ACUTE AND CHRONIC EFFECTS OF COLD TREATMENT ON PHYSIOLOGICAL VARIABLES AND NEUROMUSCULAR FUNCTION DURING A SHORT TRAINING PERIOD IN MEN

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ABSTRACT

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Introduction. Recovery following various physical exercises is a complicated process. The exercise session can involve strong demands on both muscle structure and energy production (aerobic and anaerobic). Many exercise sessions also induce muscle damage (exercise induced muscle damage; EIMD). Different recovery methods attempt to alleviate or prevent EIMD and its associated symptoms, such as muscle soreness and swelling, and reduction in muscle function. One of the used strategies is cold treatment (cryotherapy), which decreases tissue temperature and subsequently effects on e.g. blood flow, metabolism and neural conductance velocity. To date, there is conflicting evidence to support the use of cryotherapy following a single exercise, and only few studies have examined the effect of it over a longer time period. Thus, the aim of this study was to assess the effects of cryotherapy on recovery and on the amount of EIMD both after an anaerobic running exercise and over a weeklong training period. Moreover, a recently developed cooling technique called cold mist shower (Amandan®, Amandan Healthcare Oy Ltd., www.amandan.fi, Finland, 2015) was tested.
Methods. A total of eight male subjects (age 22.9 ± 1.4 years, height 1.77 ± 0.06 m, weight 79.1 ± 7.6 kg, and fat percentage 13.3 ± 2.9 %) participated in the study, which consisted of an acute phase of 48 h and a weeklong training period. All subjects completed the study protocol both with (COLD) and without (CONTROL) cryotherapy (2 min at 10–15°C) in a random order. The acute responses were measured after an anaerobic running (10 x 20 m shuttle running twice, with a 3 min break). Cryotherapy was applied immediately, and 12, 24 and 36 h after the running exercise. The training period consisted of three hypertrophic strength training sessions and three anaerobic interval running sessions on alternating days, and cryotherapy was applied once after every training session. Muscle damage (myoglobin, creatine kinase, CK), inflammation (C-reactive protein), endocrine responses (testosterone, SHBG and cortisol) and perception of muscle soreness (DOMS) were measured before (Pre) and immediately (0 min), 30 min, 60 min, 24 h and 48 h after the running exercise, and 4 d after the training period (Post). Anaerobic metabolism (lactate) was evaluated at Pre, 0, 30 and 60 min. Maximal voluntary isometric force of the knee extensors, jumping ability (a countermovement jump), and 10 x 20 m maximal sprint running were evaluated at Pre, 48 h and Post.
Results. The intensive anaerobic running exercise (peak blood lactate in CONTROL 16.3 ± 3.6 vs. in COLD 16.2 ± 3.4 mmol/l) induced a small increase in DOMS, myoglobin and CK levels in both groups, and there were no differences between the groups ($P > 0.05$). Following the training period, myoglobin concentration was significantly ($P < 0.05$) lower in COLD compared to CONTROL (COLD 24.64 ± 5.63 ng/ml vs. CONTROL 36.35 ± 14.02 ng/ml). In COLD, the Post-value was also significantly ($P < 0.05$) lower compared to the Pre-value both in myoglobin (Pre 29.95 ± 6.90 ng/ml vs. Post 24.64 ± 5.63 ng/ml) and in CK (Pre 251.00 ± 143.83 U/l vs. Post 168.13 ± 91.90 U/l). In physical performance variables there were no significant differences between the groups.
Conclusion. The finding in myoglobin provides some evidence that the cold mist shower might decrease the amount of EIMD over a weeklong training period. However, no cold mist effects on the acute recovery after anaerobic running exercise were observed.

Keywords: cold treatment, cryotherapy, anaerobic exercise, fatigue, recovery, muscle damage, training period

LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
CK	creatine kinase
Cr	creatine
CRP	C-reactive protein
CWI	cold water immersion
E-C coupling	excitation-contraction coupling
EIMD	exercise-induced muscle damage
FAD	flavin adenine dinucleotide
IMP	inosine monophosphate
LDH	lactate dehydrogenase
NAD^+	nicotinamide adenine dinucleotide
NH_3	ammonia
PCr	phosphocreatine
P_i	inorganic phosphate
ROS	reactive oxygen species

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1 INTRODUCTION

Muscle fatigue can be described as any exercise-induced decline in the ability to produce muscle force or power, and the changes in all levels of the motor pathway from cortex to skeletal muscles possibly contribute to it. Fatigue can be classified as either central or peripheral depending on its cause. Central fatigue is a state in which the altered muscle function is due to a progressive reduction in voluntary activation of muscle, whereas peripheral fatigue is a consequence of the failures located at or distal to the neuromuscular junction. Peripheral and central fatigue may appear separately or combined depending on the specific situation. (Gandevia 2001.)

Some of the metabolic factors contributing to fatigue have also a role in the development of exercise-induced muscle damage (EIMD). The severity of EIMD is adjusted by the type, intensity, and duration of exercise being performed. (Byrne et al. 2004.) EIMD is an especially frequent phenomenon as a consequence of exercises including a large amount of eccentric muscle actions (Gibala et al. 1995) or following an unaccustomed performance of exercise with an increased intensity or duration (Thompson et al. 1999). Natural human movement rarely consists entirely of one form of muscle action, and eccentric muscle action is rather followed by a concentric one (stretch-shortening cycle, SSC). However, the mechanical effects of fatiguing SSC exercise have similar consequences to pure eccentric exercise (Komi 2000), and EIMD is a common phenomenon during prolonged or intense SSC exercise, such as marathon running (Avela et al. 1999) and resistance training (Byrne & Eston 2002a).

After the initial events of muscle damage, the inflammatory response mediated symptoms emerge as fluid and proteins leak from the capillaries into the interstitial space. These symptoms include e.g. muscle swelling, soreness and stiffness. (Merrick et al. 1999.) From the athletes' point of view, the greatest concern of EIMD is the temporary reduction in muscle function, which possibly accompanies muscle damage during the days after the exercise (Byrne et al. 2004). Thus, different recovery methods after exercise have attempted to alleviate or prevent EIMD and its associated symptoms. The treatment and recovery strategies used, either singly or in combination, include e.g.

rest, sleep, nutrition, massage, active recovery, contrast temperature water immersion, compression garments, stretching, and cold treatment. (Barnett 2006; Bompa & Haff 2009, 107–115.)

Cold treatment i.e. cryotherapy is a technique where different forms of topical cooling are used to treat acute traumatic injury and promote post-exercise recovery (Barnett 2006). The fundamental cold therapy causes is a decrease in tissue temperature, which subsequently exerts its effects on blood flow, cell swelling and metabolism and neural conductance velocity. Cryotherapy has been suggested to cause vasoconstriction, which limits fluid diffusion into the interstitial space and decreases the diffusion of myoproteins into the extracellular space, consequently diminishing oedema and the acute inflammatory response. (Herrera et al. 2010; Vaile et al. 2011; Yanagisawa et al. 2003a.) The other consequences include e.g. lowered peripheral metabolism (Ihsan et al. 2013), and decreased muscle soreness (Saeki 2002).

Previous research has focused on the role of cryotherapy on indices of muscle damage following either an eccentric exercise (Eston & Peters 1999) or a single dynamic whole-body exercise (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009). There is conflicting evidence to support the use of cryotherapy following a single exercise, and to date there are only few studies examining the effect of cold treatment over a longer time period (Halson et al. 2014; Yamane et al. 2006). Thus, the aim of this study was to investigate the effects of a cold mist shower on the recovery from both an anaerobic running exercise and a weeklong training period. The cold mist shower is a new cooling method, which is portable and an easy alternative e.g. for athletes to have cryotherapy always available post-exercise. It was hypothesised that using cryotherapy would result in enhanced recovery compared to passive recovery both following one training session and following one training microcycle.

2 METABOLIC RESPONSES TO EXERCISE AND THE ROLE OF METABOLITES IN IMPAIRED MUSCLE FUNCTION

Cells store only a small quantity of adenosine triphosphate (ATP) and therefore the body must maintain a continuous ATP supply through different metabolic pathways. ATP is resynthesized at its rate of use and the building blocks of ATP synthesis are the by-products of its breakdown: adenosine diphosphate (ADP) and inorganic phosphate (P_i). Moreover, the metabolic responses occurring as part of normal muscle activity can be considered as contributors to fatigue as they have a significant influence on the contractile function of muscle. (Lindinger 1995; Robergs et al. 2004.) This chapter will first outline the major energy metabolism pathways in muscles, and next describe the underlying mechanisms of fatigue development.

2.1 Energy metabolism in exercise

At the start of intensive exercise, the body cannot deliver oxygen to the muscles fast enough to commence the complex chemical reactions, which occur during aerobic metabolism. Therefore, the body depends on anaerobic processes for the first couple of minutes. The anaerobic system can be divided into two further systems: ATP-creatine phosphate (PCr) and lactic acid system. Aerobic system is used for long-term, steady paced exercise and it can be broken down into three sections: glycolysis, citric acid cycle and electron transport chain. All energy systems work concurrently, but the intensity and type of activity determine which system is predominant. (Robergs et al. 2004.)

2.1.1 Anaerobic and aerobic metabolism

Immediate energy from the ATP-PCr system. PCr provides a quick source to produce ATP during the onset and initial seconds of muscle contraction. In this reaction, ADP combines with P_i from the PCr, forming ATP and a creatine (Cr) molecule (Figure 1). Creatine phosphate stores, like ATP stores, are limited and it is used just for short-term

activities. Since PCr can transfer energy only interchangeably with ATP, new ATP is synthesised as long as PCr remains left. This keeps the concentration of ATP at nearly constant high level and allows the metabolic reactions to continue. (Guyton & Hall 2006, 882; Lindinger 1995; Robergs et al. 2004.)

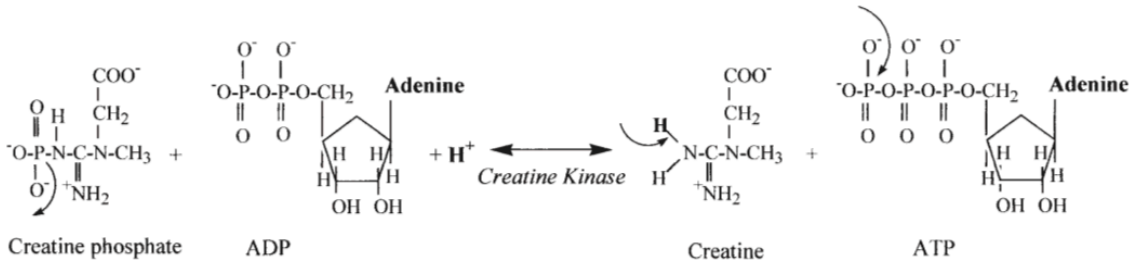

Figure 1. ATP-PCr reaction is reversible and can proceed in either direction, depending on the immediate need for ATP within the muscle cell (Robergs et al. 2004).

