by SERCA (105). It is, however, not completely clear what effect P\(_{i}\) will have on RyR as an inhibitory effect that is larger at higher [Mg\(^{2+}\)] is also reported (106). The proposed precipitation of Ca\(^{2+}\)-P\(_{i}\) in SR during later stages of fatigue is controversial (109). If this occurs, the pool of releasable Ca\(^{2+}\) in SR will decrease and contribute to the reduction in force.

3.3.2 Alterations in metabolites

ATP is utilized (ATP → ADP + P\(_{i}\)) mainly by the myosin ATPase and SERCA during muscle stimulation. The creatine kinase reaction (CrP + ADP ↔ ATP + Cr) restores the ATP level. Nevertheless, during intense fatigue ATP declines from 7 to 1.2 mM and CrP from 30
to 2.5 mM. The decrease in CrP is matched by a stoichiometric increase in $P_i$ (205), so this metabolite can increase from about 2 to 30 mM, while ADP increases from 10 to 200 μM (13). Intracellular lactate concentration may reach 30 mM (316) from resting values of about 2 mM. Intracellular pH can get as low as 6.2 from 7.05 in resting muscle (405), but a typical pH change in marked fatigue is 0.5 units (88).

ADP will increase force but slow down velocity by impeding the detachment of cross-bridges (77, 86) (see Figure 3). Acidic pH lowers Ca$^{2+}$ affinity of SERCA contributing to elevated [Ca$^{2+}$], (409) that also could be important in explaining prolongation of the relaxation commonly seen in fatigued muscle. [Mg$^{2+}$] in the mM range will decrease Ca$^{2+}$ release from SR (253), but the pump is most inhibited by combining increased [Mg$^{2+}$] and [ADP] with reduced [ATP] (42, 110). Lower pH will also reduce velocity (255), as ADP does, but the molecular basis for this effect has not been established.

### 3.3.3 Reactive Oxygen Species

Active muscle produces ROS (97, 307) and a number of studies have suggested that elevated concentrations of ROS contributes to fatigue. There is no consensus about the major sources of ROS production (13) but one presumable contributor is mitochondria that produce superoxide relative to O$_2$ consumption (350). Activity can boost O$_2$ consumption 100-fold (61) and thereby produce ROS quite extensively. Further, a blockade of the mitochondria electron transport chain reduces superoxide production to zero (384), substantiating the mitochondria’s position as ROS producers during activity. Experiments done were skeletal muscle are exposed to ROS or ROS scavengers suggest that ROS has an effect on force production, Ca$^{2+}$ sensitivity and Ca$^{2+}$ handling (264, 296, 322), but the main effect of ROS on skeletal muscle is still unclear.
3.3.4 Posttranslational modifications

Myosin Light Chain

Modulations of the structural components of the sarcomere are not part of the battery of cellular changes during activity that traditionally are thought to contribute to the development of fatigue. Over the recent years a growing body of evidence points to regulation of myosin, or more specifically phosphorylation of MLC (mandatory for contraction in smooth muscle (161, 190)), as important in regulating contractions as well as in skeletal muscle. In particular, it is proposed that MLC phosphorylation explains twitch tension potentiation (358), especially since mice lacking MLCK only have a tiny potentiation (417). Phosphorylation of MLC moves the myosin head closer to actin (366), increasing both force and contraction and relaxation rates (146). Because it has been reported that MLC phosphorylation increases 50% after sustained activity (366), and during fatigue can rise even more (385), it is hypothesized that MLC phosphorylation could be of importance in fatigue. Recently, Cooke et al found that MLC phosphorylation inhibits contraction velocity in conditions that mimic fatigue (133, 192). It seems, however, that MLC modifications play a greater physiological role in fast twitch skeletal muscle than in slow (80, 265), maybe due to lower MLCK and greater phosphatase activity in slow isoform (265). Also what happens to MLC phosphorylation status during shortening contractions is not known.

Myosin binding protein - C

MyBP-C is located as a “loop” around myosin in the A band (Figure 1). The protein can be phosphorylated, and regulation of the protein could alter its positioning relative to actin. In cardiac muscle, MyBP-C is phosphorylated by PKA and to a lesser extent by CaMKII (162). Phosphorylation moves myosin cross-bridges closer to heavy chain backbone, reducing likelihood of cross-bridge formation and force production (71). Despite structural differences, both the fast and slow skeletal muscle isoform of MyBP-C are phosphorylated by CaMKII (128). The skeletal isoforms has only one phosphorylation site while the cardiac has three (56). There are only limited reports about the physiological significance of MyBP-C regulation.
in skeletal muscle, but it is intriguing to consider that phosphorylation of this structural protein could work in concert with MLC phosphorylation, and thus have implications for skeletal muscle function during activity.

**Additional candidates with implications for the development of fatigue**

Titin could function as a biological spring (189) and contribute to “passive force enhancement” (167). TnI and TnT are also intriguing candidates for explaining skeletal muscle alterations seen during activity. It is known that regulation of these proteins could have both beneficial and negative effects on cardiac muscle function (346). Most likely a myriad of proteins are regulated. Each of them may have limited effects on function, but put together they might have the ability to tilt function in either a positive or negative direction.

### 3.4 Heart failure

One commonly-used definition of heart failure (HF) states that it is a condition where the heart no longer is able to supply the metabolizing tissue with sufficient amounts of adequately oxygenated blood, or can do so only by increasing ventricular filling pressure (48).

Heart failure is one of the largest public health problems in Western countries. In Europe, HF is evident in about 2-3% of the population (98). It is the leading cause of hospitalization in people older than 65 years (204). A study of HF in the Scottish population showed that hospital discharge rates for HF increased by almost 60% between 1980 and 1990 (251). The incidence of HF rises sharply at about 75 years of age (191, 250, 310). The median age of the European population is estimated to rise from 30 years in the 1950s (when 8% of the population was above 65 years) to 45 years in 2030 (with 22% of the population above 65 years) (297, 298). Therefore, a higher proportion of elderly people can probably account for some of the increased incidence of HF. The mortality of HF is similar to aggressive malignant diseases (354), so the long term prognosis is poor (79). It is estimated that 50% of patients with HF will die within four years, and within one year if the HF is severe (310). Patients
diagnosed with HF report a low quality of life (354). In fact, they report a lower quality of life than sufferers of other major chronic illnesses, like diabetes and arthritis (87). The HF patients also represent a great cost on the society. Between 1-2% of total annual health care budget in western industrialized countries is related to the care of these patients (159), mostly due to the cost of hospital admissions. Economic analyses from both Sweden (315) and the Netherlands (252) suggest that this portion is rising.

Several diseases are associated with an increased risk of developing HF. Most significant is hypertension, accounting for about 40% of HF events in men and 60% in women. Myocardial infarction (MI) accounts for 34% in men and 13% in women, and valvular disease stands for a little less than 10% (191, 216). Although the exact prevalence is uncertain, cardiomyopathy – a disease of the myocardium leading to deterioration of heart function – is also considered an important risk factor for HF, and is the number one indication for heart transplantation worldwide (302).

**Treatment; Non-pharmacological management**

In daily life there are several parameters the HF patients can influence to control the severity of HF. These include weight and diet control, restricted fluid intake and reduced consumption of alcohol and tobacco (98). Physical activity and exercise training are also important, and these will be discussed in more detail.

The common belief prior to the late 1970s was that a HF patient should remain inactive, and not unnecessarily stress their failing heart. About this time, it became evident to physicians that HF patients tolerated physical activity quite well, and even seemed to benefit from it (72, 211, 215, 224). We now know that physical inactivity accelerates the severity of heart failure (194, 270). Exercise reduces mortality, hospitalization episodes and increases quality of life (3). The single best predictor of cardiac deaths among patients with cardiovascular disease is maximal oxygen consumption (VO₂max) (194, 270), and systemic exercise training elevates VO₂max in these patients (see section 3.7), potentially reducing
morbidity and mortality (68). For most patients, training will also increase quality of life (31, 69, 407), although this effect is not as pronounced in all patients (144), perhaps because different training regimes affect the individual HF patient differently. It is, in fact, not clear what kind of exercise yields optimal effect (129). A recent study found that exercise intensity was an important factor in reversing some of the HF-associated skeletal muscle alterations (407). They found that aerobic interval training was superior to moderate continuous training. Four minute intervals at 95% of the peak heart rate were well tolerated in the HF group, without adverse effects. Recent case studies seem to be in agreement with these results (275), but there still does not exist a consensus on what kind of physical activity HF patients will benefit the most from. It is not unlikely that the future will show that individually tailored training regimes are most beneficial.

**Treatment; Pharmacological management**

Over the last decade, the model used to explain heart failure has changed, from merely a “failure to pump” in the 1950s to a more complex model involving both neurohumoral and cardioinflammatory factors. Every explanation model for HF is associated with its own treatment regime. Over time, numerous randomized, placebo-controlled trials have been carried out, each yielding hope for the possibilities of newer medication or old medications with altered administration regimes. In effect, physicians find themselves in a pharmacological jungle when it comes to treatment of the HF patient. The existing guidelines for the American College of Cardiology recommends that CHF routinely should be managed with a combination of three classes of drugs, ACEi, β-blockers and diuretics. Recently The European Society of Cardiology (ESC) published their guidelines (98) which stand more or less in agreement with the American guidelines when it comes to treatment.

ACEi is a blocker of the Renin-Angiotensin-Aldosterone-system (RAAS). It has been shown to reverse remodeling of the failing myocardium (193), and prevent apoptosis (145). Significantly, it has also been shown to have effects in skeletal muscle (see below). ATII receptor blockers are alternate drugs for those that do not tolerate ACEi. Some studies
indicate that ATII receptor blockers are not as effective as ACEi in reducing mortality, while others argue that for some patients a combination of both drugs might reduce the number of hospitalization days (310). The beneficial effect of β-blockers has been documented in a number of randomized trials (177). It is, however, worth noting that the advantageous consequence of β-blockers is not a class-effect, in that some β-blockers have failed to show the same effect as bisoprolol, carvedilol and metoprolol (370). It has been shown that blockage of the overactive sympathetic nervous system has several favorable effects: β-adrenoceptor blockers reverse HF specific alterations in gene expression and inhibit the proapoptotic and pronecrotic effect of β-adrenoceptor stimulation. β-blockers also improve cardiac energy balance in HF (241), and are known to partly normalize the heart muscle cellular Ca²⁺ handling (295). This probably explains some of their antiarrythmic effect (364). The European guidelines suggest that ATII blockers should be administered to HF-patients that remain symptomatic despite optimal treatment with ACEi and β-blockers. A diuretic drug should be administered to patients who display signs of water and salt retention. It has also been proven favorable to add an aldosterone receptor antagonist, but since oral potassium supplements are less effective in maintaining stable potassium levels during diuretic treatment (115), this requires a monitoring of the patients’ serum K⁺ values.

### 3.5 Heart failure and skeletal muscle function

There is no general agreement as to how and to what degree the skeletal muscle of heart failure patients is dysfunctional. The variability in reported results might be due to several factors. Patients vary in HF etiology, in what kind of medication they use and in their daily activity level. In experimental studies, different research groups induce MI and define HF differently. There is also no gold standard when it comes to which muscle groups are being investigated and the intensity of exercise performed and how skeletal muscle function should be assessed also varies. In the following sections, the main findings from human and experimental studies are presented.
3.5.1 Human studies

A major complaint of heart failure patients is increased fatigability. One obvious explanation is limited perfusion of the exercising muscle due to reduced cardiac output. This may in particular contribute to fatigue development when engaging a large muscle mass. However, several studies report a poor correlation between resting haemodynamic indexes of cardiac function (such as ejection fraction (EF)) and exercise intolerance. Furthermore, interventions that improve central haemodynamics, such as dobutamine infusion, have no effect on exercise duration, oxygen extraction or pH in the exercising muscle (240, 404) and the reduced exercise capacity seems to persist even after cardiac transplantation (357).

Thus, the reduced fatigue resistance could reside locally, maybe in the muscle itself. In fact, numerous studies report increased fatigability in CHF patients when engaging only a small muscle mass. The muscle mass in these studies is so limited that even a failing heart will be able to increase CO to meet their metabolic demand (Table 2). When larger muscle mass is engaged, however, as in whole-body exercise, it could be that oxygen demand exceeds the CO of the failing heart, leaving the working skeletal muscle under-perfused (213, 363). This effect will then be experienced in addition to the intrinsic skeletal muscle dysfunction.

The most common findings from human studies will be discussed in more detail in the following section. Morphology, metabolites and electrolytes are the most commonly investigated aspects of muscle health. Note that results regarding Ca²⁺ handling in the skeletal muscle of human heart failure patients are lacking.