Robergs et al. (2004) suggest that the formed Cr acts as a cellular buffer because a proton (H^+) replaces the phosphate group of PCr, which would make the creatine kinase reaction alkalizing to the cell. The traditional view considers both the protons consumed by ATP-PCr reaction and the protons produced by the hydrolysis of the ATP supplied at the expense of PCr, and combines them into the net proton consumption. I.e. some of the processes of proton generation are canceled out by those of proton consumption, which Robergs et al. (2004) do not take into account and may overestimate the total muscle buffer capacity. The subsequent hydrolysis of the ATP supplied at the expense of PCr results in the production of the inorganic phosphate (P_i). (Kemp 2005; Westerblad et al. 2002.) However, Robergs et al. (2005) still argue that it is not valid to couple the ATP hydrolysis and ATP-PCr reactions together, and that coupling separate reactions leads to misunderstanding of proton balance.

Glycolysis. Glycolysis is a 10 step metabolic process occurring in cytosol, in which one molecule of glucose is split to two molecules of pyruvate. Along the way four molecules of ATP are formed, and two are expended to cause the initial phosphorylation of glucose to get the process going. The net gain of glycolysis is two molecules of ATP. In addition, energy-rich electrons (H^+) removed from glucose are passed to nicotinamide adenine dinucleotide (NAD^+), generating NADH. In metabolism, NADH serves as a reducing agent and carries electrons from one reaction to another. Similar to NAD^+ is flavin adenine dinucleotide (FAD), which can be

reduced to $FADH_2$. Glycolysis does not require oxygen and is therefore possible both in aerobic and anaerobic conditions. (Robergs et al. 2004.)

Anaerobic metabolism: the lactic acid system. The lactic acid system, like the ATP-PCr system, provides a rapid supply of ATP energy. Pyruvate, the final product of glycolysis, is converted into lactate when oxygen is absent (Figure 2). This reaction is catalyzed by lactate dehydrogenase (LDH) and concurrently NADH is converted into NAD^+. As forming lactate requires electrons from NADH, electron carriers (NAD^+) are made available to further accept the electrons removed from glucose. This thereby allows continued ATP regeneration from glycolysis and delays acidosis. Furthermore, Robergs et al. (2004) claim that for every pyruvate molecule catalyzed to lactate and NAD^+, there is a proton consumed, which would make this reaction alkalizing to the cell.

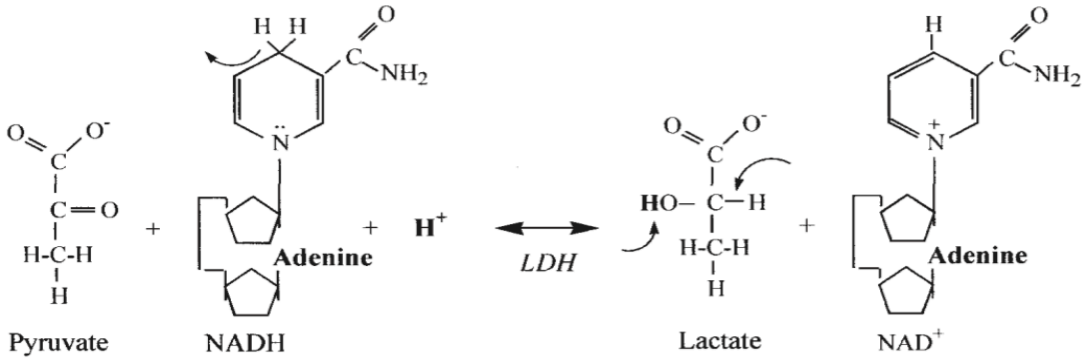

Figure 2. The lactic acid system: two electrons and a proton are removed from NADH and a proton is consumed from solution to reduce pyruvate to lactate (Robergs et al. 2004).

Since lactate is produced in anaerobic conditions, the blood lactate concentration is a useful marker of exercise intensity and adaptation to training. In fact, training accelerates lactate clearance, reduces lactate accumulation at a specified workload and results in a greater level of lactate accumulation during maximal effort. The optimal performance for aerobic exercise occurs, as the exercise intensity of maximal lactate clearance is equal to maximal lactate production. Moreover, the formed lactate provides a precursor to synthesize carbohydrate via the Cori cycle in liver and kidneys, which supports blood glucose levels and the energy requirements during exercise. Lactate may also be oxidazed back to pyruvate in other muscle cells than it is produced and used to fuel the citric acid circle. Processing lactate has a crucial role in enabling continued ATP supply from glycolysis. Therefore, for example active recovery is used after

exercise to increase blood flow through the lactate-using tissues and facilitate lactate removal. (Bompa & Haff 2009, 108; McArdle et al. 2010, 149, 163–164, 176, 232.)

Aerobic system: Citric acid cycle and electron transport chain. In aerobic conditions pyruvate formed in glycolysis is converted to acetyl-CoA, which enters the citric acid cycle in the mitochondria. In citric acid cycle acetyl-CoA is oxidized and two ATP molecules are formed for each molecule of glucose metabolized. However, most of the energy made available by citric acid cycle is transferred as electrons are passed to NAD^+ and FAD, generating NADH and $FADH_2$. In the last stage of aerobic metabolism, the electron carriers (NADH and $FADH_2$) produced either in glycolysis or in citric acid cycle pass the electrons to electron transport chain. The potential energy of the high-energy electrons delivered by electron carriers is finally converted into 32 ATP molecules. The complete breakdown of glucose in skeletal muscle equals a net yield of 36 ATPs and the whole process is represented in Figure 3. (Robergs et al. 2004.)

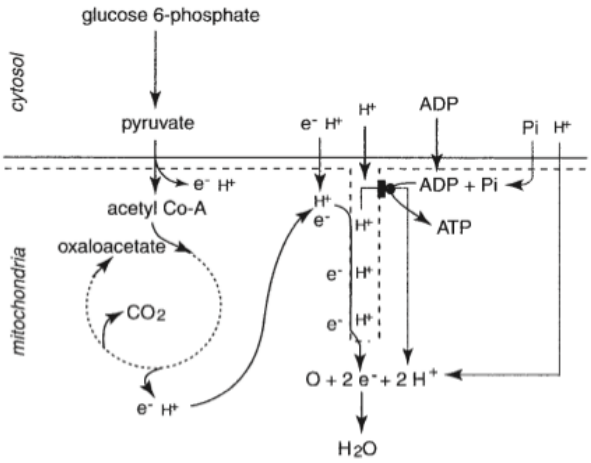

Figure 3. A summary of the main reactions to regenerate ATP: glycolysis in the cytosol and citric acid circle and electron transport chain in the mitochondria (Robergs et al. 2004).

2.1.2 ATP hydrolysis and its by-products

The chemical energy stored in the high-energy bonds of ATP is released in ATP hydrolysis, which produces ADP, P_i and a proton (Figure 4). When the capacity to re-phosphorylate ADP released from ATP hydrolysis is impaired, ADP is further hydrolyzed to adenosine monophosphate (AMP), which in turn can be de-aminated to ammonia (NH_3) and inosine monophosphate (IMP) (Karatzaferi et al. 2001). Since ADP, AMP, and IMP all have much lower affinity for Mg^{2+} than does ATP, also the

free Mg^{2+} increases during fatigue reflecting the breakdown of ATP (Westerblad & Allen 1992). If these mentioned by-products accumulate in muscles, they can modulate cross-bridge function and the excitation-contraction coupling (E-C coupling) process, and thus affect force production.

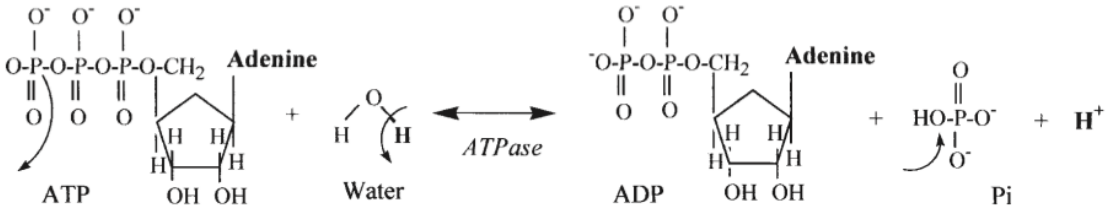

Figure 4. ATP hydrolysis releases energy and produces ADP and P_i. According to Robergs et al. (2004), the origin of the accumulating intramuscular protons and P_i is ATP hydrolysis, not creatine phosphate breakdown or lactate production which both are alkalizing to the cell. (Robergs et al. 2004.)

The key premise of Robergs et al. (2004) is that the main source of the proton arising from coupled glycolysis is ATP hydrolysis rather than lactic acid (in reality lactate) synthesis like the development of acidosis during intense exercise has traditionally been explained. They hypothesise that as exercise intensity increases there is a greater reliance on cytosolic ATP production by glycolysis and ATP-PCr system, and the rate of proton production exceeds the rate of proton transportation into the mitochondria. As a result protons accumulate causing acidosis. According to their model, the metabolic proton buffering by lactate production and PCr breakdown, as well as proton buffering by P_i, amino acids, and proteins delays the development of acidosis. They suggest that in cellular pH range from ~ 6.1 to 7.1, the P_i produced in the ATP hydrolysis has a potential to buffer the free proton that is released in the same reaction. However, the increase in intracellular P_i is not equivalent to the accumulated total of ATP hydrolysis as P_i is used further as substrates for glycolysis to regenerate ATP and also as a substrate for glycogenolysis (the breakdown of glycogen to glucose). Without other metabolic buffers this would leave the free protons to accumulate in the cytosol and result in decreased pH (Figure 5). (Robergs et al. 2004.)

However, this model has been criticised because of the potential misleading conclusions about muscle cell buffer capacity, and because it ignores the effect of pH on the stoichiometry (the calculation of relative quantities of reactants and products in chemical reactions), and focuses on individual reactions rather than taking into account

all the processes of proton generation and consumption. Robergs et al. (2004) also state that lactate is unrelated to the exercise-induced metabolic acidosis. (Kemp 2005; Lindinger et al. 2005.)

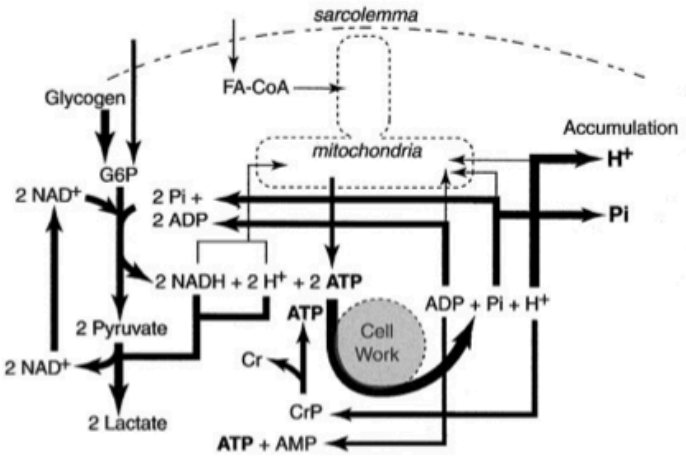

Figure 5. Energy metabolism in skeletal muscle during intense exercise leads to accumulation of metabolites (Robergs et al. 2004).

The additional model explaining decreased pH during exercise is Steward's approach, which describes the changes in H^+ concentration via changes in net strong ion difference (SID), partial pressure of carbon dioxide (pCO_2) and total weak acids (A_{TOT}). During exercise, the changes in SID are the most important causes of acidosis. For example, the accumulation of lactate within muscles contributes to intracellular acidosis since lactate is a strong anion and it alters the behavior of water i.e. leads to H^+ formation from water to maintain the electroneutrality ($[SID] + [H^+] - [HO^-] = 0$). (Lindinger et al. 2005.) Robergs et al. (2005) admit that to evaluate the acid-base changes the stoichimetric approach should be tuned by the SID method.

2.2 Fatigue development

Intense activation of skeletal muscles results in a declined performance, which is called fatigue. Fatigue may occur as a consequence of impaired α-motor neuron activation when it is called central fatigue or it may be peripheral fatigue caused by changes in intracellular environment. (Gandevia 2001.) Currently, the general agreement appears to be that fatigue during very high-intensity exercise is pH dependent (Maughan & Gleeson 2010, 91). However, recent studies have challenged the assumption of pH

dependent decline in force production, as at physiological temperatures and in whole-body exercises acidosis has not contributed to fatigue. Therefore, the effects of other fatigue factors, such as inorganic phosphate (P_i) and reactive oxygen species (ROS) have been considered and new fatigue models have been created. (Allen et al. 2008.) From the perspective of these new approaches, it is then surprising that alkalosis (e.g. through sodium bicarbonate consumption) can improve exercise performance in events lasting 1-10 minutes (Hollidge-Horvat et al. 2000; McNaughton & Thompson 2001).

In the first subsection, the focus is on proposed contractile processes contributing to fatigue, and the role of Ca^{2+} handling is underlined. Although less attention is paid to metabolic and neural processes causing fatigue, they will be discussed briefly in the second subsection when supplements delaying fatigue are presented. Most of the referred studies are conducted with fast glycolytic fibers, which are not as fatigue resistant as slow oxidative muscle fibers and display more pronounced metabolic and force changes.

2.2.1 Contractile processes contributing to fatigue

Muscle contraction is initiated when an action potential (AP) originating in the central nervous system gets to α-motor neuron and causes voltage-dependent Ca^{2+} channels to open. Sequentially, Ca^{2+} influx into the axon terminal triggers acetylcholine (ACh) release from the motor neuron into the synaptic cleft (Figure 6A). When ACh binds to its receptors on muscle fiber, the ligand-gated sodium (Na^+) channels in the cell membrane open. (Delbono 2003.) The following influx of Na^+ into and efflux of potassium (K^+) from the muscle fiber results in the development of the end-plate potential, which can lead to a generation of a muscle fiber AP. (Rossi & Dirksen 2006.)

Once the muscle fiber AP has been generated, it is then spread across the sarcolemma and down the T-tubules. In the T-tubules, AP activates voltage-sensitive receptors, which causes rapid release of Ca^{2+} from the sarcoplasmic reticulum (SR) into the cytosol via ryanodine receptors (RyRs). In cytosol Ca^{2+} binds to troponin initiating cross-bridge cycling which produces force. In order for contraction to end, Ca^{2+} ions are removed from the cytosol by the action of the SR calcium transport ATPase (SERCA)

pumps, which restore Ca^{2+} back into the SR. In this way, Ca^{2+} declines back to resting levels, force declines and relaxation occurs. These processes are known as E-C coupling and are described in Figure 6B. (Rossi & Dirksen 2006.)

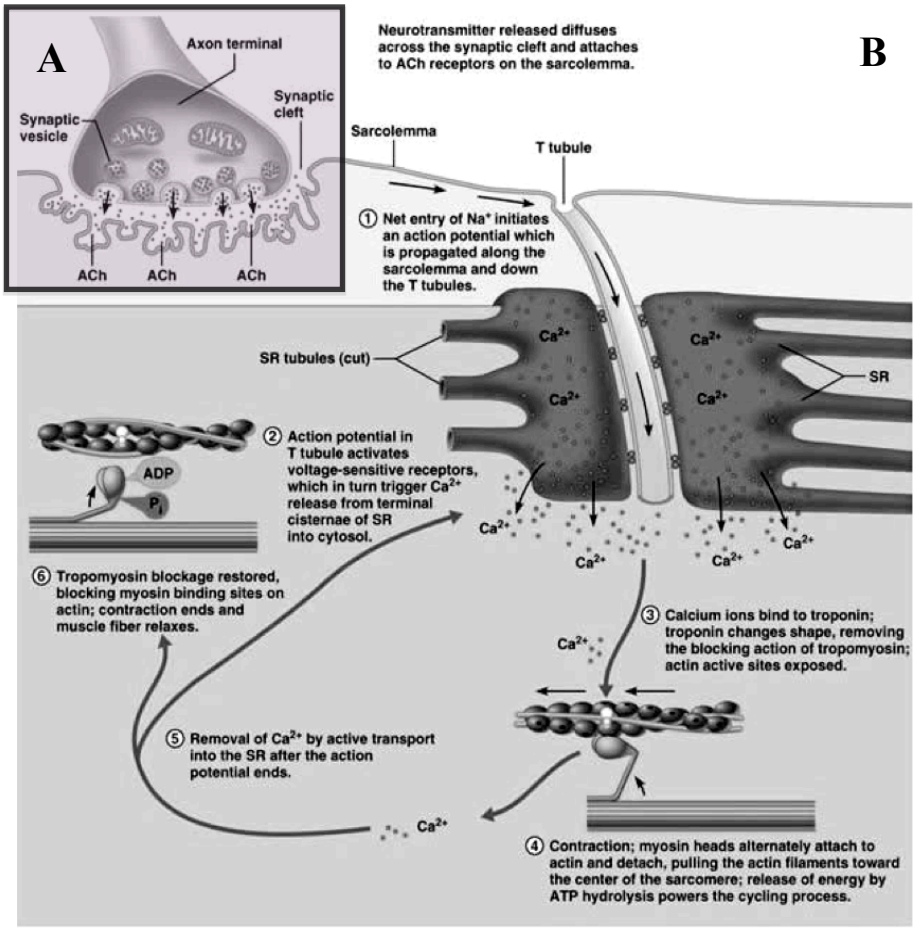

Figure 6. Excitation-contraction coupling is the sequence of events by which an action potential on the sarcolemma results in the sliding of the myofilaments (modified from Carter 2009).

At relatively low temperatures, the presence of elevated P_i and acidosis have been studied to inhibit force production by direct action on cross-bridge function in skinned skeletal muscles. However, near physiological temperatures the direct effect of P_i and acidosis on force production is likely rather small. (Cairns 2006; Coupland et al. 2001; Millar & Homsher 1990.) In turn, both P_i and acidosis affect Ca^{2+} regulation, which has a crucial role for muscle function as it largely determines the contraction and relaxation of muscles. The impairment of Ca^{2+} handling contributes to fatigue, as the amount of Ca^{2+} is not sufficient to facilitate cross-bridge cycling (Allen et al. 2008).

In fact, fatigue can be divided in three phases according to produced force and the free tetanic myoplasmic $[Ca^{2+}]_i$ (Figure 7). At first, there is a fast decline of tetanic force by ~10% with accompanied increase in $[Ca^{2+}]_i$. The force decrease during early fatigue is likely caused as P_i released from cross-bridges inhibits the further transition of myofibers to high-force cross-bridge states. The first phase is followed by a period of relatively stable tetanic force. Finally, there is a rapid decline of tetanic force caused by a decrease in tetanic $[Ca^{2+}]_i$ and reduced myofibrillar Ca^{2+} sensitivity. (Dahlstedt et al. 2000.) In the next paragraphs, the mechanisms disturbing Ca^{2+} handling and causing fatigue are introduced in three sections including Ca^{2+} release and reuptake, and Ca^{2+} sensitivity of myofibers.

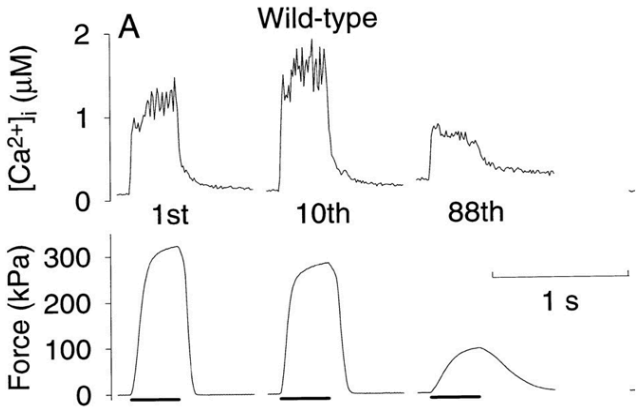

Figure 7. Tetanic $[Ca^{2+}]_i$ and force records at various phases of fatigue obtained during a low-intensity fatiguing stimulation of fast twitch fibres (flexor brevis muscle) from a wild-type mouse. Phase 1 (1^{st}–10^{th} tetanus): an early increase in tetanic $[Ca^{2+}]_i$ accompanied by 10% decrease in force. Phase 3 (88^{th} tetanus): A quick decrease of tetanic $[Ca^{2+}]_i$ and force. The picture is lacking the stable force production phase 2. (Dahlstedt et al. 2000.)

SR Ca^{2+}release. Elevated P_i as a consequence of exercise may act on ryanodine receptor (RyR1) on the SR membrane and mediate Ca^{2+} release in skeletal muscles. This may cause the early increase in free tetanic myoplasmic calcium $[Ca^{2+}]_i$ during fatigue. (Balog et al. 2000.) During the late fatigue, a phosphate permeable channel provides a route for P_i to enter the SR (Laver et al. 2001). Within the SR the entered P_i possibly precipitates with the sarcoplasmic Ca^{2+}, consequently decreasing the amount of free Ca^{2+} available in the SR for release. This possibly causes a failure of Ca^{2+} release and together with a decreased myofibrillar Ca^{2+} sensitivity it results in the final rapid decrease of tetanic force as cross-bridge activation is declined. (Dutka et al. 2005.)

Within the SR calsequestrin isoform 1 (CSQ1) may reduce the calcium phosphate precipitation by acting as a Ca^{2+} buffer and keeping the free [Ca^{2+}] sufficiently low, which enables Ca^{2+} release to continue for a longer time. The increased leak of Ca^{2+} from the SR during exercise highlights the role of these Ca^{2+} buffers. Murpy et al. (2009) found in their study that the high concentration of CSQ1 keeps the free sarcoplamic reticulum [Ca^{2+}] sufficiently low in fast twitch fibers, which decreases the Ca^{2+} leakage through the SR Ca^{2+} transport ATPase (SERCA1) pumps. (Murphy et al. 2009.)

The effect of P_i on force production has been studied to be smaller early in fatigue but is enhanced as fatigue progresses and P_i reaches high levels, and [Ca^{2+}] is decreased (Figure 8) (Millar & Homsher 1990). In end-stage fatigue, the combined effects of declined [ATP] and sudden rise in [Mg^{2+}] possibly make the inhibitory action of P_i on SR Ca^{2+} release more pronounced (Blazev & Lamb 1999; Duke & Steele 2001). The accompanying build-up of AMP and IMP as a consequence of ATP break down possibly amplifies the effect by competing with the ATP for the stimulatory binding site on the Ca^{2+} release channel (Blazev & Lamb 1999).