Table 2. Controlled studies assessing fatigue in small muscle groups of CHF patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>Muscle studied (unilaterally)</th>
<th>HF etiology i</th>
<th>Fatigue parameter</th>
<th>Main findings in CHF patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brassard et al., 2006 (47)</td>
<td>Knee extensors</td>
<td>IHD, ICM</td>
<td>Time to exhaustion during isometric contraction at 60% of MVC</td>
<td>Reduced isometric endurance</td>
</tr>
<tr>
<td>Schulze et al., 2004 (328)</td>
<td>Knee extensors</td>
<td>NYHA: II, III(10), IV(6)</td>
<td>Decrease of force during 20 s maximal isometric contraction</td>
<td>Increased fatigability of maximal isometric contractility</td>
</tr>
<tr>
<td>Reference</td>
<td>Muscle studied (unilaterally)</td>
<td>HF etiology N: CHF/contr</td>
<td>Fatigue parameter</td>
<td>Main findings in CHF patients</td>
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<td>---------------------------------</td>
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<tr>
<td>Sunnerhagen et al., 1998 (365)</td>
<td>Knee extensors Plantar flexors</td>
<td>IHD, ICM, VHD NYHA: II(10), III(6) N: 16/112</td>
<td>Knee extensors - Isometric endurance; time to hold 40% of MVC Isokinetic endurance; Fall in peak torque Plantar flexors - Standing heel rise test</td>
<td>Knee extensors - Increased isometric and isokinetic fatigability Plantar flexors - Increased fatigability during dynamic work</td>
</tr>
<tr>
<td>Massie et al., 1996 (247)</td>
<td>Knee extensors</td>
<td>IHD, CM NYHA: I(2), II(7), III(7), IV(2) N: 18/8</td>
<td>Reduction in peak torque from the first 3 to the last 3 of 15 maximal isokinetic knee extensions</td>
<td>Increased fatigability during dynamic work</td>
</tr>
<tr>
<td>Harridge et al., 1996 (160)</td>
<td>Plantar flexors Knee extensors</td>
<td>IHD, CM NYHA: II(5), III(1) N: 6/6</td>
<td>Knee extensors - Fatigue index = Peak torque from the 3 best of the first 5 / peak torque from the 3 best of the last 5 of 50 consecutive maximal concentric contractions Plantar flexors (Electrical stimulation) - Fall in peak torque from the first 3 to the last 3 isometric contractions</td>
<td>Knee extensors - Increased fatigability during dynamic work Plantar flexors - No difference</td>
</tr>
<tr>
<td>Magnusson et al., 1996 (236)</td>
<td>Knee extensors</td>
<td>Not specified NYHA: II(8), III(3) N: 11/11</td>
<td>Fatigue index = Peak torque from the 3 best of the first 5 / peak torque from the 3 best of the last 5 of 50 consecutive maximal concentric contractions</td>
<td>Increased fatigability during dynamic work</td>
</tr>
<tr>
<td>Yamani et al., 1995 (415)</td>
<td>Knee extensors</td>
<td>IHD, CM NYHA: I-V N: 11/10</td>
<td>1. Time to reach 60% of MVC during a sustained voluntary maximal isometric contraction 2. The number of isokinetic contractions required for peak torque to decline to 60% of its initial value</td>
<td>1. Reduced isometric endurance in the CHF group 2. Increased fatigability in the CHF group during isokinetic work</td>
</tr>
<tr>
<td>Minotti et al., 1992 (259)</td>
<td>Foot dorsiflexors</td>
<td>IHD, CM NYHA: I(1), II(3), III(5) N: 9/8</td>
<td>1. Time to reach 60% of MVC during a sustained voluntary maximal isometric contraction 2. Number of contractions to reach 60% of MVC during intermittent isometric contractions</td>
<td>Increased fatigability both during sustained isometric contractions and during intermittent isometric contractions</td>
</tr>
<tr>
<td>Buller et al., 1991 (54)</td>
<td>Knee extensors Adductor pollicis</td>
<td>IHD NYHA: Not specified Mild/moderate (5), Severe (5) N: 10/5</td>
<td>Knee extensors - MVC after 20 min of intermittent isometric contractions as a percentage of the initial MVC Adductor pollicis - Decline in isometric force during repetitive supramaximal tetanic ulnar nerve stimulation, both with and without circulatory occlusion</td>
<td>Knee extensors - Increased isometric fatigability in a subpopulation of CHF patients (severe heart failure) Adductor pollicis - No differences between groups, with or without circulatory occlusion</td>
</tr>
</tbody>
</table>

CM = Cardiomyopathy; ICM = Idiopathic cardiomyopathy; IHD = Ischemic heart disease; VHD = Valvular heart disease; NYHA = New York Heart Association classification of clinical signs of CHF.
Morphology and Metabolism

Several studies report reduced oxidative capacity in the skeletal muscle of CHF patients as compared to controls (102, 245, 320, 361). Increased atrophy (239, 260), increased apoptosis (5, 124, 221, 393) and a fiber type switch towards a less fatigue resistant phenotype (320, 361, 400) have also been described, along with a reduced number of capillaries per muscle fiber (101, 107, 108, 236, 320, 361, 400). However, studies estimating the ratio of capillaries to cross-sectional fiber area found no difference between CHF patients and healthy subjects (225, 361, 400), although reduced (102) as well as increased (238) ratios also have been reported. Deconditioning can undoubtedly contribute to decreased exercise tolerance, but several researchers point to important changes in muscle performance which may originate in the muscle itself (239, 245, 260, 340) and thus distinguish the heart failure-associated skeletal muscle dysfunction from deconditioning. First, the enzymatic and mitochondrial abnormalities in heart failure skeletal muscle is also established in the diaphragm (which in fact has an increased load in CHF) (89), while disuse affects only postural muscles (38). Furthermore, while energy transfer through CK in skeletal muscle is limited in heart failure (102), it is preserved in deconditioning (38). Stroke patients who have atrophy due to immobilization have a fiber type switch toward type I fibers (391). Although these patients were bed-bound for over a year, and thus perhaps not representative for deconditioning in CHF, the fiber type switch is qualitatively different from CHF patients who have a switch toward faster fiber types. It has been proposed that neurohumoral or neuromuscular signals might contribute to the fiber type switch (360). This suggests that heart failure per se causes different changes in the fiber type composition than the alterations that mainly result from inactivity and deconditioning.

Changes in muscle metabolism may clearly contribute to increased fatigability. The amount of work needed to reach the same level of CrP depletion is noticeably reduced in CHF patients (243), even though resting levels of CrP and ATP are similar in CHF and healthy controls (321). Increased glycolytic metabolism (246) and decreased oxidative
metabolism have also been reported, along with an early increase in blood lactate (62). Similar results have also been reported by Wilson et al (403), but several researchers show less lactate accumulation and CrP depletion at peak exercise in CHF patients compared to controls (321, 362). Increased glycolytic enzyme activity also fails to be unanimously reported (236, 238). One problem faced in interpreting these results is that both digitoxin (lower oxidative enzyme activity (320)) and ACEi (increase LD (319) and other enzyme systems (418)) seem to modulate skeletal muscle metabolic status. In addition, both the ATII receptor blocker (Losartan) and ACEi (Enalapril) can partly reverse the fiber type switch associated with heart failure (396).

To sum up, deconditioning can contribute to the reduced exercise capacity in CHF, but there are skeletal muscle changes in these patients that cannot be explained by deconditioning alone. Since heart failure patients as a group are heterogeneous, and because drugs used only by some patients seem to have direct effects locally on the skeletal muscle, it is hard to speculate how much of the observed fatigability is due to the failing heart. It seems, however, that alterations in metabolism exist in these patients, which led Ventura-Clapier to suggest the term “metabolic myopathy” (389) to describe these changes.

**Electrolytes**

Membrane excitability is highly dependent on the tight regulation of electrolytes across the cell membrane, especially the active transport of Na+ and K+, most importantly by the NKA (66). The importance of an optimal ion regulation is demonstrated by Green et al, who found that NKA concentration correlated with CHF patients’ ability to perform exercise (149). However, the literature is equivocal when it comes to the question of whether CHF patients have a reduced number of pumps. Some find a reduction in number of pumps (276, 293) while others do not (149). Another notable aspect of the NKA is that diuretics and digitoxin (both drugs commonly used by HF patients) can affect the pump concentration negatively (100, 324). Deficiencies in both Mg2+ and K+ have been noted in human patients (132, 226), and this could decrease NKA concentration (277). β-adrenergic stimulation usually increases
NKA pump activity (65). Increased sympathetic nerve activity in heart failure (244) could desensitize the β-response, as reflected in decreased NKA pump concentration (293).

Regardless of NKA concentration, reduced K⁺ and increased Na⁺ are the most prominent findings in skeletal muscle biopsies, while K⁺ in plasma is reduced in CHF patients (52, 111, 112, 398). Neurohumoral activation in HF (increased activation of the RAAS system and levels of circulating catecholamines) could account for some of these changes, but the same changes are found experimentally in rats treated with furosemide (45) – a diuretic often used in the treatment of HF – suggesting that also drugs could alter electrolyte status in these patients. At the same time these changes could be a reason for increased fatigability seen in patients.

### 3.5.2 Experimental heart failure studies

Heart failure patients constitute a heterogeneous group which could make experimental data difficult to interpret. Therefore, one important rationale for using animals in research on skeletal muscle function in heart failure is that there are less confounding factors in animal models compared to humans. For example, animals do not use drugs, they are not engaged in any organized training, and have the same diet. Also, in animals, heart failure can be induced in a standardized manner, whereas in humans the pathophysiological etiological behind the heart failure condition varies. Heart failure can be induced in several ways in animals; by coronary artery ligation (377), by aortic banding (90), spontaneously in hypertensive rats (290), by rapid atrial pacing (402), by feeding salt to salt-sensitive rats (81) and finally by monocrotaline injections (395). However, there are limitations to some of the models, such as the monocrotaline model. Monocrotaline is a poison that is injected into the circulation and has toxic effects on the pulmonary artery (326). Pulmonary hypertension develops, along with right sided heart failure (208, 394). However, the possibility that the skeletal muscle also is influenced by monocrotaline cannot be ruled out.
In many studies the degree of heart failure in the animals is important to assess. E.g., in post MI models it is important to separate the failing animals from those who simply have MI without HF. Three parameters have proven useful in making this distinction; EDP > 15 mmHg (231), left atria dilatation and reduced posterior wall shortening as evaluated in M-mode echocardiography (343). In addition, common post-mortem findings in animals with both MI and HF include pleural effusion and increased heart and lung weight.

**Skeletal muscle morphology**

Researchers disagree on whether skeletal muscle from HF patients is atrophied or not. Some report decreased muscle weight suggesting increased atrophy (290) and cross-sectional area of muscles (197) while other report unchanged skeletal muscle morphology (413). Six weeks following coronary artery ligation, Perrault *et al* (289) did not find any atrophy of EDL, but 8 weeks after ligation, Simonini *et al* (339) demonstrated significant reductions in plantaris and soleus muscle mass. Possibly, atrophy only occurs late in the development of HF. Apoptosis and elevated concentrations of proinflammatory cytokines have not been found in coronary artery ligation models of heart failure, although reported in monocrotaline studies (82, 208). The shift from slow to fast fiber type, however, is a common finding across different HF models (90, 93, 208, 392). This is similar to the skeletal muscle phenotype shift following denervation (187) and muscle unloading (38). This could imply, again, that muscle alterations simply are a detraining phenomenon. To test this, rats’ activity levels were been monitored, finding neither differences between CHF animals and controls (229) nor correlation between EDP or infarct size and activity (341). Skeletal muscle alterations are thus probably a part of heart failure pathophysiology and not only due to inactivity or deconditioning (369).

**Blood flow**

Nusz *et al* found decreased vascular rarefaction and endothelial apoptosis in a coronary artery ligation model of heart failure. They hypothesized that this may contribute to the skeletal muscle abnormalities (278). Following this line of thought, reduced skeletal muscle
blood flow were measured in HF rats running on a treadmill (268). Treadmill running engages large muscle masses, and blood flow will probably be compromised because of the reduced CO of the failing heart. This was also noted by the authors, as they found that the reduction of blood flow was dependent on MI size. However an in situ model demonstrated both a lower microvascular O₂ pressure and a more rapid lowering of this pressure after contraction (29). It could be, then, that limitation in blood flow when smaller muscle masses are engaged also contributes to increased fatigability. The flow abnormalities could result from sympathetic vasoconstriction or by increased levels of ROS that could influence NO synthase (26, 371, 378). It has been shown that administration of agents that reduce NO compromises blood flow in sham animals, but that this effect was blunted in CHF animals (171). Even though blood flow and the number of capillaries perfused can be reduced in working skeletal muscle relative to the mass of the muscle working, Kindig et al found that this can be compensated for by a reduction in RBC velocity, allowing a more complete oxygen extraction by the skeletal muscle (197). Consequently, there is no clear agreement if blood flow and oxygen delivery to working skeletal muscle really is limited in CHF when small muscle mass is used.

**Metabolism**

Glycolytic capacity is frequently reported as maintained in skeletal muscle from CHF animals (19, 53, 93). Nevertheless, several groups have also reported a reduced oxidative capacity (particularly a reduction in CS (53, 342), but also in MDH and HADH (93, 291)) and altered mitochondrial regulation (90). This reduction is seen in all fibertypes (93), but is dependent on the degree of HF with no alteration in animals with MI without failure (53). These energetic abnormalities could be due to reduced mitochondrial gene transcription. PGC-1α seems to be an important modulator (136). Although there are no differences in resting concentrations of ATP, CrP, Pₐ, pH and lactate in skeletal muscle from CHF animals compared to both sham and MI (59, 373), there is increased breakdown of CrP during stimulation in skeletal muscle from the CHF animals compared to control. It is also shown
that CrP concentration has a prolonged recovery for both MI and CHF animals compared to sham operated animals (53, 373), which further strengthens the hypothesis that CHF animals have a reduced mitochondrial capacity. CK expression is also altered in skeletal muscle in a fiber type-specific manner (90) and could clearly contribute to the “metabolic myopathy” seen in heart failure.