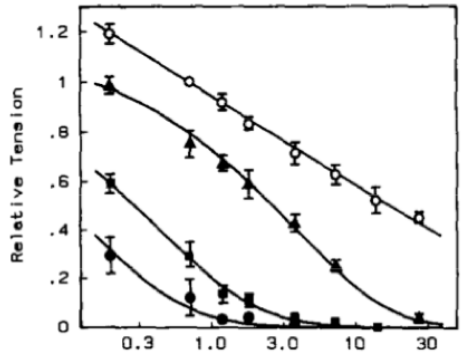

Figure 8. The effect of P_i on isometric tension at different Ca^{2+} concentrations. The Ca^{2+} is decreased during fatigue and P_i increases. (Millar & Homsher 1990.)

Ca^{2+} sensitivity of myofibers. In later stages of fatigue the reduced myofibrillar Ca^{2+} sensitivity may have a significant impact on the force production. The affinity of troponin C (TnC) for Ca^{2+} may be reduced due to acidosis, which may inhibit the cross-bridge function. The increased H^+ concentration possibly causes changes in TnC structure and H^+ competes with Ca^{2+} for binding to TnC, and this way the number of force-generating cross-bridges is decreased. Similarly, the fatigue-induced increase in P_i

can reduce myofibrillar Ca^{2+} sensitivity by lowering the Ca^{2+} affinity of TnC. (Millar & Homsher 1990; Palmer & Kentish 1994.) Additionally, Palmer and Kentish (1994) stated that an increase in Ca^{2+} promotes transition of cross-bridges from weak to strong (force production) attachment states, whereas increase in P_i could result in opposite transition and decrease the Ca^{2+} sensitivity of myofibers.

The current knowledge indicates that the contractile proteins together with Na^+-K^+ pump are the most susceptive components to ROS under physiological conditions. For example, Moopanar and Allen (2005) showed that ROS decrease the myofibrillar Ca^{2+} sensitivity and thus possibly decrease force production. On the other hand, ROS scavengers have been studied to diminish the reduction in Na^+-K^+ pump activity and the rise in plasma K^+, which both occur as a consequence of exercise. Attenuating ROS production by N-Acetyl-cysteine delayed fatigue and improved exercise performance in humans. (McKenna et al. 2006.) On the contrary, experiments with skinned muscle fibers have shown that lactate even at concentration up to 30 mM has only a small inhibitory effect on force production and Ca^{2+} sensitivity of myofibers. (Dutka & Lamb 2000; Posterino et al. 2001.)

SR Ca^{2+}reuptake. The reuptake of cytosolic Ca^{2+} into the SR by the SERCA pumps and sarcolemmal Ca^{2+} transporters determines largely the rate of muscle relaxation. It has been shown that depletion of PCr, which occurs rapidly after the onset of fatiguing stimulation, reduces SR Ca^{2+} uptake (Duke & Steele 1999). Additionally, Duke and Steele (2000) showed that during fatigue when the presence of PCr is low and P_i increases, the Ca^{2+} efflux pathway is activated by reversal of the SR Ca^{2+} pump. Thus, activation of a Ca^{2+} efflux pathway by P_i may contribute to the reduced net Ca^{2+} uptake and results in increased resting $[Ca^{2+}]_i$ and prolonged relaxation time. Also acidosis inhibits the Ca^{2+} uptake by the SR and increases the cytoplasmic free Ca^{2+}. Thus, the total amount of Ca^{2+} binding to TnC may not decrease as a consequence of acidosis, although the affinity of TnC for Ca^{2+} may be reduced like mentioned earlier. (Wolosker et al. 1997.) The increase in resting $[Ca^{2+}]_i$ has a role in exercise-induced muscle damage, which will be discussed in the next chapter.

Nakamura et al. (2002) studied that ATP binding in the SERCA pumps concurrently improves the Ca^{2+} transportation across the SR membrane, and as ATP decreases during

exercise, it results in reduced reuptake of Ca^{2+} into the SR (Figure 9). This likely explains the reduced relaxation of tetani as a consequence of ATP depletion. However, at pH 6.23 Ca^{2+} binding of the SR pump is already decreased by acidosis and reducing ATP has little if any further effect. (Nakamura et al. 2002.) Also the elevation of ADP reduces the ability of the SR to store Ca^{2+}. Increased ADP causes passive leak of Ca^{2+} from the SR and decreases the rate of the SERCA pump. In this way ADP plays an important role in determining Ca^{2+} movements and modulating SR function during exercise. (MacDonald & Stephenson 2001.)

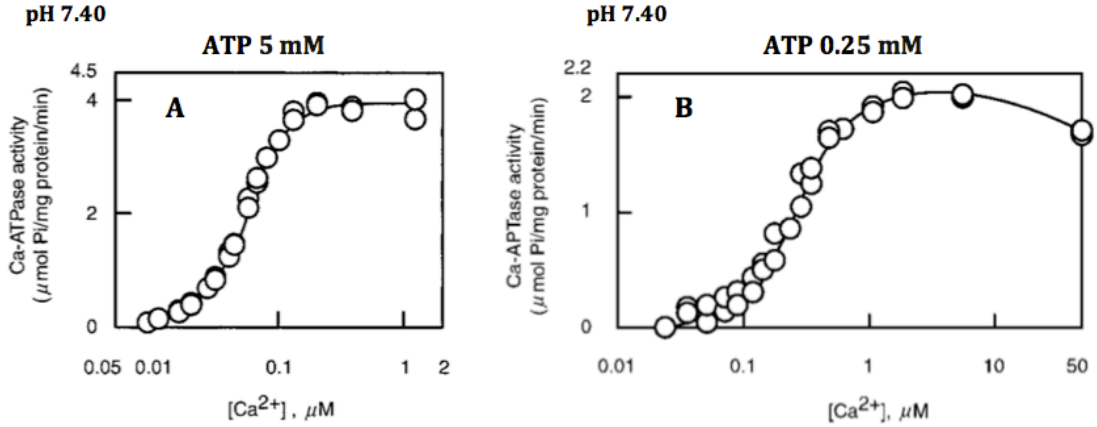

Figure 9. The calcium dependence of the total Ca^{2+} pump (Ca-ATPase) activity of the two forms of ATP binding sites at 5 mM ATP (A) and at 0.25 mM ATP (B), pH 7.40. ATP increases the calcium affinity of the Ca-ATPase molecule. (Modified from Nakamura et al. 2002.)

2.2.2 Other processes contributing to fatigue and techniques used to delay fatigue development

During intense exercise, the buffer capacity of the body is exceeded and H^+ begin to accumulate in the muscles. In the previous chapter the mechanisms (including acidosis) causing impairments in the contractile processes were described, but modifying pH can cause fatigue via other processes as well. These include metabolism, blood oxygen saturation and unloading, cardiac and local vasculature function, and central nervous system drive (Cairns 2006; Allen et al. 2008), which are discussed next. Additionally, the supplements used to improve muscle function are briefly considered.

Study of Jubrias et al. (2003) demonstrated that acidic pH inhibits oxidative ATP supply during exercise as even a mild acidosis (pH 6.8–6.9) prevents mitochondrial function.

They also showed that this occurs despite a substantial rise in [ADP]. When phosphofructokinase (a key enzyme in glycolysis) is isolated, acidosis can inhibit its function. However, in whole-body exercise various other enzyme activators may oppose the effect, why glycolysis is not inhibited by the decreased pH. Other metabolic processes potentially contributing to fatigue include decreased ATPase and glycogen phosphorylase activity as pH falls. (Cairns 2006.) Moreover, under acidic conditions haemoglobin binds more H^+ and liberates oxygen, which impairs the oxygen delivery and may affect performance (Dersjant-Li et al. 2002). Sodium citrate mediated alkalosis, in turn, has been studied to aid membrane excitability and delay the onset of fatigue via the H^+ sensitive K^+ channels. Therefore, alkalosis may prevent the increase in extracellular $[K^+]$, which would occur during intense exercise and which is involved in the development of fatigue. (Sostaric et al. 2006.)

Creatine (Cr) supplementation is widely used among athletes to provide extra Cr in muscles. On the muscle cell level, Cr supplementation results in an increased PCr concentration, which provides better ATP supply during the onset of intense activity. It also retards the increases in ADP that might slow cross-bridge cycling and SR Ca^{2+} pumping. Thus, a high power output of approximately 10 s can possibly be sustained for a slightly longer time. Moreover, in these short exercise bouts the inhibitory effect of energy metabolites, such as P_i, is limited. (Allen et al. 2008.) Increased body weight is commonly associated with Cr supplementation, since PCr/Cr is osmotically active and causes water accumulation in muscle cells (Terjung et al. 2000). Although increased body weight may be harmful in some sports, the increased water amount in muscles might improve myofibrillar Ca^{2+} sensitivity and maximum Ca^{2+} activated force production via decreased ionic strength (Murphy et al. 2004).

Other supplement used to improve muscle performance and delay the fatigue development is β-alanine, which has been studied to increase muscle carnosine concentration (Harris et al. 2006; Hill et al. 2007; Suzuki et al. 2002). Carnosine has multiple beneficial physiological actions, including proton buffering, anti-oxidization, membrane stabilizing, increasing Ca^{2+} sensitivity of myofibers and potentiating Ca^{2+} release. Thus, carnosine can aid in maintaining a better force production during the later stages of fatigue when Ca^{2+} release declines and H^+ builds-up. (Dutka & Lamb 2004.) Using sodium bicarbonate may increase extracellular bicarbonate concentration, aid in

proton buffering and increase the efflux of H^+ from the muscles. This may reduce the fall in muscle cell pH, delay fatigue development and improve performance via enhanced anaerobic glycolysis. (Hollidge-Horvat et al. 2000; McNaughton & Thompson 2001.)

3 DEVELOPMENT OF EXERCISE-INDUCED MUSCLE DAMAGE (EIMD) AND ITS EFFECTS ON PHYSICAL PERFORMANCE

The impaired recovery and muscle function seen after intense exercise is likely due to complex structural damage to the muscle cells. These factors include such as disrupted sarcomeres (Brockett et al. 2004; Brughelli et al. 2010), and damage to the components of the excitation-contraction coupling system (Ingalls et al. 1998; Takekura et al. 2001; Yeung et al. 2002a), cytoskeleton (Zhang et al. 2008; Verburg et al. 2005) and sarcolemma (Duncan & Jackson 1987; Mason et al. 1997). This chapter describes the factors causing exercise-induced muscle damage (EIMD), and how EIMD affects athletic performance.

3.1 Mechanical and metabolic muscle damage

The exercise-induced mechanical and metabolic stress in muscles increases sarcolemmal permeability, alters contraction kinetics when damaging the E-C coupling system, activates inflammation process, promotes oedema formation and causes soreness and stiffness (Duncan & Jackson 1987; Mason et al. 1997; Morgan 1990). The ultrastructural changes and the immediate consequence of cellular necrosis are referred to as primary injury, whereas secondary injury refers to damage, which is caused by the physiologic responses to primary injury (Merrick et al. 1999).

3.1.1 Disrupted sarcomeres and T-tubules

Muscles consist of myofibrils, in which individual sarcomeres are in series. In an early study of Fríden (1981) overstretched sarcomeres and wavy Z-lines were found throughout the affected fibers following eccentric contractions. These changes were thought to explain the immediate muscular weakness and the subsequent muscle damage. (as cited in Allen 2004.) Later on, stretch-induced muscle damage is suggested to be a consequence of a non-uniform lengthening of sarcomeres when the active

muscle is stretched beyond its optimum length. Stretching a sarcomere makes it weaker, until the rising support of the passive structures compensates for falling active tension i.e. usually the length beyond filament overlap. When the muscle relaxes some of the overstretched sarcomeres may not re-interdigitate properly and become disrupted. During repeated contractions the region of disruption increases, which may cause membrane damage and ultimately some of the fibers may even die. (Morgan 1990.)

Damage to sarcomeres and failure to produce active tension may necessarily not influence on the maximal force production. Alternatively, damaged sarcomeres possibly increase the compliance, resulting in a shift to the right of the optimal angle for force generation (length-tension relationship) to achieve the same myofilament overlap (Figure 10). (Morgan 1990.) The presence of disrupted sarcomeres has been proved both in human (Brockett et al. 2004; Brughelli et al. 2010) and animal (Yeung et al. 2002a) experiments by a shift in the muscle's length-tension curve in the direction of longer muscle lengths.

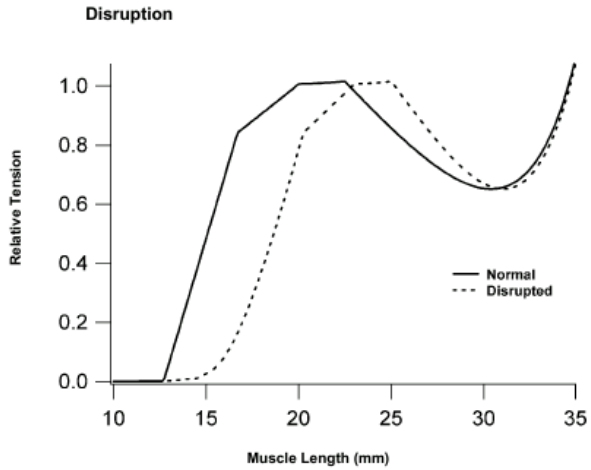

Figure 10. A computer-simulated curve of changes in sarcomere length-tension relationship following a series of eccentric contractions (Proske et al. 2004).

Brockett et al. (2004) examined if previously injured muscles are more prone to eccentric damage than un-injured muscles, as one may assume from the high re-injury rates observed among athletes. They used the optimum angle for torque as an indicator of susceptibility for the damage from eccentric exercise. It was found that mean optimum angle in the previously injured muscles was at a significantly shorter length, which suggests that the injured muscles were more vulnerable to eccentric damage and

thus more prone to strain injuries than un-injured muscles. However, a regular program of eccentric exercise may lead to adaptive changes, which have a potential to protect against further injury and therefore should be used to eliminate injuries.

Takekura et al. (2001) showed morphological damage to T-tubular system following eccentric exercise. The formation of abnormal membrane systems included e.g. increase in the number of longitudinally oriented T-tubule segments and forming of pentad and heptad contacts between T-tubule and SR terminal cisternae instead of normal triads. These disruptions of membrane systems possibly account for the functional failure of the E-C coupling, but also interference with Ca^{2+} dynamics described later. The study of Yeung et al. (2002a) suggested the T-tubule rupture to be related to the non-uniform lengthening of sarcomeres. The shearing stress is greatest between the sarcomeres of different lengths, which possibly cause the T-tubules at the overlap of thick and thin filaments to rupture (Figure 11).

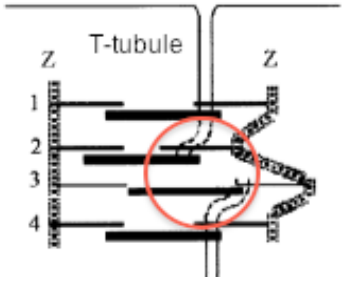

Figure 11. Sarcomere inhomogenities in an eccentrically damaged fibre and a disruption of a T-tubule within the red circle (Modified from Yeung et al. 2002a).

The T-tubule damage raises intracellular sodium $[Na^+]_i$, which is accompanied by osmotically equivalent water and contributes to oedema. The elevated Na^+ activates Na^+-K^+-pump and as the extra Na^+ has to be pumped from the cell. When the volume load of Na^+ and water exceeds the capacity of T-tubules it causes vacuole formation, which can be seen in T-tubules following stretched contractions. Other consequences of T-tubule rupture include increase in resting $[Ca^{2+}]_i$ and disruption of action potential due to disturbed ionic distribution across the membrane (Yeung et al. 2002b), which may contribute to the failure of the E-C coupling process.

Ingalls et al. (1998) established the presumably site for the E-C coupling failure after eccentric exercise to be located at the interface of the T-tubule and the SR Ca^{2+} release channel. Immediately after the exercise, the E-C coupling impairment seemed to be

responsible for over 50 % of the reduction in maximal isometric tetanic force leaving only a small part of the primary damage occurring at the level of the sarcomeres. Since the resting $[Ca^{2+}]_i$ has been studied to rise immediately following eccentric damage, the role of Ca^{2+} in E-C uncoupling and in the reduced force production has been of interest and will be discussed next. (Ingalls et al. 1998.)

3.1.2 Metabolic damage triggered by calcium

The elevated $[Ca^{2+}]_i$ can possibly hinder E-C coupling in many ways (Figure 12). The elevated resting $[Ca^{2+}]_i$ is a result of increased influx of Ca^{2+} and impaired intracellular Ca^{2+} regulation by SR, mitochondria and cytosolic proteins. Thus, it could be of importance to target for lowering Ca^{2+} entry and/or preventing the Ca^{2+}-activated pathways causing ultrastructural and membrane damage. Normally, situations which lead to an elevation of resting $[Ca^{2+}]_i$ produce a prolonged reduction in tetanic $[Ca^{2+}]_i$ which reduces force (Yeung et al. 2005). Similarly, the force recovery has been studied to be faster at low $[Ca^{2+}]_i$ (0.65 mM) than at high $[Ca^{2+}]_i$ (2.54 mM) (Ramer Mikkelsen et al. 2004).

Activated by eccentric contractions, the influx of Ca^{2+} is thought to occur mainly through stretch-activated channels (SAC) (Yeung et al. 2005; Zhang et al. 2012). Because it is likely that SAC are composed of TRPC1 protein (Maroto et al. 2005, Zhang et al. 2012), they could also be called TRP (transient receptor potential) channels, which mediate Ca^{2+} entry in response to depletion of intracellular Ca^{2+} stores (Kurebayashi & Ogawa 2001). Also a shearing damage to T-tubules (Yeung et al. 2002a) and sarcolemmal damage by Ca^{2+}-dependent degradative pathways (Duncan & Jackson 1987; Mason et al. 1997) may lead to passive influx of Ca^{2+} from the extracellular fluid. Ramer Mikkelsen et al. (2004) showed that during 60 min stimulation the last 15 min Ca^{2+} influx was rather through the leaks in the membrane than through different channels and dependent on excitation. In the next paragraphs Ca^{2+}-mediated muscle damage will be discussed in two sections: mitochondrial Ca^{2+} uptake produces reactive oxygen species (ROS), and Ca^{2+} activates phospholipases and proteases.

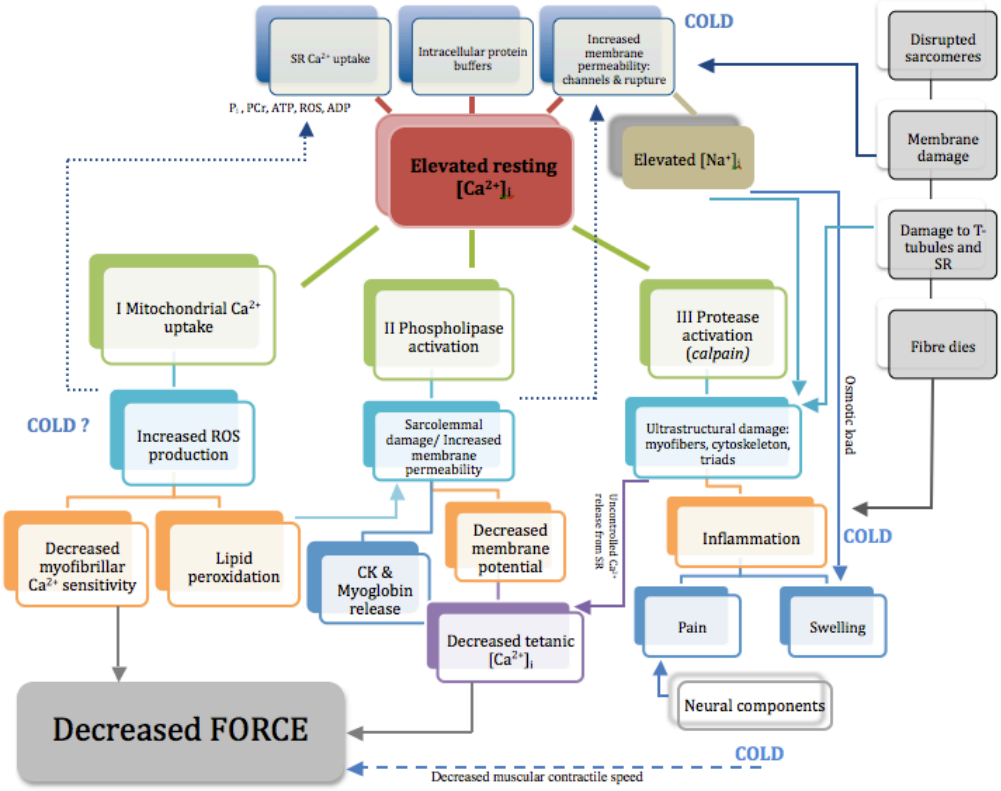

Figure 12. The role of calcium in triggering damage and the effect on force production. Also the possible sites for cold treatment to affect muscle damage are marked in the picture (modified from Carlsen & Villarin 2002; Proske & Morgan 2001; Yeung et al. 2002a).

ROS production. Once entering mitochondria, Ca^{2+} can be both beneficial and harmful for its function. Elevated mitochondrial matrix Ca^{2+} $[Ca^{2+}]_m$ up-regulates oxidative phosphorylation, and Ca^{2+} is needed to stimulate mitochondria to work faster during exercise. However, as the metabolism increases also more ROS are produced as by-products. ROS may serve as a pathological stimulus for Ca^{2+} turning it from a physiological effector to a detrimental one. (Brookes et al. 2004.)