**Ca\textsuperscript{2+} handling and Electrolytes**

Studies have been conducted to uncover alterations in SR function and Ca\textsuperscript{2+} handling but the results are not conclusive. Some report increased Ca\textsuperscript{2+} uptake and release (348, 401) while others find a reduced release and reduced rate of removal (229, 289) in muscles consisting predominantly of fast twitch fibers. These alterations could be due to alterations in SERCA, SR storage or RyR. In addition, NKA is important for Ca\textsuperscript{2+} homeostasis. This pump is essential in restoring and maintaining membrane potential, so adjustments in pump function could have implications on muscle function. It is also indirectly important to other transport systems in the cell, for example NCX. A reduction in NKA concentration in skeletal muscle is reported in some studies (269, 276, 293) whereas other report no changes (230). It remains a possibility that the reason for literature disagreement on pump concentration in both human and experimental studies is based on muscle type heterogeneity (in human studies) and the variety of examined muscle (in experimental studies), as recent findings suggest that alterations in the NKA is muscle-specific and property-specific (25).

The Dhalla group showed that SERCA activity in skeletal muscle was reduced in severely failing animals following coronary artery ligation (8, 9, 333). This was later confirmed by others (290). Reduction in both protein and mRNA expression of SERCA2a (338) and SERCA1 (290) has been reported, but there are contradictory findings also. Spangenberg et al. did not find any differences in either isoform SERCA1 or SERCA2a in failing animals, using the same HF model as Dhalla et al. (348). Others yet have reported elevated levels of SERCA in skeletal muscle from HF animals (230). It is probable that the rats in this study
were only moderately failing, and with this in mind it is interesting that it has been suggested that alterations in SERCA are dependent on the severity of heart failure (333).

RyR regulation by PKA could be defective in heart failure, thus contributing to the decreased fatigue resistance. PKA activation leads to hyperphosphorylation of the RyR complex (309, 397) and is thought to lead to “leaky” channels attenuating Ca²⁺ release. Also of interest is myofibrillar Ca²⁺ sensitivity, and one recent study suggests that modulation of sensitivity could contribute to increased fatigability (230). The same group also reported that Ca²⁺ handling properties in single skeletal muscle fibers from failing animals during exercise seemed to be different compared to single fibers from sham operated animals.

In conclusion, Ca²⁺ handling in skeletal muscle is probably altered in HF, but no clear understanding has emerged from experiments so far.

3.6 Triggers of skeletal muscle dysfunction

Skeletal muscle fails when the heart fails (37), suggesting a connection between the two organs. It could be a humoral factor produced in the heart, or some other signal, like hypoxia or malnutrition, constantly maintaining the dysfunctional skeletal muscle. Training of one muscle group has beneficial effects on skeletal muscle function (see below). Restoring ideal nutrition does not have the same beneficial effect (51). This indicates that oxygen delivery and/or inactivity contributes to the skeletal muscle dysfunction while limited nutrient supply plays a minor role in the pathophysiology. Heart transplantation does not reverse skeletal muscle dysfunction (357), which could imply that an initiating factor triggers a self-containing vicious circle within the skeletal muscle, independent of further stimulation from the circulation. Results from in situ experiments indicate that skeletal muscle dysfunction is not dependent on external stimuli. If this is the case, the task of identifying the initial signal is even harder, simply because it may not be there anymore.

Heart failure is characterized by elevated plasma levels of several pro-inflammatory cytokines, for example, TNF-α, IL-6 and IL-18 (138, 202, 271), among others. Since plasma
levels of cytokines correlates with both disease severity (96) and the magnitude of muscle apoptosis (207), it is likely that the cytokines not only are important in heart failure pathogenesis but also play a role in skeletal muscle function. Cytokines produced in the heart could act as humoral signalling molecules, thereby affecting the skeletal muscle. In murine models, TNF-α seems to depress maximal force by blunting myofilaments’ response to Ca²⁺ activation (308) as well as inducing muscular protein loss and reactive oxygen-mediated NF-κB activation (220). Furthermore, cytokine production possibly increases in the skeletal muscle in response to cytokine signals from the failing heart. If so, skeletal muscle cytokine production might have a negative impact both on the heart (58, 332, 408) and on the skeletal muscle itself (6, 137, 327), creating a vicious cycle. Interestingly, the term “myokine” has now been introduced in the literature (286) reflecting the growing belief that skeletal muscle is a cytokine-producing organ (10, 352, 353).

Based on the observation that muscle fibers from CHF animals appeared more fragile than muscles from sham animals (229), Schiøtz Thorud et al reported increased MMP activity in muscle from CHF rats (323). MMPs control ECM turn-over, modulate immune responses and may also influence the bioavailability of cytokines. Although increased MMP activity also could change ECM composition, and thereby the muscle’s mechanical properties, no direct evidence of such an effect has been reported. Similarly, triggers of increased MMP activity and possible alterations of major structural components and signalling molecules in the ECM have not been identified.

### 3.7 Beneficial effects of training on skeletal muscle in HF

As mentioned before, prior to the mid-1980s, heart failure patients were advised to live sedentary lives. Today, however, it is known that physical activity slows down the severity of heart failure (194, 270), reduces number of admissions to hospital and mortality (294) and is thus feasible for patients (67). Quite clearly, physical activity should be part of the treatment regime for such patients. But why is training advantageous?
Systemic training

Many of the studies in heart failure patients are done systemically (Table 3). Cycling, stair climbing or bilateral knee extensor training consistently leads to a higher VO\textsubscript{2max} in CHF patients (32, 137, 155, 318). Several factors may account for this improvement (Table 3), and again, improved oxidative capacity is a candidate factor (4, 137, 154, 379, 407). This type of training also reduces oxidative stress and the level of inflammatory cytokines in the skeletal muscle (138, 221), and results in skeletal muscle hypertrophy (32, 380) and reduced apoptosis (221). Training has also been reported to induce a fiber type shift towards a more fatigue resistant phenotype (153), but the result was not confirmed in later studies (151, 158, 196, 380). The same ambiguity is true for capillarization, as one study reported increased capillary to fiber ratio after training (318) whereas others did not (32, 195, 196).

**Table 3. Effects of systemic exercise training on skeletal muscle of CHF patients**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patient characteristics</th>
<th>Endpoints to evaluate</th>
<th>Effect of training</th>
</tr>
</thead>
</table>
| Wisløff et al., 2007 (407) | IHD
NYHA: 2.5 (mean)
N: 27, of which 9 served as controls | PGC-1α
Ca\textsuperscript{2+} handling | Increased PGC-1α in response to interval training
Increased rate of Ca\textsuperscript{2+} reuptake in response to interval training |
| Harjola et al., 2006 (158) | IHD, ICM
NYHA: I(2), II(7), III(5)
N: 17, of which 9 served as controls | Fiber type distribution | Not altered by training                                                             |
| Gielen et al., 2005 (137) | IHD, ICM
NYHA: II(18), III(2)
N: 20, of which 10 served as controls | Systemic aerobic capacity
Oxidative capacity | Increased VO\textsubscript{2max}
Reduced iNOS and nitrotyrosine content paralleled by an increased Cytochrome c oxidase activity |
| Hambrecht et al., 2005 (155) | IHD, ICM
NYHA: II(16), III(2)
N: 18, of which 9 served as controls | Systemic aerobic capacity
IGF-1 | Increased VO\textsubscript{2max}
Increased IGF-1 and reduced IGF-1 receptor levels |
| Linke et al., 2005 (221) | IHD, ICM
NYHA: II(20), III(3)
N: 23, of which 11 served as controls /12 | Systemic aerobic capacity
Local inflammation | Increased VO\textsubscript{2max}
Reduced oxidative stress and apoptosis
Red. expression of TNFa and IL1β |
| Keteyian et al., 2003 (195) | Not specified aetiology
NYHA: II-III
N: 15 | Systemic aerobic capacity
Capillarization
Oxidative capacity
Fiber type distribution | Increased VO\textsubscript{2max} in men (- in women)
No change in endothelial cell/muscle fiber ratio
Unaltered enzyme activity
Increased MHC1 in men (- in women) |
| Gielen et al., 2003 (138) | IHD, ICM
NYHA: II(18), III(2)
N: 20, of which 10 served as controls /10 | Systemic aerobic capacity
Local inflammation | Increased VO\textsubscript{2max}
Red. mRNA level of TNFα, IL-1β and IL-6
Red. iNOS expression both in mRNA and protein level |
Table 3. Effects of systemic exercise training on skeletal muscle of CHF patients (Continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patient characteristics</th>
<th>Endpoints to evaluate</th>
<th>Effect of training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larsen et al., 2002</td>
<td>Not specified aetiology</td>
<td>Fiber type size, area and distribution</td>
<td>Trend towards increased thickness for all fiber types</td>
</tr>
<tr>
<td>(210)</td>
<td>NYHA: II-III</td>
<td></td>
<td>Trend towards an increase in type Iib and decrease in type I fiber area</td>
</tr>
<tr>
<td>Ennezat et al., 2001</td>
<td>IHD, &quot;non-ischemic&quot;</td>
<td>Antioxidant enzyme-related genes</td>
<td>Uregulation of genes encoding copper zinc superoxide dismutase and glutathione peroxidase</td>
</tr>
<tr>
<td>(119)</td>
<td>heart disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NYHA: III(14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N: 14, of which 4 served</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>as controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiilavuori et al.,</td>
<td>IHD, ICM</td>
<td>Rate limiting metabolic enzymes</td>
<td>PFK activity increased; Key aerobic enzymes unaltered</td>
</tr>
<tr>
<td>2000 (196)</td>
<td>NYHA: II(15), III(12)</td>
<td>Fiber type distribution Capillarization</td>
<td>No change in fiber type distribution</td>
</tr>
<tr>
<td>Scarpelli et al.,</td>
<td>IHD, ICM</td>
<td>Systemic aerobic capacity Capillarization</td>
<td>Unaltered capillary/fiber ratio</td>
</tr>
<tr>
<td>1999 (318)</td>
<td>NYHA: II(5), III(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N: 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyni-Lenne et al.,</td>
<td>IHD, ICM</td>
<td>Fiber type distribution Fiber size</td>
<td>No effect on fiber type distribution</td>
</tr>
<tr>
<td>1997 (380)</td>
<td>NYHA: II(9), III(7)</td>
<td></td>
<td>Increased cross sectional area of all fiber types</td>
</tr>
<tr>
<td></td>
<td>N: 16, of which 8 served as controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hambrecht et al.,</td>
<td>IHD, ICM</td>
<td>Fiber type distribution Mitochondrial ultrastructure</td>
<td>Increase in type I fibers; Decrease in type II fibers</td>
</tr>
<tr>
<td>1997 (153)</td>
<td>NYHA: II(10), III(8)</td>
<td></td>
<td>Increased surface density of cytochrome c oxidase positive mitochondria, inner mitochondrial membrane and mitochondrial cristae</td>
</tr>
<tr>
<td></td>
<td>N: 18, of which 9 served as controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyni-Lenne et al.,</td>
<td>IHD, ICM</td>
<td>Oxidative capacity</td>
<td>Increased activity of CS and LDH</td>
</tr>
<tr>
<td>1997 (379)</td>
<td>NYHA: II(9), III(7)</td>
<td></td>
<td>Increased VO$_{2\max}$ during bilateral knee extension</td>
</tr>
<tr>
<td></td>
<td>N: 16, of which 8 served as controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belardinelli et al.,</td>
<td>IHD, ICM</td>
<td>Systemic aerobic capacity Capillarization</td>
<td>Increased VO$_{2\max}$ despite unaltered peak cardiac output</td>
</tr>
<tr>
<td>1995 (32)</td>
<td>NYHA: II(17), III(10)</td>
<td>Fiber size</td>
<td>Unaltered capillary/fiber ratio</td>
</tr>
<tr>
<td></td>
<td>N: 27, of which 9 served as controls</td>
<td></td>
<td>Hypertrophy of type I and type II fibers</td>
</tr>
<tr>
<td>Hambrecht et al.,</td>
<td>IHD, ICM</td>
<td>Systemic aerobic capacity Oxidative capacity</td>
<td>Increased VO$_{2\max}$</td>
</tr>
<tr>
<td>1995 (154)</td>
<td>NYHA: II(12), III(10)</td>
<td></td>
<td>Increased volume density of mitochondria</td>
</tr>
<tr>
<td></td>
<td>N: 22, of which 10 served as controls</td>
<td></td>
<td>Increased cytochrome c oxidase-positive mitochondria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased maximal leg oxygen consumption</td>
</tr>
<tr>
<td>Adamopoulos et al.,</td>
<td>IHD</td>
<td>Oxidative capacity</td>
<td>Reduced CrP depletion during exercise</td>
</tr>
<tr>
<td>1993 (4)</td>
<td>NYHA: II(7), III(5)</td>
<td></td>
<td>Reduced CrP recovery half-time</td>
</tr>
<tr>
<td></td>
<td>N: 12/15</td>
<td></td>
<td>Decreased ADP during exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased exercise tolerance</td>
</tr>
</tbody>
</table>

CM = Cardiomyopathy; HT = Hypertension; ICM = Idiopathic cardiomyopathy; IHD = Ischemic heart disease; NYHA = New York Heart Association classification of clinical signs of CHF; VHD = Valvular heart disease.
Training of one muscle group

Skeletal muscle dysfunction could be part of the heart failure pathophysiology independent of reduced CO and limited local blood supply. Therefore, studies should be designed so that the pump capacity of the heart does not limit exercise (as it will in systemic training). These conditions have been met in five studies (142, 151, 235, 282, 356) (Table 4). Training of one muscle group increases peak work load (235) and endurance (142, 356). Oxidative capacity of the muscle is also improved, as reflected either by increased activity of key aerobic enzymes (142, 151, 235) or by an increase of intracellular pH and PrC levels, both at rest and during work (282, 356). Magnusson et al also reported an increased capillary-to-fiber ratio, as well as an increased peak work rate during one-legged knee extensions (235).