Increased $[Ca^{2+}]_m$ may activate programmed cell death (apoptosis) both directly and via increased ROS production. The direct detrimental effect of increased $[Ca^{2+}]_m$ is possible via prolonged opening of a mitochondrial Ca^{2+} efflux pathway, permeability transition (PT) pore. This releases cytochrome *c* (cyt *c*), which is an intermediate in apoptosis. Additional cyt *c* release is possible via positive feedback process as the cyt *c* released from PT pores may cause endoplasmic reticulum Ca^{2+} release, which further increases the $[Ca^{2+}]_m$. Besides, more ROS are produced as a result of opening the PT pore. ROS

can promote cyt *c* release via oxidation of the inner mitochondrial membrane lipid called cardiolipin, but they can also induce PT pore opening and act as a pathological stimulus for Ca^{2+}. (Brookes et al. 2004.)

ROS have been studied to decrease the myofibrillar Ca^{2+} sensitivity and thus possibly decrease the force production of muscles (Moopanar & Allen 2005). Additionally, Mason et al. (1997) showed that generation of ROS might lead to changes in membrane structure. ROS may cause peroxidation of membrane lipids and thus membrane defects are developed increasing the permeability. (Mason et al. 1997.)

Phospholipase and protease activation. The increased $[Ca^{2+}]_i$ may activate phospholipase, which cause membrane damage and increase the membrane permeability. More precisely, the activation of phospholipase A2 leads to release of arachidonic acid from the phospholipid membrane and causes sarcolemmal damage. This allows the loss of large intracellular proteins, such as creatine kinase into the blood stream, but also further enables influx of extracellular Ca^{2+}. (Duncan & Jackson 1987.) These intracellular proteins can be used as indirect markers of muscle damage.

The activation of proteases by elevated $[Ca^{2+}]_i$ may result in cytoskeletal and protein degradation. The cytoskeletal proteins (desmin, dystrophin and titin) maintain the structure of the myofiber and are involved in the transmission of the generated force from the sarcomere to the fiber surface. A study of Verburg et al. (2005) showed that elevated $[Ca^{2+}]_i$ activates proteases (likely calpain-3) resulting in proteolysis of titin. As a calpain inhibitor, leupeptin, inhibited the E-C uncoupling and titin damage the researchers assumed calpain activation contribute to muscle damage. A later study of Zhang et al. (2008) showed that Ca^{2+}-activated calpain proteolysis is predominantly responsible for the cytoskeletal damage after eccentric exercise which can be seen in the altered immunostaining of desmin, dystrophin and titin. However, the prevention of cytoskeletal damage by removing extracellular Ca^{2+} or by application of leupeptin had only moderate effects on the muscle force production. Other Ca^{2+}-dependent structural changes resulting in uncoupling phenomenon include e.g. distorted triads, Z-line alterations and SR vesiculation, some of which may be caused by proteolysis (Lamb et al. 1995).

3.2 Consequences of exercise-induced muscle damage

The previous chapter described the underlying mechanisms causing EIMD, whereas this chapter describes the consequences of muscle damage. The first part of the chapter will briefly describe the role of inflammation following exercise, and the most common symptoms of EIMD, whereas the second part goes through the effects of EIMD on athletic performance.

3.2.1 Symptoms of EIMD

The findings of large changes in circulating leucocytes (e.g. neutrophils), inflammation-related substances (e.g. C-reactive protein, creatine kinase, cytokines) and myofibrillar-bound proteins (e.g. myosin heavy chain, MHC) in blood and muscle post-exercise support the fact that exercise activates inflammatory response (MacIntyre et al. 2001). The immune system plays a role in the de-generation and re-generation process of muscle and surrounding connective tissue after EIMD, and is thus essential in adaptation. Inflammation is caused by multiple tissue products, such as histamine, bradykinin, serotonin and prostaglandins, and is characterized by (Guyton & Hall 2006, 434.):

(1) Vasodilation of the local blood vessels, with consequent increase in blood flow.
(2) Increased permeability of the capillaries, which allows leaking of fluid into the interstitial spaces.
(3) Clotting of the fluid in the interstitial spaces due to excessive amounts of proteins leaking from the capillaries and increasing the osmotic pressure of the interstitial fluid.
(4) The loss of fluid increases the concentration of red blood cells in small vessels and increases viscosity of the blood.
(5) Migration of granulocytes, principally neutrophils, and monocytes into the tissue.
(6) Swelling of the tissue cells.

Rapidly after exercise, neutrophils are mobilized into the circulation and migrate towards chemoattractants released from the site of the injury. For example, the Ca^{2+}-activated calpain is possibly associated with neutrophil chemotaxis, thus localizing neutrophils to the injured site post-exercise. This hypothesis is supported by the study Cuzzocrea et al. (2000) who showed that the degree of inflammation is diminished in

calpain inhibitor I-treated rats. Furthermore, arachidonic acid released from sarcolemma by Ca^{2+}-activated phospholipase A2, can also be converted to potent inflammatory mediators, the eicosanoids (Smith et al. 2000).

Within a day after damaging exercise, neutrophils are replaced in damaged muscle tissue by macrophages. Both neutrophils and macrophages are phagocytes and contribute to the degradation of damaged muscle tissue, which is important in repair and re-generation of muscles. (Peake et al. 2004.) However, there are studies suggesting that neutrophils and macrophages also produce ROS and thus inflammation to have a role in secondary muscle damage (Nguyen & Tidball 2003a; Nguyen & Tidball 2003b). Additionally, the activation of proteases (Lamb et al. 1995; Verburg et al. 2005; Zhang et al. 2008) and phospholipases (Duncan & Jackson 1987) may lead to damage to the cell structures and contribute to secondary damage.

Oedema. Exercise-induced muscle oedema is biphasic, such that an initial increase in intracellular volume occurs acutely (0–2 h) post-exercise and a sub-acute increase occurs 24–96 h post-exercise. The sub-acute oedema is possibly a result of inflammatory response mediated membrane leakage, whereas the acute oedema is caused by increased osmolality of the cell due to accumulation of metabolites and an elevation in muscle enzymes and/or degraded protein components (Robergs et al. 2004; Yanagisawa et al. 2003a). Additionally, exercise builds up the local blood flow (Guyton & Hall 2006, 195) and causes initial damage to sarcolemma (Morgan 1990), which may contribute to acute oedema as well.

The breakdown products of dead and dying cells cause further damage (the secondary enzymatic injury) and a local inflammatory response associated with the sub-acute oedema and soreness. By degrading the cell membranes secondary injury may lead to loss of resting membrane potential and hydropic swelling of the cell. Furthermore, swelling of cells can occlude the vasculature, providing a source for ischemia and cause further cell death. The hypoxic period prevents the ATP production via oxidative phosphorylation and if not enough ATP is produced, membrane ion pumps may fail causing again hydropic swelling and cellular necrosis. The secondary ischemic muscle damage may be caused by multiple haemodynamic changes as well, including bleeding from damaged vessels, reduced blood flow from the inflammation induced increase in

blood viscosity, and increased extravascular pressure from an expanding hematoma (collection and pooling of blood outside the blood vessels) and muscle spasm. (Merrick et al. 1999.)

DOMS. Together with oedema and stiffness, DOMS is one of the well-documented symptoms of muscle damage. The increase in muscle soreness measured following exercise is known to occur in two phases, like the oedema formation described above. The immediate soreness is due to accumulation of metabolic by-products, where as DOMS is associated with the inflammatory response and muscle damage. Actually, DOMS is caused by the events described earlier in this chapter. In the first phase of DOMS development, the high tensile forces, often associated with eccentric exercise, disrupt muscle tissue and connective tissue. Damage to sarcolemma leads to a disturbance in calcium homeostasis, and increase intracellular calcium inhibits cellular metabolism and e.g. activates proteases, which further cause damage to cell structures. This is followed by an acute inflammatory response including oedema formation and inflammatory cell infiltration. (Cheung et al. 2003.)

It is thought that the tissue breakdown products of damaged and dying cells sensitise nociceptors so the muscle is tender to local palpation, stretch and contraction (Proske & Morgan 2001). Additionally, Weerakkody et al. (2001) proposed that large-fibre mechanoreceptors contribute to DOMS. A more recent study of Murase et al. (2010) found that bradykinin released from exercising muscle may trigger development of muscular mechanical hyperalgesia (an increased sensitivity to pain) and nerve growth factor (NGF) produced in the muscle after eccentric contraction has a central role in pain maintenance by sensitizing nociceptors to mechanical stimulation. The study of Svensson et al. (2003) supports the importance of NGF in DOMS as they showed intramuscular injection of NGF to induce prolonged muscle tenderness in humans. In addition, TRP ion channels and a pathway from cyclooxygenase-2 to glial cell line-derived neurotrophic factor (GDNF) may serve as potential mechanisms for generating DOMS after eccentric exercise (Fujii et al. 2008; Murase et al. 2013).

3.2.2 Effects of EIMD on athletic performance

EIMD results in immediate and prolonged functional impairments, e.g. reductions in strength and power. From the athlete's point of view, especially the ability to generate power output after EIMD is important, as athletic events are associated with movements with high angular velocities. These complex sport-specific movements are less studied, whereas force-generating capacity has been studied through isometric and dynamic isokinetic testing modalities. (Byrne et al. 2004.) The inability of eccentrically exercised muscle to generate an initial high force and power could be explained by the selective damage of fast-twitch fibres, which has been proved in many studies (Cairns et al. 2009; Piitulainen et al. 2012; Takekura et al. 2001; Vijayan et al. 2001). In such case, there is a lack of the marked rise and rapid decline in force and power, thus the eccentrically exercised muscles appearing less fatigable but unable to act powerfully (Byrne & Eston 2002b). This chapter will focus on studies measuring power output after damaging exercise, but will also briefly describe studies using isometric strength as a muscle function determinant. The psychological effects of EIMD, such as fear of pain, on performance are not considered.

Following eccentric muscle action, Byrne and Eston (2002b) reported instant and prolonged reductions in peak power assessed during a 30-second Wingate cycle test and in isometric strength using an isokinetic dynamometer. Even though peak power and isometric strength were both declined, they followed different temporal patterns of recovery. Whereas isometric strength demonstrated a linear recovery, peak power declined further at days 1 and 2 post-exercise before starting to recover. These results suggest that muscle power, unlike strength, may be affected by DOMS and the inflammatory response associated with EIMD. On the contrary, Semark et al. (1999) showed there are no evident effects of EIMD on a single sprint performance. They used 5, 10, 20 and 30m sprints and there was no evidence to suggest that muscle damage and DOMS caused by a series of drop jumps impaired sprint time or acceleration. As subjects had severe DOMS at the time of post-exercise tests (12, 24, 36, 48, 60 and 72 h) it was assumed to negatively effect on the sprint performance.

Byrne and Eston (2002a) investigated the effect of EIMD following eccentric exercise on vertical jumping performance with and without the use of the SSC. They measured

both immediate and up to four days lasting reductions in jumping performance, however, the jump method affecting the result. Vertical jump performance was particularly affected in the squat jump (no SSC) compared to the countermovement or drop jump (with SSC). Thus, the way in which strength is utilized appears to be an important determinant of performance and the SSC possibly attenuates the detrimental performance effects related to EIMD. In the same study, the extent and rate of recovery for isometric, concentric and eccentric muscle actions was similar. The reduction in strength persisted for four days in all muscle actions. Similarly, Avela et al. (1999) demonstrated that following long-lasting SSC exercise (marathon) muscle force production capacity is declined up to two days. The recovery occurred in a bimodal pattern, as the immediate decline was followed by an early recovery and secondary reduction in performance at 2 days post-exercise, possibly due to secondary damage (Figure 13).