Table 4. Effects of local exercise training on skeletal muscle of CHF patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>N: CHF (healthy controls/ baseline comparison)</th>
<th>Endpoints to evaluate</th>
<th>Effect of training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gustafsson et al., 2001 (151)</td>
<td>IHD, ICM, HT NYHA: II(4), III(4) N: 8</td>
<td>Fiber type distribution Oxidative capacity VEGF</td>
<td>Unaltered fiber type distribution Increased CS activity. Anaerobic enzymes unaltered mRNA and protein level of VEGF increased</td>
</tr>
<tr>
<td>Ohtsubo et al., 1997 (282)</td>
<td>ICM NYHA: II(6), III(1) N: 7/7</td>
<td>Oxidative capacity</td>
<td>Increase in intracellular pH and CrP level without changes in blood flow</td>
</tr>
<tr>
<td>Magnusson et al., 1996 (235)</td>
<td>IHD, ICM NYHA: II(5), III(5), IV(1) N: 11</td>
<td>Oxidative capacity Capillarization</td>
<td>Increased activity of CS and HAD. PFK unaltered Increased VO_{2max} and peak work load during one-legged exercise Increased capillary/fiber ratio</td>
</tr>
<tr>
<td>Gordon et al., 1996 (142)</td>
<td>IHD, ICM, HT NYHA: II(8), III(8) N: 16</td>
<td>Oxidative capacity</td>
<td>Increased CS activity; PFK unaltered</td>
</tr>
<tr>
<td>Stratton et al., 1994 (356)</td>
<td>IHD, ICM, VHD NYHA: II(2), III(7), III(1) N: 10</td>
<td>Oxidative capacity</td>
<td>Increased intracellular pH, both during rest and work Reduced CrP utilization during exercise Increased CrP resynthesis rate Increased exercise duration</td>
</tr>
</tbody>
</table>

CM = Cardiomyopathy; HT = Hypertension; ICM = Idiopathic cardiomyopathy; IHD = Ischemic heart disease; NYHA = New York Heart Association classification of clinical signs of CHF; VHD = Valvular heart disease. HAD = Hydroxyacyl-CoA dehydrogenase PFK = Phosphofructokinase. VEGF = Vascular endothelial growth factor.

None of the studies on the effects of exercise training on skeletal muscle of CHF patients have a control group for any other possible confounding elements other than baseline
characteristics. Also, this field lacks results regarding Ca\(^{2+}\) handling in human skeletal muscle. This is discussed further in Paper 4.

4 Problems addressed in the thesis

Skeletal muscle fatigue has been studied extensively in both humans and in animal models. Although shortening contractions (muscle shortens during activation) have been used to investigate fatigue in humans (316), this method is not commonly used on animals (15). Here, muscles are most often stimulated isometrically and at room temperature. This means that the fatigue mechanisms known from animal models are mostly obtained in muscles that did not shorten upon stimulation. This is surprising, since locomotive muscles usually shorten when they are stimulated. Fatigue mechanisms could be different in an isometric contracting muscle compared to a shortening muscle. Consequently, it is not necessarily possible to conclude which are the most important determinants of fatigue in shortening human muscle based on isometric experimental studies alone. Furthermore, it is unknown what role posttranscriptional regulation of proteins plays in the development of fatigue through the shortening of slow-twitch muscle.

Several researchers report that skeletal muscle from HF patients has increased fatigability. There are, however, limited reports on how skeletal muscle from HF patients differs from normal skeletal muscle regarding Ca\(^{2+}\) handling. This is unexpected considering the large number of articles pointing to altered Ca\(^{2+}\) handling as a key player in the development of skeletal muscle fatigue. The trainability and potential reversal of dysfunction with training is also scarcely investigated.
5 **Aims**

**Main aims:**
- Investigate the skeletal muscle function of heart failure animals and patients
- Explore fatigue mechanisms in skeletal muscle from normal rats and rats with heart failure
- Investigate the effect of training of one muscle group on skeletal muscle in heart failure patients

**Specific aims:**
- Investigate the fatigue development following shortening contractions in slow twitch rat skeletal muscle
- Explore cellular mechanisms contributing to fatigue development in shortening slow twitch rat skeletal muscle
- Elucidate development of fatigue in rat slow twitch skeletal muscle during shortening contractions in experimental heart failure
- Uncover whether CHF patients have altered skeletal muscle $Ca^{2+}$ homeostasis compared to healthy peers
- Explore effects of local skeletal muscle training on $Ca^{2+}$ handling in CHF patients and healthy peers
6 Methods

6.1 Animal model

The experimental work (Paper 1 and 2) used male Wistar Hannover rats (Taconic, Lille Skensved, Denmark). These rats are outbred and therefore have an undesired genetic variability but were still used for several reasons: First, our institute has extensive experience with this rat, and our experiments were to a large extent based on previous physiological and pathophysiological results from this strain. Second, it has a suitable homogeneity in the soleus muscle (SOL), consisting of about 94% type I fibers (347). Lastly, these rats have a favorable size and price. All animals were caged one week prior to surgery. They were housed in standard cages (12/12 hr day/night light cycle, temperature: 22 ± 2°C, humidity: 55 ± 5 %) with access to rat food and water ad libitum. The experimental procedures conformed to the European Convention for the Protection of Vertebrate Animal Used for Experimental and other Scientific Purposes, and protocols were approved by the Norwegian Animal Research Authority.

Post infarction rat model

A coronary artery ligation model was used as a model of acute human MI to induce CHF. Weighing about 300 g (70 days old) the rat was put in a chamber and anaesthetized with a mix of 30 % O₂, 70 % N₂O and 5 % isoflurane (Forene®). After anaesthesia the rat was intubated and placed on a respirator (Zoovent, Triumph Technical Services LTD, London, UK). The anaesthesia gas mixture was the same except that isoflurane content was reduced to 2 – 2½ %. The ligation and induction of MI has been described by Tønnessen et al (377), but in short, the pericardium is opened and the heart gently exteriorized after a left sided thoracotomy. Although no arteries were visible, a ligation was made using a silk suture, 3-4 mm from the base of the left artery on the posterior wall of the heart. The heart was then replaced and the thorax closed. Heart exteriorization was also done on sham animals, but without ligation. The animals were given 0.2 mg·kg⁻¹ buprenorphine (Temgesic®, Schering-
Plough, NJ, USA) postoperatively. During the six weeks from primary to secondary operation, the animals were kept under daily surveillance. Survival rate was about 80%.

A recent study argues that the coronary ligation model may be less biologically useful than previously assumed to investigate the mechanisms of skeletal muscle fatigue relevant for CHF patients (306). Indeed, there are several problems with this method: MI is induced in young and otherwise healthy animals, whereas MI in humans typically strikes the elderly. Since the coronary occlusion is acutely introduced in rats, and in humans the development is gradual, one could speculate that compensatory mechanisms found in human CHF will be absent in rats. Of particular interest are signals produced in early stages of HF, maybe during long-standing ischemia. These could be, for instance, cytokines that could affect skeletal muscle negatively. Frequently, heart failure patients also have several risk factors for developing HF. In the Framingham study, 57% of patients with coronary artery disease as the primary cause of HF also had a history of hypertension (163). Lastly, MI is an unnatural condition for a rat of any age. Thus, the coronary ligation model does not ideally reflect human CHF, but in rats it is probably as close to the pathophysiology we can get.

Both unoperated animals (Paper 1) and operated animals (Paper 2) were used in the exercise protocol. To assess whether the induced MI had developed into CHF, a pressure sensitive catheter was used (Cardiovascular catheter SPR-407, Millar Instruments, Houston, TX, USA). It was led retrograde from right carotid artery, past the aortic valves and positioned in the left ventricle. As described by Sjaastad et al. (343), a cutoff at EDP > 15 mmHg discriminates well between MI rats with and without HF. In Paper 2 only sham animals and animals with EDP > 15 mmHg were used.

**In situ muscle preparation**

A rat weighing about 380 g (100 days old) was anaesthetized and intubated as described above, and placed on a heated (37°C) operating table (modified version of Model 806A In situ Rat Test Apparatus, Aurora Scientific, Ontario, Canada). The gas mixture was the same
as during artery ligation, with isoflurane content at 2 – 2½ %. The right leg was skinned from the knee and down. Connective tissue covering the muscle groups on the leg was split both on medial and lateral side, starting distally between the Achilles tendon and crus going almost up to the knee. Triceps surae was isolated and a suture was placed in the distal end, just proximal to the calcaneus bone. A smaller portion of calcaneus was cut, loosening triceps surae’s distal attachment. The thin fascia covering this muscle group was carefully cut on either side of the soleus muscle (SOL), making it feasible to tie a 3.0 surgical thread between gastrocnemius and SOL, isolating it. Great care was taken not to damage or pull the blood supply coming from gastrocnemius entering the SOL near the middle of the belly of the soleus muscle. Except for its continued connection to the blood supply, and the accompanying nerves surrounded by thin connective tissue, the SOL was dissected and cut completely free of adjacent tissue (Figure 5) and left attached only proximally to tibia and fibula.

Figure 5. Soleus separated from nearby tissue. Note that the blood supply is intact.
The thread used to separate the SOL from gastrocnemius was tied tightly to the lever arm of a combined force and length transducer (model 305B, Aurora Scientific, Ontario, Canada). The lever arm was positioned so that the SOL was parallel to the tibia. This ensue a physiological movement of the fibers upon stimulating. Platina electrodes were placed proximally and distally on the muscle. Since the SOL is 5° pennated (44) and has rather long aponeurosis in both ends, electrodes were carefully placed on muscle fibers and not on the connective tissue, ensuring proper propagation of current. The muscle was kept moist and warm by constantly bathing it with preheated (39°C) 0.9% NaCl solution. Entering laterally on the thigh, the sciatic nerve (or the tibial and common peroneal nerve) was identified and cut. The free nerve endings were isolated with water-free vaseline to prevent retrograde transmission of current during stimulation of the SOL.

**Dynamic fatiguing protocol**

Before starting the protocol, the ideal sarcomere length was identified as the length associated with highest developed force when stimulated at 1 Hz. Stimulation voltage was adjusted accordingly, thus identifying the voltage that resulted in the highest developed force. Stimulation voltage was set one volt higher than this identified voltage during experiments (8V). Increasing it at any time during exercise did not lead to increased force production or shortening. Pulse duration was 1 ms. Maximal isometric force (\(F_{\text{max}}\)) was obtained, and during the dynamic protocol a load corresponding to 1/3 of \(F_{\text{max}}\) was used as afterload. It was chosen because the muscle works most effectively at this load (331). SOL is a postural predominantly slow twitch muscle and the motoneuron firing rate probably rarely exceeds 30 Hz (166). Therefore, this stimulation frequency was used. The muscle was stimulated for one second, followed by one second of rest, for 15 minutes. Force, shortening, blood pressure and superficial muscle temperature were displayed and sampled at 2000 Hz by a custom-made LABview program. Analysis of tracings was performed on a MATLAB program (MathWorks Inc., Natick, MA, USA), except for analysis of the time constant of isometric relaxation. This analysis was done in the first contraction cycle, after 100 s, 5 min and
15 min. Two time constants were estimated by fitting the tracings to a double exponential decay curve \( y = a + b \exp(-x/c) + d \exp(-x/e) \) using SigmaPlot (Systat software, Norway, 2006). Constant \( c \) and \( e \) from the equation were defined as \( \tau_1 \) and \( \tau_2 \), respectively.

**Slack test**

Despite the fact that skeletal muscle rarely shortens against zero loads, a slack test, introduced by Edman in 1979 (113), is the method of choice to evaluate maximal shortening velocity \( (V_0) \). The SOL was isolated and mounted as described above. Starting from optimal sarcomere length, the muscle was stimulated at 100Hz \((8V, 1 \text{ ms pulse duration})\), fully saturating the myofilaments with \( \text{Ca}^{2+} \). After reaching maximal isometric, the length of the muscle was abruptly reduced \((2-6 \text{ mm})\) at a speed greatly exceeding the muscle’s own shortening velocity, so that developed force dropped. As the muscle was continuously stimulated, the force picked up again. Initially, the redevelopment of force rose discreetly and it was hard to identify objectively exactly when it started. A custom-made Matlab program was used to identify the start of rise and the time from the force dropped due to shortening till the force begun to rise was plotted vs. shortening. This plot was fitted to the linear equation \( y = ax + b \). The rise of this line \( (a) \) was used as the muscle’s unloaded shortening velocity \( (\text{mm}*\text{ms}^{-1}) \).

**6.2 Procedures on human test subjects**

Three groups of human test subjects have been used in Paper 4; 1) Heart failure patients (CHF), 2) Patients with coronary artery disease (CAD) but with normal heart function and 3) Healthy controls (HS). All the CHF patients had post infarction heart failure, and the CAD group was included to have a control group that also used medication. The two patient groups were recruited from the Department of Cardiology at Ullevål University Hospital, Oslo, Norway, either by searching journals or results from echocardiography, or meeting candidate patients in the out-patients’ department. Healthy subjects were recruited through charitable organizations. All subjects were informed about the nature of the research project. They received a written information leaflet, and were informed prior to agreeing to participate that
leaving the project was possible at all times without any consequences. The human studies were approved by the regional ethics committee.

**Spirometry**

\( \text{VO}_2\text{max} \) was measured (Vmax 229, SensorMedics, Yorba Linda, CA, USA) with test subjects on an ergometer bicycle (Schiller ERG911, Baar, Switzerland). Sampling was done every 20 s. The heart failure patients started to cycle on 50 W. The two other groups started at 100 W. The load increased 10 W/min. All subjects were verbally encouraged to work hard, and the test was terminated at exhaustion. For the test to be valid a Borg score \( \geq 18 \) at exhaustion was required. Work was also stopped at angina suspect chest pain or at ECG alterations indicating ischemia.

To ensure that a reduced lung capacity was not limiting the \( \text{VO}_2\text{max} \) measurements, all subjects tested maximal voluntary ventilation (MVV) by breathing as fast and heavily as possible for 12 s. MVV values were extrapolated from these recordings.

**Echocardiography**

Echocardiography examination was done using a Vivid 7 echocardiograph (GE-VingMed, Horten, Norway) with 1.7MHz probe (M3S9) in second harmonic mode. Left ventricular ejection fraction (EF) was calculated *ad modum* Simpson (351). This method uses combined measurements from two two-dimentional planes, both running through apex and the mitral ostium, to calculate a three dimensional volume with acceptable accuracy. The calculation of EF is highly dependent on the operator. This is part of the reason why all but a few subjects had EF evaluated by the same physician. Additionally, all patients needed to meet the inclusion criteria (described in Paper 3).

**Computer tomography of the quadriceps muscle**

CT scans were performed on a HiSpeed or a LightSpeed scanner (General Electric, Paris, France). Tube voltage was 120 kV and the current 150-230 mA. The examination was
a helical scan with a 5 mm collimation and a table movement of 30 mm per rotation, from spina iliaca anterior inferior through patella on both thighs. The rotation time was 0.7 s and 0.8 s for the HiSpeed and LightSpeed scanner respectively. 5 mm slices were reconstructed every 5 mm. For reconstruction a standard algorithm and a 512 X 512 matrix were used and the scan field of view was 35-47 cm, resulting in a pixel size of 0.47 – 0.84 mm². Skeletal muscle has an average density of 30-80 HU but different studies have used different thresholds for HU (254, 262). However, when taking into account noise and streaky artifacts from the adjacent femoral bone, a threshold of -29 to150 HU has been found to agree best with skeletal muscle (262). The quadriceps muscle volume was calculated using an Advantage Workstation (General Electric, Paris, France). The muscle was outlined manually on selected slices and automatically interpolated on the interspaced slices by the software. The outlining was then corrected and confirmed by the operator. The calculated volume can be recalculated to weight by multiplying with the specific weight of muscle (1.049 kg/l).

**Skeletal muscle biopsy**

Percutaneous needle biopsy of the vastus lateralis part of the quadriceps muscle was done in sterile conditions. After anaesthetizing the skin locally (Xylocain adrenaline, 10 mg/ml + 5 μg/ml, AstraZeneca), a 10 mm long incision was made laterally on the thigh where the muscle belly was largest. A 6 mm Pelomi needle (Albertslund, Denmark) with manual suction was used to take the muscle samples in three different directions (proximally, distally and medially). The biopsy mass varied greatly between the test subjects, approximately 30 to 150 mg per biopsy. The biopsies were rinsed as quickly as possible in saline. Fat and connective tissue were carefully removed. Muscle samples for immunohistochemistry were frozen in isopentane on dry ice, while handling of samples for preparation of SR vesicles for measurements of Ca²⁺ uptake and release are described in section 6.5. All preparations were stored at -80°C until analysis. After the procedure the incision was compressed and taped.
The vastus lateralis musculature is not homologous, and there is various fiber type composition in different parts of the muscle (217). Other qualities are also probably unevenly distributed. This is an important limitation, as different analyses are done on tissue from different locations.

**Training protocol**

One-legged dynamic knee extensor exercise was done on an ergometer, designed according to Andersen *et al.* (17) and modified by Hallén *et al.* (152). This setup restricts contractions to the quadriceps muscle unilaterally (17), leaving the other leg as a control. Also, the limited muscle mass engaged will not increase CO, so that a reduced CO that characterizes the heart failure patients does not limit the localized muscle training (237). High intensity (HI) exercise was defined to the workload where the test person was exhausted after 20 min of exercise. Low intensity (LI) exercise (~70% of HI) consisted of 60 min exercise while moderate intensity (MI; ~80% of HI) consisted of a 20 min warm up at LI followed by a 40 min exercise at MI. Both these workloads led to tiredness, but not exhaustion. The 20 min of exhausting exercise at HI was initiated by 10 min warm-up and concluded with 10 min cool down, both at LI. Two LI and one MI and HI exercise sessions were done on a weekly basis. All exercise was performed at 60 rounds per minute, with visual feedback provided on a computer screen.

Voluntary work is to a large extent dependent on peer response. All test subjects were verbally encouraged during the test situation, but it is of course possible that all subjects did not receive the same amount of cheering, or that daily motivation for individual subjects were low. This could lead to seemingly poorer performance.

**Peak torque and peak power**

In Paper 4, maximal voluntary isokinetic strength (peak torque) was tested before and after the training period on an isokinetic dynamometer (REV9000, Technogym®, Italy). The range of motion was set to a knee angle from 20° to 90°, and the angle speed to 60° s⁻¹.
Warm up was done on an ordinary cycle ergometer (50 W, 10 min). Peak torque was defined as the highest torque from the 3 trails.

Peak power was tested on both the trained and untrained leg on the knee extension ergometer already described. The tests were performed after the 6 week exercise period on two consecutive days. A stepwise incremental protocol was used and start load was individually adjusted to cause exhaustion between 4 to 10 min after the start of testing. The load was increased by 2 W-min\(^{-1}\) until exhaustion. A physician was always present during the testing of CHF patients. At exhaustion researchers registered the time period of how long the last load was sustained, and included this information when peak power was calculated.

It is not customary to use one leg only on an ordinary cycle ergometer, and there is a great deal of learning involved the first several times it is done. This could represent a problem, but the subjects in Paper 4 were well acquainted with this kind of exercise from the practice built into the exercise protocol. Also, the trained and untrained leg was tested one after the other alternately on the first day.

6.3 **Metabolites**

In the soleus muscle from rats, the muscle content of ATP, ADP, AMP, NAD, CrP, and lactate were analyzed (Paper 1 and 2). Muscle tissue was pulverized in a mortar cooled with liquid N\(_2\), as described by Lowry and Passonneau (228). Metabolites were extracted from the muscle powder by incubation in ice cold 3 M perchloric acid (PCA). After being neutralized with KHCO\(_3\), the samples were stored at -80°C before enzymatic analysis or analysis by high-performance liquid chromatography (HPLC).

Using a fluorometric method on micro titer plates, lactate was determined in the neutral extract by measuring increase in NADH fluorescence when incubating the sample with an excess amount of NAD\(^+\) and lactate dehydrogenase:
To pull the reaction to the right, a second step is added:

\[
\text{Lactate} + \text{NAD}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{Pyruvat} + \text{NADH} + \text{H}^+
\]

HPLC was used to quantify muscle ATP, ADP and CrP content. This method, described by Sellevold et al. (330), is used for separation and quantification of a wide variety of substances and utilizes a column that holds chromatographic packing material (stationary phase). The sample is injected into the flowing stream of liquid (mobile phase) that sweeps the sample through the stationary phase. Substances bind to the stationary phase with different affinity as the mobile phase continues to flow through the column. The period before the different substances in the sample reach a detector showing the retention time varies in length depending on the interactions between the stationary phase and the molecules being analyzed. The different metabolites are represented by individual peaks on the chromatography.

Breakdown of CrP leads to a stoichiometric increase in Pi (205). The relative increase in Pi in exercised muscle can thus be calculated by estimating the reduction in CrP in the exercised leg relative to the resting contralateral control muscle. By assuming a [Pi] in resting slow twitch skeletal muscle, the actual concentration of Pi can be estimated in the exercised muscle. In Paper 1 this concentration was set to 9.98±0.47 mmol*kg⁻¹ dry weight (104). In a series of experiments (n=18) the water content in SOL was estimated to 75.48±0.82%. This fraction was used to determine the Pi concentration in wet muscle.

### 6.4 Citrate synthase activity

Enzyme activity was determined according to an established spectrophotometric assay (349). Human vastus lateralis biopsies (Paper 4) were homogenized in 0.5 ml 50 mM Tris-HCl at pH 8.1. 20 μl 10% Triton X-100 was added in the homogenization process. 10 μl
20 mM DTNB (5,5′-ditiobis-(2-nitrobenzoic acid)) was also added before the reaction was initiated by adding 5 μl 10 mM acetyl-CoA and 5 μl 100 mM oxaloacetat.

\[
\text{Acetyl-CoA} + \text{Oxaloacetat} \xrightarrow{\text{citrate synthase}} \text{Citrate} + \text{CoA-SH} + H^+ 
\]

The added DTNB reacts with the sulfhydryl group in CoA-SH (335), producing a free mercaptide ion that was measured at 412 nm.

### 6.5 Ca\textsuperscript{2+} uptake, release and leak

Measurements were done on crude homogenates from human vastus lateralis (Paper 4), based on methods originated by O’Brien (279), and modified by Li et al. (219). After quickly removing fat and connective tissue, the muscle was weighted and homogenized (Polytron 1200, Kinematica AG, Luzern, Switzerland) in an ice cold buffer (1:10 w/ w, vol/ vol, pH 7.9) containing sucrose (300 mM), sodium azide (NaN\textsubscript{3}, 5 mM), EDTA (1 mM), L-histidine (40 mM) and Tris HCl (40 mM) at 25000 rpm for 3 x 20 s with a 20 s break between bursts. The buffer was supplied with a phosphatase inhibitor (P2850, Sigma-Aldrich, Oslo, Norway).

The Ca\textsuperscript{2+} uptake and release were measured in an assay buffer, pH 7.0 containing KCl (165 mM), Hapes (22 mM), oxalate (7.5 mM), NaN\textsubscript{3} (11 mM), TPEN (5.5 μM), MgCl\textsubscript{2} (4.5 mM) and Tris HCl (9 mM). Ca\textsuperscript{2+} fluxes were monitored using the Ca\textsuperscript{2+}-binding dye indo-1 (pentapotassium salt, Molecular Probes, Eugene, OR, USA). Indo-1 is a ratiometric indicator, meaning that [Ca\textsuperscript{2+}], can be determined from the ratio of two emission wavelengths. Fluorescence was measured using a luminescence spectrometer (LS50B, Perkin Elmer Ltd, Beaconsfield, Buckinghamshire, United Kingdom). Heated (37°C) assay buffer (2.2 ml) was added to a plastic cuvette. 1.3 μM indo-1 was added after 80 μl of just-thawed vortexed homogenate. The mix was illuminated by a xenon lamp with light at 349 nm. Emitted light was measured at 405 and 495 nm. The 405 to 495 nm ratio was sampled at 25 Hz, smoothed using Savitzky-Golay (approx. 0.7%), splined (order:3, knots:10) (Figure 6).
(TableCurve 2D, version 5.01, Systat Software Inc, San Jose, CA, USA) and then used to calculate $[\text{Ca}^{2+}]$ from the following equation (150):

$$[\text{Ca}^{2+}] = K_d \left( \frac{(R - R_{\text{min}})/(R_{\text{max}} - R)}{S_{\text{f2}}/S_{\text{b2}}} \right)$$

where $K_d$ is the dissociation constant of indo-1 and $S_{\text{f2}}/S_{\text{b2}}$ is the ratio measured fluorescence intensity at 495 nm when indo-1 is $\text{Ca}^{2+}$ free or saturated respectively. $R_{\text{min}}$ is the ratio at very low $[\text{Ca}^{2+}]_i$ and $R_{\text{max}}$ is the ratio at saturating $[\text{Ca}^{2+}]_i$, obtained by adding 3.3 mM EGTA and 4.8 mM CaCl$_2$ respectively to the cuvette at the end of each experiment. 1.0 μm free $\text{Ca}^{2+}$ and 1.1 mM MgATP were added to initiate $\text{Ca}^{2+}$ uptake by SERCA, and 1.5 μM thapsigargin was used to block the pump after uptake had leveled off. 5.5 mM 4-chloro-<em>m</em>-cresol (4-CmC) initiated $\text{Ca}^{2+}$ release through RyR. The 405 to 495 nm ratio recordings between the addition of thapsigargin and 4-CmC were fitted to the linear equation $y = ax + b$ (TableCurve 2D, version 5.01, Systat Software Inc, San Jose, CA, USA) and the slope of this line represented the leak of $\text{Ca}^{2+}$ from a fully loaded SR vesicle. The derived $[\text{Ca}^{2+}]$ curve after adding MgATP and 4-CmC was the $\text{Ca}^{2+}$ uptake and release rates respectively. $\text{Ca}^{2+}$ uptake was calculated as rate at $[\text{Ca}^{2+}]_i = 0.3 \mu M$ and release as maximal rate.