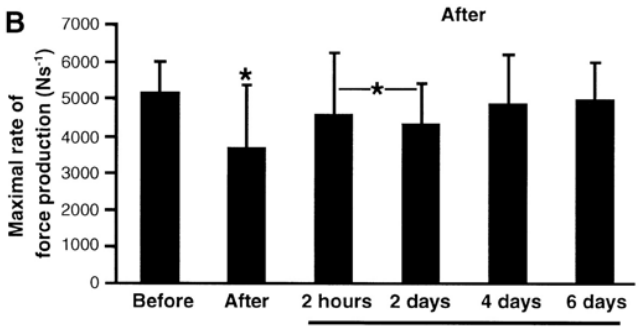

Figure 13. Force production capacity is reduced immediately after exercise (*). The early recovery of force production occurs at two hours post-exercise and the secondary reduction at two days post-exercise (*). (Avela et al. 1999.)

4 PROMOTING RECOVERY AND TRAINING ADAPTATION WITH COLD TREATMENT

Recovery from an intense exercise occurs in multiple phases as some of the components are treated immediately after the exercise, whereas proper sleep and nutrition should be part of everyday life. The recovery time depends on the exercise, since for example the immediate energy stores (ATP and PCr) recover quickly in 2–5 min, whereas the recovery of muscle damage may take up to 8 days. Immediately after the exercise, it may be beneficial to facilitate the removal of metabolic by-products by e.g. active recovery. The instant consumption of proteins and carbohydrates, in turn, activates protein synthesis and restores glycogen stores. During the first couple of days following the exercise, recovery can be aided by e.g. massage and stretching, which may reduce muscle tension and soreness. (Bompa & Haff 2009, 108–117.)

Especially important is the treatment of EIMD, since it is a long-term limiting factor for muscle performance. One of the treatment strategies used to alleviate or prevent EIMD and its associated symptoms, such as oedema and muscle soreness, is cold treatment (cryotherapy). Cryotherapy means a technique where cold water immersion (CWI), applying ice or other forms of cooling are used to treat acute traumatic injury and promote post-exercise recovery. (Barnett 2006.) Cryotherapy reduces muscle temperature, which eventually causes other physiological changes. Although the main focus of the next chapter is on the treatment of EIMD, also factors associated with recovery, such as the rate of post-exercise protein synthesis, post-exercise glycogen synthesis and the role of inflammation are considered.

4.1 Temperature decline induces physiological responses

Since many of the therapeutic effects of cryotherapy are temperature-dependent, the magnitude of tissue temperature change seems to be of importance when using cryotherapy. For example, increasing the thermal gradient by applying colder water (Mawhinney et al. 2013) and using cold treatment for longer duration (Peiffer et al.

2009) results in greater tissue temperature change. Furthermore, it is possible that deeper tissues will not reach their lowest temperature during the actual cooling period, but rather during the post-cooling period when the heat from them is used to re-warm the cooler superficial tissue (Figure 14) (Enwemeka et al. 2001). This supports the rationale for using intermittent cooling strategies to achieve lower muscle temperature and avoid cold-caused damage to the superficial tissues.

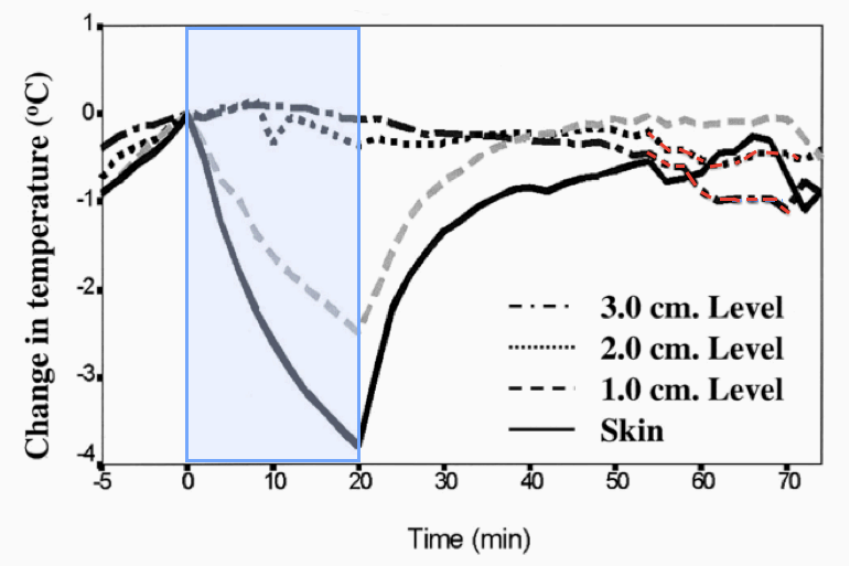

Figure 14. The temperature gradient at skin level and 1.0 cm, 2.0 cm, and 3.0 cm below the skin before, during, and after cold pack therapy (treatment period in blue: time 0–20 min). There is a sharp fall in the temperature of the skin and the 1.0 cm level during the treatment, whereas the temperatures at the two deeper levels fell progressively following the treatment (in red). (modified from Enwemeka et al. 2001.)

Although the beneficial effects of CWI can be associated with reducing hyperthermia as elevated core temperature has been studied to reduce voluntary activation during maximal isometric force production (Morrison et al. 2004), this chapter will focus on what kind of physiological changes altering tissue temperature causes.

4.1.1 Cardiovascular effects and changes in blood flow

When human body is exposed to cold it intends to minimize the heat loss via cutaneous vasoconstriction i.e. narrowing of the blood vessels. Whole body skin cooling has been studied to induce reflex vasoconstriction, in which the release of norepinephrine and sympathetic co-transmitters, such as neuropeptide Y, results in decreased skin blood flow. (Stephens et al. 2001; Stephens et al. 2004.) In turn, the vasoconstriction with

local skin cooling is provoked by a postsynaptic up-regulation of α_{2c}-adrenoceptors and by prevention of the nitric oxide system (Johnson & Kellogg 2010). Reflex and local vasoconstriction often interact during cold exposure to maximize vasoconstriction, however, the combined effect being less than the sum of the results from independent local and reflex cooling (Alvarez et al. 2006).

CWI following exercise has been suggested to reduce muscle blood flow via vasoconstriction. Vaile et al. (2011) showed that 15 min full-body CWI (15°C) reduced the blood flow to the limbs supporting the idea that CWI leads to a redirection of blood flow from the periphery to the core. Mawhinney et al. (2013) studied if there is difference in the physiological responses when using 8°C and 22°C water for immersion post-exercise. The CWI at 8°C decreased the tissue temperature more, but there was no further reduction in muscle or cutaneous blood flow compared to immersion at 22°C. At rest, 8°C and 22°C have resulted in similar limb blood flow, however, at 8°C skin blood flow being increased, thus suggesting decreased muscle blood flow in colder water (Gregson et al. 2011). According to these studies, the distribution of blood may differ if exercising before applying cryotherapy.

Using cold treatment following exercise is suggested to prevent oedema formation and decrease the extent of muscle cell damage (Yanagisawa et al. 2003a). The acute exercise-induced oedema formation is thought to be limited via decreased microvascular blood flow around the injured area as the capillaries constrict (Vaile et al. 2011), which attenuates perfusion elevation following exercise (Ihsan et al. 2013; Yanagisawa et al. 2004). As oedema formation is attenuated the degree of mechanical compression of capillaries and mechanical stress on cell structures are decreased as well, which could otherwise cause further cell death. Additionally, decreased oedema also improves oxygen delivery, since the route between muscle cells and capillaries is shorter for oxygen and waste product transportation (Swenson et al. 1996 as cited in Yanagisawa et al. 2003a.) Also the blood and interstitial fluid exchange between superficial and deep tissues, when trying to equalize the temperature gradient, can facilitate the removal of accumulated fluid and metabolites from damaged superficial tissue, and reduce pain and oedema (Enwemeka et al. 2001).

Although restricting blood flow may be beneficial to recovery, its long-term effects are controversial. For example, Roberts (2014) suggests decreased blood flow to have detrimental effects on adaptation to training via attenuated amino acid delivery. Decreased amino acid delivery may consequently decrease protein synthesis, which is especially essential when the target is muscle growth (hypertrophy). (Roberts 2014.) The role of protein synthesis is discussed further in the context of training adaptation (4.2.2).

If a training session results in glycogen depletion, the next session may be limited if the re-loading of glycogen post-exercise is not sufficient. Especially, if more than one training session or competition are performed per day, the rapid glycogen synthesis is of importance in the early hours post-exercise. (Jentjens & Jeukendrup 2003.) Enhanced muscle blood flow in trained athletes has been studied to correlate with glycogen synthase activity, which increases glucose delivery to the muscle and may lead to increased glycogen synthesis rate compared with sedentary individuals (Ebeling et al. 1993). Since cryotherapy targets mediating post-exercise blood flow and metabolism, it could alter glucose availability to the muscle during recovery and negatively effect on glycogen re-synthesis. However, Gregson et al. (2013) demonstrated that the glycogen synthesis rate is the same following cooling than without it. Thus, athletes should not be concerned with inhibitory effects of the cryotherapy-associated reductions in muscle blood flow on the restoration of the glycogen stores.

Šrámek et al. (2000) studied cardiovascular changes during 1-h head-out immersion at 14°C. Cooling decreased rectal temperature, and activated the sympathetic nervous system, which increased metabolic rate and blood pressure via vasoconstriction. The things are vital to occur in order to aid the body to maintain the core temperature. Nevertheless, little is known about how cryotherapy affects vasomotor activity during the post-exercise period. Buchheit et al. (2009) showed that 5 min of CWI (14°C) applied following supramaximal exercise in the heat causes faster and greater post-exercise parasympathetic re-activation compared with a non-immersed condition. These results are supported by the later study of Al Haddad et al. (2010) who showed that a 5 min water immersion, regardless of water temperature (CWI: 14–15 °C, TWI: 33–34 °C), seems to speed up post-exercise parasympathetic activation, as inferred from heart rate and heart rate variability indices.

4.1.2 Changes in cell metabolism

Following exercise muscles have an increased oxygen demand as they e.g. restore ion gradients, repair structural damage and remove metabolic by-products (Børsheim & Bahr 2003). The study of Ihsan et al. (2013) showed that applying CWI following exercise increased the tissue oxygenation index (TIO), which may reflect reduced muscle metabolic activity following cooling, and thereby the O_2 supply matching better to its demand. The authors were unsure whether the lowered metabolic demand is a result of reduced muscle blood flow or reduced muscle temperature. However, when the muscles are encountering less metabolic stress, the depressed mitochondrial energy production may result in decreased ROS production and limited muscle damage following exercise (Brookes et al. 2004). There are no human studies investigating the effect of cryotherapy on ROS production, but a study of Carvalho et al. (2010) examined the effect of cooling on ROS-mediated damage in rats. They found that applying cold might reduce the ROS-mediated damage by modulating the inflammatory response, since the intensity of inflammation seems to be an important factor involved in the development of oxidative damage. The role of ROS in adaptation to training will be discussed later on (4.2.2).