![Figure 6. Ca$^{2+}$ handling. Ratio in black and grey from a typical trial.](image)

Ratio was smoothed using Savitzky-Golay (red) and spline estimation (green). See text for details.
The fluorophore indo-1 was chosen because it has a $K_d$ in the range of interest ($[Ca^{2+}] = 130 \text{ nM (375)}$. Widely used $Ca^{2+}$ indicators, like Fluo-3 and Fluo-4, were not used. This is partly because their $K_d$ is somewhat higher than optimal (about $[Ca^{2+}] = 350 \text{ nM for both}$), and because there are no filter sets that correspond to the emission qualities of these indicators and fit with the luminescence spectrometer that was used. Another ratiometric indicator, fura-2, has both an acceptable $K_d$ ($[Ca^{2+}] = 224 \text{ nM (150)}$) and can be analyzed with the luminescence spectrometer, but was not used either. Indo-1 and fura-2 are generally considered to be interchangeable, so indo-1 was chosen mainly because the institute has experience in using this fluorophore.

With this method, there is no good way to ensure that you have a similar amount of SR vesicles. Differences in rates and leak can thus be due to different SR vesicle content. However, each sample analyzed contains an identical mass of protein, probably with the same SR density. Furthermore, the left and right leg before exercise showed no differences in any parameter measured with this method, thus confirming its accuracy.

6.6 Protein immunoblot

This is a semi-quantitative method that makes use of selective antibodies which bind to their corresponding antigen on a specific protein. Proteins are first separated by sodium-dodecyl-sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) before being blotted over to a membrane and then incubated with the desired antibody (see Table 5). The amount of antibody binding was determined by the binding of an enzyme-linked secondary antibody that gave rise to a luminescent product detectable by a light sensitive video camera (LAS-1000 or LAS-4000, Fujifilm, Stockholm, Sweden). Density and area of the bands or spots were calculated using commercial software (ImageQuant, GE Healthcare, Oslo, Norway).

Some methodological considerations regarding protein immunoblot include the following:
1. Antibody-antigen affinity is specific to each antibody, so the amount of protein loaded on the gel must be adjusted adequately for each antibody used.
2. The appearance of a single band indicates a high specificity of the antibody since other proteins are not labeled.

3. It is not usually possible to calculate protein concentrations; however, relative amounts can be compared in two groups.

4. Comparisons should always be made on the same blot due to large blot-to-blot variability.

5. Small changes in immunolabelling should be interpreted with care.

Table 5. Primary and secondary antibodies used for protein immunoblot

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Product nr</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti-SERCA1</td>
<td>MA3-912</td>
<td>Affinity Bioreagents, CO, USA</td>
</tr>
<tr>
<td>anti-SERCA2</td>
<td>MA3-919</td>
<td>Affinity Bioreagents, CO, USA</td>
</tr>
<tr>
<td>anti-Phospholamban total</td>
<td>MA3-922</td>
<td>Affinity Bioreagents, CO, USA</td>
</tr>
<tr>
<td>anti-PLB phosphor-Ser16</td>
<td>A010-12</td>
<td>Badrilla, Leeds, UK</td>
</tr>
<tr>
<td>anti-PLB phosphor-Thr17</td>
<td>A010-13</td>
<td>Badrilla, Leeds, UK</td>
</tr>
<tr>
<td>anti-MLC2</td>
<td>ALX-BC-1150-S</td>
<td>Alexis, AH Diagnostics, Oslo, Norway</td>
</tr>
<tr>
<td>Secondary antibodies</td>
<td></td>
<td></td>
</tr>
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<td>antimouse HRP-conjugate</td>
<td>NA-931</td>
<td>Amersham, Oakville, Ontario, Canada</td>
</tr>
<tr>
<td>antirabbit HRP-conjugate</td>
<td>NA-934</td>
<td>Amersham, Oakville, Ontario, Canada</td>
</tr>
</tbody>
</table>

6.7 Phosphoprotein gel stain

After separating proteins on glycerol/SDS-PAGE gel (see above) the gel was incubated with Pro-Q Diamond Phosphoprotein gel stain (Molecular Probes, P33300, invitrogen, Oslo) for 90 min and scanned in a Typhoon laser scanner (excitation/emission: 532/580 nm, Typhoon 9410, GE Healthcare, Oslo, Norway). This stain binds to all phosphorylated amino acid residues in a protein, both serine, threonine and tyrosin. Other proteins with the same molecular weight could therefore yield false results, but MLC and RyR were quantified (4-15% gradient gel), both making distinct bands, clearly separated from other proteins. To quantify total protein content, the same gel was then incubated with SYPRO Ruby (Molecular Probes, S12000, invitrogen, Oslo) overnight, and scanned again (excitation/emission: 457/610 nm). Quantification of bands was done using ImageQuant (GE Healthcare, Oslo,
Norway). The amount of phosphorylation was then related to total protein content. As with protein immunoblots, this is a semi-quantitative method, so it is not possible to calculate protein concentrations, and comparisons should always be made on the same blot. This method has also gained some criticism in regard to the binding of Pro-Q to acidic residues in the bands on the gel, which increases fluorescence. However, this is a minor problem, as the ratio between intervention and the control sample were always calculated so that potential background fluorescence would most likely be cancelled out.

6.8 Near-infrared spectroscopy

Rat SOL (Paper 1) were harvested after 100 s and 15 min of dynamic exercise and frozen in liquid nitrogen within 10 s. The non-working soleus muscle of the contralateral leg served as control. Five animals underwent the operation and stimulation protocol but with the blood supply to soleus severed. These animals served as ischemic controls (ISCH).

Immediately prior to analysis, samples were carefully thawed in ice cold paraffin oil. Diffuse reflectance near infrared spectra were collected in the spectral range of 400-2500 nm (32 scans) using a FOSS XDS™ near infrared analyzer (Foss NIRSystems Inc., Silver Spring, MD, USA) equipped with a OptiProbe module featuring a reflectance probe. Spectras were obtained at a spectral resolution of 0.5 nm, and reference scans using the built-in internal reference. Five replicate spectra were collected for each muscle. They were obtained by making contact between the reflectance probe and the muscle that was placed on aluminium foil and kept at 0°C. The probe was thoroughly washed between every sample measurement. Reflectance (R) was defined as R=I/I₀, where I is the intensity of reflected light and I₀ is intensity of light emitted to the muscle. Oxygenation status of hemoglobin (HbO₂) and cytochrome aa₃ (Figure 7) was measured according to Wray et al. (410):

\[
\begin{align*}
\text{HbO}_2 &= -0.499*A_{778} - 1.756*A_{813} + 2.577*A_{867} \\
\text{HbR} &= 1.768*A_{778} - 0.877*A_{813} - 0.421*A_{867} \\
\text{Cyt. aa₃ redox} &= -0.559*A_{778} + 1.659*A_{813} - 0.949*A_{867}
\end{align*}
\]

where \( A_n \) are absorption measurements at wavelength \( n \).
Figure 7. Near-infrared spectroscopy. Representative tracings of resting control fibers, working fibers after 100 s and 15 min, and working ischemic fibers.

Myoglobin (Mb) and hemoglobin has similar absorption spectras (123), so the measured HbO₂ level more accurately reflects skeletal muscle oxygen saturation as a whole (combined Hb and Mb oxygenation; SO₂). SO₂ in resting skeletal muscle tissue and in exercising ISCH muscle was set to 70% and 10% respectively (30). SO₂ in normoxic exercising muscles (100 s and 15 min) where calculated relative to these assumptions. During oxidative phosphorylation cytochrome aa₃ (cytochrome c oxidase; terminal member of the respiratory chain) reduces 90% of the oxygen in skeletal muscle (292). Monitoring the redox state of cytochrome aa₃ therefore reflects oxygen availability in the tissue.

There are several issues to consider regarding this methodology:

1. It can be argued that the oxygen saturation is not maintained at end-of-exercise levels when analyzing. This would be a problem foremost in fibers with relatively high SO₂ and not so much in tissue depleted of oxygen, simply because there is less O₂ to lose. The measurements in tissue with high SO₂ would be artificially low, meaning that the difference in SO₂ between working muscle and ISCH in reality is even bigger.

2. Blood covering the muscle tissue would interfere with reflectance, so every muscle was carefully wiped free of blood before being plunged into liquid nitrogen. This was done as quickly as possible.
3. Other components of the soleus muscle could contribute to the reflectance at the wavelengths of interest. This is, however, a factor that probably would be similar in every muscle tissue sample. Since ratios are presented, this will not interfere with the results.

4. The soleus muscle has a broad tendon part on either side. If the probe was placed over this part of the muscle, it was readily identified in the resulting scan. When this happened, the scan was discarded and a new one obtained.

6.9 Statistics

Data are presented as means ± SEM. Any p value less than 0.05 was considered to be statistically significant. In Paper 1, changes over time between pre- and post-recovery protocols were tested by comparing the means of the area under the curve with a Student’s paired t test (248). In Paper 4 we have a repeated measures design, where measurements are repeated for each individual, specifically by measuring pre and post training for both trained an untrained leg. Repeated measures ANOVA analysis with training (pre/post) and leg (trained/untrained) as factors was considered. In our approach we instead related (either as ratios or as delta values) the post training measurements directly to the corresponding pre training value and then performed t-test between the legs. The pre/post ratios were log-transformed to approximate the data to normal distribution before t-test was performed. Our approach gives the same power and specificity as the repeated measurements ANOVA. Dealing with humans, with presumable large genetic variability between individuals, multiple testing correction like Bonferroni or even less strict corrections like Benjamini Hochbergs False Discovery Rate (FDR) approach (34), can make it very hard to detect differences. As the purpose of the study in Paper 4 was to screen for the potential contribution of altered Ca\textsuperscript{2+} handling to the skeletal muscle dysfunction in heart failure patients, the main results were presented without such multiple testing correction. Statistics were performed on either Statistica (StatSoft, Inc. (2007). STATISTIC, version 8.0, Tulsa, OK, USA) or Microsoft Excel 2007.
7 Results and discussion

7.1 Introductory comments

Isotonic and isometric muscle stimulation

Reduced $F_{\text{max}}$ is traditionally considered to be the main indicator of fatigue during isometric muscle activity (13, 20, 117, 126). If only $F_{\text{max}}$ and other traditional fatigue parameters, like shortening velocity, were evaluated, these parameters would indicate that 15 min of dynamic exercise do not lead to fatigue (Paper 1). This assumption, however, is contradicted by the muscles’ marked reduction in shortening capacity; consequently, we argue that the traditional definition of fatigue is inadequate. Few experimental fatigue studies have examined muscle shortening, and as a result, a knowledge gap exists regarding muscle shortening and fatigue; however, the most recent definition of fatigue is less loyal to traditional definitions in stating that fatigue is “any decline in muscle performance associated with muscle activity” (13). Only when a muscle is shortening against a load is it actually doing work, because work ($W$) is defined as force ($F$) multiplied by distance ($W = F*d$) ($[N][m] = [Nm] = [J]$). The classic work by Fenn (121, 122) stated that “whenever a muscle shortens upon stimulation and does work in lifting a weight, an extra amount of energy is mobilized which does not appear in an isometric contraction”. Although every aspect of “the Fenn-effect” is not generally accepted (263, 303), there is an agreement that a shortening and working muscle liberates more energy than a muscle that does not shorten upon stimulation. To bring about shortening of a muscle, myosin must undergo a conformational change leading to lever arm movement (see section 3.1.3). This process is probably different from what happens during isometric contractions, but as Swenney and Houdusse point out: “although […] studies support a role for swinging of the lever arm in the generation of force and movement, they do not address whether or not force production is directly coupled to lever arm movement” (367). Consequently, shortening and force production does not necessarily reflect the same cellular mechanisms and surprisingly few experiments are done where the two processes are compared.
To illustrate that shortening and development of force by the skeletal muscle do not reflect the same qualities, a muscle was stimulated isometrically and isotonically at different frequencies (Figure 8). The most striking result from these pilot experiments is that maximal isometric force rises from 1 to 10Hz stimulation frequency without a corresponding increase in maximal shortening capacity.

Figure 8. Shortening and force production in soleus muscle of rat

Soleus muscles (n=4) were prepared as described in section 6.1 and stimulated at different frequencies against a very low afterload (about 25-30 mN) to test maximal shortening capacity, and analyzed isometrically to test maximal force production. Representative tracings of selected frequencies are shown in Panel A-F. Black lines are force, gray are shortening. G, Force and shortening pair obtained from different frequencies. H, Force-frequency (black) and length-frequency (gray) relationships. Dots represent the ratio between shortening and force.
When fatigue is studied experimentally, the power of the skeletal muscle is frequently reported. Power (P) is defined as work per time (t) ((F*d)/t = W/t = P) ([Nm/s] = [J/s] = [W]). The conventional way to measure power has been to base calculations on the force-velocity curve. This curve is obtained by load clamping a tetanically stimulated muscle preparation at different loads and measure the resulting shortening velocities (46). Velocity is plotted against load and fitted to the Hill equation (F + a)(V + b) = (F₀ + a)b (169) where a and b are constants, F is force, V is velocity and F₀ is peak isometric force. Power is calculated by multiplying the force and the corresponding velocity (d/t), (F*(d/t) = (F*d)/t = W/t = P) ([N*m/s] = [Nm/s] = [J/s] = [W]) (Figure 9).

**Figure 9. Calculation of power.**

A-D, shortening (upper panels) and force development (lower panel) of *in situ* stimulated (100Hz) soleus muscle at variable afterload (75, 50, 33 and 25% are shown). Gray line in lower panel A represents maximal isometric force (F_max). Maximal shortening velocities are shown in straight lines in upper panels (A-D), and plotted against afterload in Panel E. F, power plot obtained by multiplying velocity with corresponding load from Panel E. G, actual work done by the muscle during 1 s of stimulation (shortening multiplied by afterload). H, power plot based on the work plot, G (work divided by time).
The limitation to the traditional way to calculate power lies in the fact that it only indirectly takes the work performed by the muscle into consideration. In addition, power will be unaffected by the time used in contraction phase. If shortening speed is constant (as in the work by Edman et al. (114)) it can be argued that peak power (maximal power at a given load) can be calculated as described, but it is confusing since power per definition is “work per time”. Also, shortening contractions do not usually start at fully Ca$^{2+}$ loaded myofilaments, and conversely, shortening speed will not necessarily be constant throughout the shortening phase. We propose that a calculation of power based on actual work will reflect muscle function more closely (Figure 9). Further, since locomotive muscles normally shorten upon stimulation and because force production and shortening seem to be regulated differently (Figure 8), shortening and work should be included as standard fatigue parameters.

**Maintaining appropriate temperature**

Muscles are slower and weaker at low temperatures and tend to fatigue faster at lower (e.g. 21°C) compared to higher (37°C) temperatures (304, 312). Moreover, the effects of different factors on muscle mechanics are also temperature dependent. More specifically, the effect of $P_i$ on cross-bridge force development seems to be less marked as temperature increases (78, 91), whereas the effect on myofibrillar Ca$^{2+}$ sensitivity is larger at 30°C compared to 15°C (92). Deleterious effects of high H$^+$ on force production diminishes with increasing temperature, while the effect on $V_0$ (200) and contribution of ROS to fatigue increase with higher temperatures (116, 264). Recently it has also been shown that phosphorylation of MLC2 in fast twitch fibers limits shortening velocity more at 30°C compared to at 10°C (192). Also MLC phosphorylation has been found to increase tension of skeletal muscle fibers more at 23°C than at 15°C (366). The effects of MLC2 phosphorylation could partly be due to a disorientation of myosin heads that will increase the probability of myosin – actin crossbinding (366). At lower temperatures, myosin heads already are disordered (414). Thus, the effects of MLC phosphorylation would be expected to be more evident at high compared to low temperatures. Posttetanic twitch potentiation (a
phenomenon attributed to MLC phosphorylation) is also greater at higher temperatures (203).

As many of the hypothesized key players in skeletal muscle fatigue seem to be temperature dependent, choosing an appropriate temperature in the experimental setup is crucial when it comes to interpretations of the results. The temperatures in Paper 1 and 2 are set to give the appropriate skeletal muscle core temperature of an exercising muscle.

7.2 Fatigue in shortening skeletal muscle

To our knowledge, very few other animal studies have examined muscle fatigue development during controlled shortening contractions (15, 388), and thus, causes of fatigue in shortening slow twitch muscle during aerobic conditions at physiological temperature may have escaped detection. Some of these mechanisms have been studied further in Paper 1.

During isotonic muscle activity, fatigue is seen as a gradual reduction of shortening ($S_{max}$), which is in striking contrast to the maintained $F_{max}$. Reduction of $S_{max}$ is temporally paralleled by reduced phosphorylation of MLC2s. There is a transient decline of the rate of isometric relaxation and the unloaded shortening velocity peaking at 100 s. This takes place without a reduction in muscle tissue oxygen saturation but is paralleled by lactacidosis and a reduction of CrP and ATP. During a repeated exercise protocol following 15 min of recovery, muscle performance was significantly superior to the initial fatigue protocol. This effect was lost after 1 hour.

Metabolites

Several experimental NMR studies report a 50% reduction in [CrP] following 2 min of isometric low frequency stimulation (19, 374). Considering that the energy cost from dynamic muscle performance is assumed to be about 2.7 greater than the energy cost from isometric contractions (299) and thus creates a higher oxygen demand, it is not surprising that the concentrations of ATP and CrP already drop during the initial 100 s of exercise (Paper 1 and 2). However, in Paper 1 there is a trend towards regeneration of CrP during stimulation.
is unexpected, since ATP production that is dependent solely on muscle metabolism without CrP buffering taking place at the same time is thought to be too slow to maintain ATP during contractions (383). The surprising tendency towards an increase in CrP during stimulation may be interpreted in terms of differences between isometric and shortening contractions.

It is important to maintain sufficient skeletal muscle blood supply during exercise. This ensure a sufficient oxygen delivery to and removal of waste products from the muscle metabolism. Isometric stimulations compromises blood flow during stimulation, and depending on the duty cycle, blood supply could be noticeably limited during exercise. This could contribute to fatigue during isometric stimulation protocols (24, 344). In submaximal dynamic exercise, however, there could be fewer restrictions on blood supply, leaving the cell fully saturated with oxygen throughout the exercise period (NIR experiments, Paper 1). This constitutes a potential difference between isometric and isotonic exercise that could affect the fate of [CrP] during exercise.

The activation of mitochondrial enzymes is slowed in comparison to the elevated work rate. This is embodied in the “oxygen deficit” concept, and it has been shown that the accumulation of lactate and utilisation of CrP can be reduced by activating PDH prior to exercise (376). This can also be achieved by high intensity exercise prior to activity. NIR experiments have shown that the muscle tissue and the respiratory chain are fully oxygenated during the shortening contractions in Paper 1. At 100 s, the mitochondria will thus have a high oxidation capacity, but at the same time will have only limited access to acetyl-CoA due to a possible lag in activation of PDH. The energy consumption from shortening contractions is higher compared to isometric contractions, so it could be speculated that shortening contractions activate PDH to a greater extent and thus increase oxidative phosphorylation and production of high energy metabolites to a rate superior to the consumption rate. This would stabilize and ultimately even increase levels of CrP in the muscle.
As shortening capacity drops, paralleled by a reduced phosphorylation of MLC2s, so does the oxygen demand. It could be speculated that the regulation of MLC2s represents a “physiological brake” on the muscle. Skeletal muscle shortening could be reduced due to dephosphorylation of MLC2 thereby dramatically reducing energy expenditure. This makes possible a continued aerobic metabolism despite a high motoneuron firing rate, and ultimately increases endurance. In this way, dephosphorylation of MLC2s has a protective effect on type I fibers, making them even more fatigue resistant to shortening in comparison to isometric contractions. With reduced shortening capacity, total energy consumption also decreases, to some extent explaining how [CrP] nominally increase even during contraction (Paper 1).

![Figure 10](image.png)

**Figure 10. Schematic diagram illustrating the work intensity and aerobic metabolism during dynamic exercise.**

In shortening slow twitch skeletal muscle *in situ* at physiological temperatures, reduced CrP and ATP and accumulation of Pi and lactate seem to affect contractile parameters, both of force production and shortening.

**Ca\(^{2+}\) handling**

Since the Ca\(^{2+}\) ion plays a key role in activation of the myofilaments (see section 3.1.2), many researchers hold altered Ca\(^{2+}\) handling to be a probable cause of fatigue. Potentially, all proteins engaged in Ca\(^{2+}\) handling could contribute to fatigue, but it has recently been
proposed that exercise can lead to phosphorylation of RyR, resulting in dissociation of Calstabin 1 from the receptor complex. This, in turn, could make the receptor leaky, so that Ca\(^{2+}\) escapes SR even through a “closed” RyR (33). During exercise there is an excitation of sympaticus because of a central command, but also due to a local exercise pressor effect (249, 313) that potentially could bring about RyR phosphorylation. Thus, exercise could be limited because an elevated resting cytosolic Ca\(^{2+}\) level triggers the contractile machinery, slowing down relaxation. The increase in base line tension (T\(_{bi}\), Paper 1 and 2) could, in this way, be a result of a leaky RyRs. Further, training in elderly healthy male subjects is correlated to a tendency toward decreased RyR phosphorylation and a lower Ca\(^{2+}\) leak, which could increase fatigue tolerance (Paper 4).

**MLC2s dephosphorylation**

MLC2s is dephosphorylated in response to shortening contractions in slow twitch skeletal muscle (Paper 1 and 2). In contrast, the literature is more or less unanimous in reporting an increased phosphorylation after stimulation. This is reported in a variety of species, like frog, rabbit and rat (23, 242, 359), and at different temperatures (23 - 37°C (242, 265)). MLC2 phosphorylation has been shown to have limited effects in slow twitch compared to fast twitch fibers, maybe due to a blunted kinase and enhanced phosphatase activity in slow twitch fibers (265). Humans, however, seem to lack the difference between the fiber types (176).

Following 100 s of dynamic exercise, MLC2s in SOL was dephosphorylated by 49% relative to control muscle in healthy animals (Paper 1) and by 23, 31 and 36% in sham, chf1 and chf2 respectively (Paper 2). It is interesting that it has been impossible to dephosphorylate MLC2s more than 75% in rats, maybe because the remainder phospho group is mechanically hidden in some way, inaccessible to phosphatases (141). The experimental setup in Paper 1 and 2 is, however, different from other experiments and could explain why our results on MLC2 phosphorylation are different from what is reported in literature. First, and maybe most importantly, the muscle in Paper 1 and 2 was allowed to
shorten when stimulated. Shortening contractions could activate regulators that will not be triggered in isometric contractions. These regulators could be mechanically stimulated. Also, such contractions are more energetically costly than isometric contractions and might trigger signaling pathways more profoundly compared to isometric contractions. Probably also very important for the differences seen in MLC2 phosphorylation is the fiber type specific relationship between kinases and phosphatases that in slow twitch fibers, are tilted in favor of dephosphorylation (265). Lastly, although probably not very important for the result, one should note that previous papers often have measured phosphorylation differently from how it is done in Paper 1 and 2. Both isotope labeling (23) and separation of phospho proteins on pH gradient gels (199) have been used.

The reduced phosphorylation of MLC2s in shortening slow twitch skeletal muscle in situ at physiological temperatures may decrease contraction rate, shortening capacity and velocity of isotonic shortening.

### 7.3 Skeletal muscle function in CHF

#### 7.3.1 Experimental studies

Unlike most experimental studies on skeletal muscle function in heart failure animals, a dynamic exercise protocol was used where the muscle shortened during stimulation (see page 47). Since CHF patients report fatigue during activity, dynamic protocols will probably reflect skeletal muscle dysfunction more fully than isometric protocols, in which shortening does not occur. Sham animals (Paper 2) and healthy animals (Paper 1) performed similarly during dynamic exercise. SOL’s isometric relaxation rate from the severely failing animals (chf2), however, decreased less during dynamic exercise, resulting in only a modest rise in baseline tension after 100 s (17±1% of afterload compared to 26±3% for sham) and tended to shorten more (do more work) during exercise (679±63 mm in total shortening compared to 591±37 mm for sham) (Paper 2). The phenotype during dynamic exercise of SOL from chf2 animals was similar to the phenotype of SOL from unoperated rats (Paper 1) in the second
session of dynamic exercise that was performed following 15 min of rest. This is reflected in the changes in the time constants describing the isometric relaxation tracings during exercise. During the first 100 s of the first exercise protocol, the time constants for healthy animals increased from 2.9 to 17.2 ms (tau1) and 38.7 to 328.1 ms (tau2). The changes in tau1 and tau2 were smaller when the dynamic exercise protocol was repeated after 15 min of rest, increasing from 3.9 to 10.2 ms (tau1) and 37.6 to 146.0 ms (tau2) after 100 s. Thus, a prior exercise bout seems beneficial to the skeletal muscle. The time constants for severely failing animals increased from 5.3 to 8.1 ms and 38.9 to 225.8 ms the initial 100 s of exercise. Although the isometric relaxation rate during the second exercise session for control muscles decreased even less than SOL from chf2 animals, it is tempting to speculate that chf2 animals were in some way primed for exercise. Surprisingly, the failing animals seemed to perform better than sham animals and failing animals with a lower EDP (chf1). Animals with CHF might have a lower sympathetic responsiveness (372), and the local sympathoexcitation is blunted in severely failing animals (218). This could limit PKA phosphorylation of RyR in skeletal muscle of chf2 animals compared to muscles from sham and control animals. As a result Ca^{2+} leaking from SR could also be reduced in chf2. This mechanism could provide some rationale as to why the isometric relaxation rate seems to be better maintained in chf2, compared to control and sham operated animals, for which the rate drops with a resulting rise in baseline tension. Maximal isometric force ($F_{\text{max}}$) showed a different time course than the isometric relaxation. $F_{\text{max}}$ deteriorated steadily during the exercise for chf2, while it recovered for the sham group (Paper 2) and healthy animals (Paper 1) during the last part of the protocol. Previous experimental studies have mainly evaluated $F_{\text{max}}$ as a marker of fatigue. These studies typically report that $F_{\text{max}}$ decreases during muscle stimulation, so by solely assessing this parameter our findings are in line with literature.
7.3.2 Human studies

The main objective of the training study (the TRUST study, Paper 3) was to examine skeletal muscle baseline characteristics, as well as fatigability and training effects in CHF patients, and compare the results with data obtained from healthy controls (HC) matched for age and activity level. Furthermore, the study was designed to investigate the molecular mechanisms responsible for possible increased skeletal muscle fatigability in these patients. Of particular interest were alterations residing within the skeletal muscle itself. The study is described in more detail in Paper 3.

This is the first training intervention study in CHF patients to include a group of matched, healthy controls beyond the purpose of comparing baseline characteristics. Only patients with post infarction heart failure were included, because this is the most common cause of heart failure and mimicked in several experimental HF models. Also, inclusion of patients with various aetiologies would introduce confounding factors and complicate interpretation of the results. Only men were included because gender differences appear to exist in skeletal muscle properties in the CHF patients (95).

Listed in Table 2 are results from human studies indicating an increased fatigability in CHF patients. As discussed, the nature of the skeletal muscle dysfunction in CHF is at the least linked to alterations in fiber type, oxidative capacity and energy metabolism. Recent publications point to activation of AMPK as important in inducing gluconeogenesis and expression of mitochondrial genes in muscle, probably mediated by PGC1α and peroxisome proliferator-activated receptor δ (PPARδ) (272). PGC1α increases after systemic exercise in healthy (21) and CHF patients (407), but ACEi administration also elevates PGC1α mRNA levels (418). Thus, dysfunctional alterations in the PGC1α pathway might contribute to the attenuated skeletal muscle function in the heart failure patient. The pathway also provides a potential link between training and medication (see below) and improvement of the impaired skeletal muscle.
In Paper 4, CHF patients had the same peak force in the quadriceps when corrected for the muscle cross sectional area, but peak power was 30% lower in the untrained leg compared to untrained leg of HC. The reduction in peak power was also present even when correcting for CSA, and indicates that the CHF patients had increased fatigability. After the training period the difference in peak power between trained CHF muscle and untrained HC muscle was reduced by 35%, suggesting that it is partly possible to reverse skeletal muscle dysfunction in CHF with training of one muscle group independent of the effects of systemic training. This is in line with conclusions presented in Table 4. There were, however, no differences in trainability between HC and CHF. The results from Paper 4 state that reversal of dysfunction is possible in CHF patients without altered Ca\(^{2+}\) handling, while the training for healthy controls was associated with a reduction in SR Ca\(^{2+}\) leak. Thus, in line with experiments in rats (230), CHF calcium handling in humans does not appear to be the site of increased fatigability.

As in experimental studies, it has also been reported that local sympthoexcitation is attenuated in heart failure in humans (273). Thus, the lower Ca\(^{2+}\) leak in CHF patients compared to HC before training could be due to lower sympathetic stimulation and lower phosphorylation of RyR in these patients compared to the control group. Also, the use of β-blockers could affect skeletal muscle and Ca\(^{2+}\) leak by reducing RyR phosphorylation and dissociation of calstabin 1.

**Training effects**

Previous studies have shown that the beneficial effects of training on small muscle groups in CHF seem to arise mainly from increased CS activity and a more efficient CrP utilization (Table 4). Effects on Ca\(^{2+}\) handling do not seem to contribute to these beneficial effects (Paper 4). However, it could be argued that the patients in Paper 4 were too healthy (NYHA average: 2.4) for researchers to detect differences in Ca\(^{2+}\) handling that may exist in the more severe stages of heart failure. However, patients in the studies included in Table 4 have NYHA classes ranging from 1.9 to 2.6, i.e. in the same range as patients in Paper 4.
The way our human study was designed (Paper 3) would probably make it impossible to include patients with NYHA class 4, simply due to practical limitations. Furthermore, the beneficial effects of training could be more easily detected following a harder training program than was used in Paper 4. It has been shown that heart failure patients tolerate even high intensity systemic exercise quite well, so local muscle training regimens could probably be more intense than those used in Paper 4. Also, as will be discussed below, medication used by heart failure patients may affect skeletal muscle. Better guidelines for treatment of patients may in this way also reduce the skeletal muscle dysfunction in parallel with other effects.

In Paper 4, muscle samples were not obtained in relation to exercise. Therefore it is impossible to explore the role of posttranslational alterations of, for example, MLC2 in limiting exercise in CHF. It is possible that reduced phosphorylation of MLC2 with exercise (Paper 2) limits exercise capacity in patients due to desensitized adrenergic receptors in CHF (372). Training could partly reverse the dysfunctional signaling system in skeletal muscle, or in some other way lead to a more adequate MLC2 response to exercise. However, exercise in CHF rats in Paper 2 had the same effects on MLC2s phosphorylation as in the sham animals. It is impossible to conclude from these results how training in humans would affect the phosphorylation status during activity, both because the rat study did not involve training and because the enzymatic profile is different in rats and humans (176).

Systemic exercise reduces cytokines like TNFα, IL-1β and IL-6 (138, 221) and elevates PGC1α (407). What effects local muscle training will have on these substances is unknown, but it remains a possibility that training of one muscle group will also reduce the production of inflammatory cytokines in the muscle ultimately reducing the circulating concentration. This could in turn explain why there are some alterations in the untrained leg after training in Paper 4.
**Pharmacological effects**

An interesting observation in Paper 4 is that the training effect on Ca$^{2+}$ handling in HC seems to already have been achieved in CHF patients before training. For these patients, training did not alter Ca$^{2+}$ handling significantly. Maybe skeletal muscle function in CHF is partly rescued by the use of medication (Table 6)?

**Table 6. Drugs commonly used in heart failure and their effects on skeletal muscle**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Drug</th>
<th>Species (MI induction/ HF etiology)</th>
<th>Effect on skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoll et al., 2006</td>
<td>ACEI (Perindopril)</td>
<td>Rat (ligation), Gastrocnemius</td>
<td>Increase in mitochondrial respiration, Recovery of depressed CS and cytochrome oxidase</td>
</tr>
<tr>
<td>Coirault et al., 1999</td>
<td>ACEI (Perindopril)</td>
<td>Rabbit (banding), Diaphragm, Soleus</td>
<td>Limiting loss of cross-bridges, preserve strength</td>
</tr>
<tr>
<td>Shah et al., 2004</td>
<td>ACEI (Captopril, imidapril, enalapril) ATII blocker (Losartan)</td>
<td>Rat (ligation), hind leg</td>
<td>Alterations in Ca$^{2+}$ uptake and release are prevented</td>
</tr>
<tr>
<td>Vescovo et al., 1998</td>
<td>ACEI (Enalapril) ATII blocker (Losartan)</td>
<td>Human (IHD, HT, CM), Medial gastroc</td>
<td>Both drugs: Shift toward slower MHC isoform</td>
</tr>
<tr>
<td>Dalla Libera et al., 2001</td>
<td>ATII blocker (Irbesartan)</td>
<td>Rat (monocrotaline), Tibialis anterior</td>
<td>Normalization of MHC distribution, Partial improvement of atrophy, Trend toward normalization of TNFα</td>
</tr>
<tr>
<td>Dalla Libera et al., 2005</td>
<td>β-blocker (Carvedilol) - Bisoprolol</td>
<td>Rat (monocrotaline), Soleus</td>
<td>Reduced protein oxidation, Normalization of twitch and tetanic tension</td>
</tr>
<tr>
<td>Darup et al., 1988</td>
<td>Diuretics (Thiazides, loop-diuretica)</td>
<td>Human (IHD, HT), Vastus lateralis</td>
<td>Potassium and magnesium deficiencies, Reduced concentration of NKAs</td>
</tr>
</tbody>
</table>

CM = Cardiomyopathy; IHD = Ischemic heart disease; HT = Hypertension; Ligation = Coronary artery ligation; Banding = Aorta banding; Gastroc = Gastrocnemius muscle

It has been proposed that digitalis could lower oxidative enzyme activity or even down-regulate the amount of oxidative enzymes (320). Although not specifically shown in CHF patients, statins could have negative effects on skeletal muscle mitochondrial ATP-producing capacity (198). Additionally the β-blocker carvedilol downregulates NF-κB activity (416) and levels of IL-6 and TNFα (368) in the plasma of CHF patients. This could constitute a
therapeutic mechanism in the treatment of atherosclerosis. As far back as 1956 it was reported that HF severity was correlated to the level of CRP in the blood (118). The Framingham Heart study also pointed to CRP as important in identifying risk candidates for developing HF (386). Other cytokines have been shown to be produced in the heart, and the patient’s individual “inflammatory fingerprint” could, in the future, guide physicians in choosing the appropriate therapy (49).

The HF condition has detrimental effects on skeletal muscle (see section 3.5). It is likely that skeletal muscle function is also influenced pharmacologically (Table 6). This makes it hard both to evaluate human skeletal muscle dysfunction in heart failure, but also obscures the effects of training. Drug-induced changes could, however, explain why Ca$^{2+}$ handling properties in CHF patients before training were phenotypically similar to the trained leg in the HC group (Paper 4).
8 Main conclusions

The present studies reveal new insight into the cellular mechanisms governing skeletal muscle fatigue. This is the first time fatigue in animals has been studied in an in situ exercise protocol where the muscle shortens during stimulation and the temperature is kept at physiological levels. New knowledge about skeletal muscle function in heart failure is also provided.

Referring to the specific aims of the study, the conclusions are:

Conclusion 1
Fatigue following shortening contractions reduces the shortening capacity of the muscle to a greater extent than the muscle’s force generating capacity, implying that different cellular mechanisms are at work in shortening and isometric contractions. During shortening contractions maximal force production initially drops and then rises again, while shortening capacity declines steadily.

Conclusion 2
Shortening contractions give rise to a drop in ATP and CrP and dephosphorylation of MLC2s. The concentration of CrP is partially restored towards the end of the protocol. Dephosphorylation of MLC2s is associated with reduced shortening capacity but not correlated to maximal force generating capacity. Sufficient nutrient supply, activation of oxidative phosphorylation and the control of contraction by dephosphorylation of MLC2s might explain why CrP builds up even during stimulation.

Conclusion 3
Maximal isometric force declines steadily for severely failing animals during shortening contractions, whereas $F_{\text{max}}$ values for sham animals tend to recover after an initial drop. The decline in isometric relaxation rate and the resulting rise in baseline tension between stimulation trains are less marked in severely failing animals compared to sham animals.
Skeletal muscle from HF animals also tends to shorten more than the sham operated animals during shortening contractions.

**Conclusion 4**

Heart failure patients demonstrate lower skeletal muscle SR Ca\(^{2+}\) leak compared to healthy peers, and have increased fatigability. This fatigability seems unrelated to Ca\(^{2+}\) handling. Several drugs used in standard treatment of HF also affect the skeletal muscle and could influence the skeletal muscle of heart failure patients favorably.

**Conclusion 5**

Skeletal muscle from CHF patients with increased fatigability profits from local muscle training similarly to healthy skeletal muscle. However, contrasting healthy subjects, the beneficial effects of training in CHF patients seems unrelated to changes in skeletal muscle Ca\(^{2+}\) handling.

**9 Future perspectives**

In the present study we propose that skeletal muscle fatigue development is dependent on the contraction modality. Our results suggest that future experiments on skeletal muscle function and fatigue should include shortening as a fatigue parameter to evaluate the functional influence on the skeletal muscle from exercise. Since fatigue in humans is most often present in shortening muscle, this could have implications for how we understand fatigue and ultimately treat pathological processes characterized by increased fatigability, e.g. heart failure. The literature is not uniform when reporting skeletal muscle phenotypes from CHF patients. Alterations seem to depend on several factors such as medication, and the field is in need of larger controlled studies to further clarify this area.
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Paper I

Causes of fatigue in slow twitch rat skeletal muscle during dynamic activity

*Am J Physiol Regul Integr Comp Physiol* 297: R900-R910, 2009
This article is removed.
Paper II

Attenuated fatigue in slow twitch skeletal muscle during isotonic exercise in rats with chronic heart failure

Submitted
This article is removed.
Paper III

Skeletal muscle fatigue and trainability in heart failure; 
Background and design of the TRUST Study

In manus
This article is removed.
Paper IV

Training effects on skeletal muscle calcium handling in human chronic heart failure

In revision
This article is removed.