Additionally, as cryotherapy reduces cell metabolism via either decreased blood flow or temperature decrement, it has a potential to minimize the secondary exercise-induced hypoxic cell death (Knight 1976 as cited in Merrick et al. 1999; Yanagisawa et al. 2003b). Thus, if a smaller degree of breakdown products of dying cells are released, the inflammatory response may be less powerful and oedema formation attenuated (Lamb et al. 1995; Verburg et al. 2005; Zhang et al. 2008).

Participation in high-intensity exercise increases the concentration of metabolic by-products within the muscle as described in chapter 2.1.2. The study of Yanagisawa et al. (2003a) showed that cryotherapy may raise the muscle pH via improved metabolic clearance of H^+ following strenuous exercise, and thus it could boost the short-term recovery. On the other hand, cooling may decrease muscle function e.g. by increasing muscle stiffness (Muraoka et al. 2007) impairing the ability of the muscle spindle to trigger the stretch-reflex (Meeusen & Lievens 1986) and decreasing acetylcholine concentration (Abramson et al. 1966 as cited in Wilcock et al. 2006), and result in short-

term decrease in functional performance, such as sprint ability (Patterson et al. 2008). This is supported by the study of Crowe et al. (2007), as they showed that an all-out 30s anaerobic cycling performance was negatively affected if CWI was applied during the 1-h recovery period between the two cycling bouts. Peak power, total work, blood lactate concentration and peak exercise heart rate were decreased in the cold trial compared to the control. Thereby, cryotherapy is not recommended prior short-term exercises and the benefit of higher pH is concealed by the reduced neuromuscular function of a cooled muscle.

On the contrary, Vaile et al. (2010) showed that CWI leads to better maintenance of performance during 35 min high intensity cycling in hot conditions (32.8°C) compared to active recovery. CWI reduced the blood flow to the limbs supporting the idea that CWI leads to a redirection of blood flow from the periphery to the core. This possibly increases venous return, cardiac efficiency and exercise performance. These findings suggest that the performance benefit of CWI may be limited to a high intensity exercise of a longer duration.

4.1.3 Way to relief pain perception

The most widely accepted mechanism associated with cooling-induced reduction of the acute pain perception is its analgesic effect. The effect is likely due to the cold temperature, which reduces nerve conduction velocity (NCV) and muscle spindle activity with a consequent blunted stretch-reflex response and decreased spasticity of muscle. These events inhibit the pain-spasm cycle and contribute to the relief of pain. (Meeusen & Lievens 1986.) Although pain relief requires relatively low temperature (10–15°C), the motor neurons are significantly affected at even lower temperatures. Thus, cooling may have an analgesic effect before muscle function is affected. (Herrera et al. 2010.)

The other mechanisms explaining the pain relief with cold application include suppression of nociceptive receptor sensitivity and decreasing the number of ascending nociceptive stimuli via cold acting as non-noxious stimuli and inhibiting the noxious inputs. The analgesic-descending pathway of the central nervous system is also a

potential mechanism to reduce pain sensation when cold is applied. For example, the release of endorphins may increase due to changes in ambient temperature. (Saeki 2002.) Additionally, reduction in metabolic enzyme activity levels and restricting oedema may decrease the pressure on pain receptors (Yanagisawa et al. 2003a).

4.1.4 Cold treatment induced endocrine responses

Exercise and cold both stress body, and thus may cause changes in circulating hormone concentrations. As hormones modulate e.g. blood flow, fluid balance and metabolism (Guyton & Hall 2006, 201, 906–907), they have a potential to affect recovery from exercise. However, the effects of cryotherapy on the post-exercise hormone response are not well studied.

Srámek et al. (2000) observed hormone changes during 1-h head-out immersion in water at temperatures of 32°, 20° and 14°C without the effect of exercise. They suggest that water immersion is capable of altering some key endocrine factors, however, the results not describing the changes in circulating hormones following exercise. They observed a trend towards decreased cortisol following both the 14°C and 20°C conditions and increased concentration of norepinephrine and dopamine following only the 14°C condition. Nevertheless, the study protocol was extreme with the whole-body exposure to cold for 1 h, and therefore the results may not reflect the changes occurring following a shorter immersion time.

Testosterone and cortisol levels are frequently measured to determine the anabolic/ catabolic effects of training and recovery. In the study of Nemet et al. (2009) a four times 250 m interval running exercise was associated with an increase in testosterone levels, but there was no significant change in cortisol level. Testosterone levels returned to baseline in the recovery (post 60 min) with no effect of the cold-pack application. Similarly, cooling had no effect on cortisol levels. However, local cooling immediately following sprint-interval training was associated with greater decreases in both pro- and anti-inflammatory cytokines (IL-1β, IL-1ra). Cooling also reduced the anabolic (IGF-I and IGFBP-3) and increased the catabolic (IGFBP-1) hormonal response. These results

suggest that the application of cold pack immediately after exercise may cause attenuation of the anabolic effects of the preceding training.

4.2 Effect of cold treatment on physical performance

Whether cryotherapy enhances or reduces adaptation to training has become a topic of interest. The improvements in performance observed in acute cold treatment studies (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009) propose cold treatment to result in an enhanced stimulus for adaptation, consequently allowing athletes to perform subsequent training sessions better. However, a certain degree of fatigue and/or inflammation post-exercise may be necessary to promote long-term adaptations to training, thus cryotherapy being harmful in long-term use (Yamane et al. 2006). This chapter separates the studies examining the effects of cryotherapy on a single exercise bout and those observing the adaptive processes over a longer time period. Also the mechanisms by which cold treatment may enhance or impair recovery and training adaptation are discussed, although some of them being still relatively unknown.

4.2.1 Recovery from a single exercise

There are many studies suggesting that cryotherapy has a potential not only to reduce EIMD and DOMS following a single exercise, but it may also promote a faster recovery of neuromuscular function (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009). Bailey et al. (2007) showed that CWI reduced the decrement in maximal isometric force following a bout of intermittent exercise, whereas Ascensão et al. (2011) reported CWI to be beneficial following a soccer match when measuring strength. More accurately, Roberts et al. (2014) demonstrated that applying cold treatment following a single high intensity training session improves strength performance during the time period of 20 min to 6 h post-exercise.

Similarly, Ingram et al. (2009) observed reduced decrements in isometric leg extension and flexion strength in the 48-h post-exercise period when CWI was used following a simulated 80 min team sports exercise. In their study, CWI also facilitated the recovery of a repeated sprint performance. Additionally, immediate CWI has been studied to

result in better next-day shuttle run performance compared to control (no treatment), and also a delayed (3 h) CWI is likely beneficial but not as efficient as immediate CWI (Brophy-Williams et al. 2011). In all of the studies mentioned above, CWI was able to reduce DOMS.

A recent study by Tseng et al. (2013) suggests cooling to delay the recovery of EIMD. They state that cooling attenuates the rate of cell turnover and muscle regeneration in the early period of recovery. As the elevation in muscle damage markers occurs during the regeneration phase, the change in the regeneration process time course concurrently delays myoglobin and CK response as well.

Commonly used indirect markers of muscle damage are myoglobin and creatine kinase (CK) concentrations measured in the blood. Myoglobin is an iron- and oxygen-binding protein found in the muscle tissue. Myoglobin has oxygen attached to it to provide extra oxygen for the muscles to keep at a high level of activity for a longer period of time. (Guyton & Hall 2006, 81.) Creatine kinase (CK) is an enzyme, which has a crucial role in energy metabolism as it catalyses the conversion of creatine and consumes ATP to create PCr and ADP. The serum CK level can rise as a consequence of metabolic and mechanical damage of the muscle tissue following exercise, and thus it is commonly used as indicator of EIMD. CK levels can remain elevated up to 7 days following an extreme eccentric exercise. (Brancaccio et al. 2007.)

However, special caution in the interpretation of CK activity as a marker of muscle damage has been advised, since large inter-individual and intra-individual variation has been noted (Statland et al. 1976; Warren et al. 1999). It has been studied that CK is released from the muscle when a fixed loading exceeds a certain limit of muscle ability and causes membrane permeability to change (break point). Consequently, CK leaks into the interstitial fluid, is taken up by the lymphatic system and returned into the circulation. (Brancaccio et al. 2007.) The high-responders, those gaining improvements in performance easiest, to training have been studied to exercise at relatively higher intensity to supply enough energy to complete the same exercise compared to low-responders, thus exercising beyond the CK break point. In the study of Totsuka et al. (2002) peak CK values were 751 ± 81 U/l in high-responders, whereas low-responders peak CK values were 184 ± 16 U/l during the same exercise.

Cryotherapy has been studied to reduce membrane permeability, fluid diffusion into the interstitial space and the diffusion of myoproteins into the extracellular space, consequently diminishing oedema and the acute inflammatory response (Eston & Peters 1999). There are multiple studies showing a lower concentration of CK and/or myoglobin if cold treatment is applied post-exercise (Ascensão et al. 2011; Bailey et al. 2007; Eston & Peters 1999), thus suggesting diminished muscle damage. Additionally, C-reactive protein (CRP) is a widely used inflammatory marker, and its concentration has been studied to be lower when cryotherapy is used following a single exercise (Ascensão et al. 2011).

4.2.2 Long-term adaptation to training

The results of a recent investigation of Halson et al. (2014) suggest that CWI completed four times per week over three weeks of increased training load does not impair adaptation to training in competitive cyclists, rather improving the performance. On the contrary, Yamane et al. (2006) reported that regular CWI of the exercised muscles over a 4- to 6-week training period resulted in an attenuated improvement in cycling and handgrip exercise performance in untrained subjects. However, the water temperature in the study by Yamane et al. (2006) was rather low (5°C and 10°C) and time for immersion long (1-2 x 20 min) potentially affecting the study outcome. Considering adaptation to strength training, CWI has attenuated the acute satellite cell response to a resistance training session, and also reduced strength and hypertrophy adaptation to resistance training during a 12-week period (Raastad et al. 2014; Roberts 2014).

One of the potential factors causing the positive effect of cryotherapy is the transcriptional co-activator PGC-1α. PGC-1α is an important regulator of mitochondrial function, oxidative metabolism, and energy homeostasis, thus it has a potential to improve performance and have a role in adaptation to training. (Lin et al. 2005; Wu et al. 2011.) An acute exercise consisting of five bouts of swimming induced increase in PGC-1α expression in rats, so that one exercise bout caused approximately two-fold increases in full-length muscle PGC-1 mRNA and PGC-1 protein (Baar et al. 2002). In humans, six weeks endurance training promoted PGC-1 α expression as well, especially in type 2a muscle fibers (Russell et al. 2003). Interestingly, the PGC-1α expression has

been studied to be temperature sensitive. As subjects undertook a 3–4 h recovery period in cold (7°C) compared with room temperature (20°C), it resulted in a larger elevation in PGC-1α mRNA expression following a 1 hour cycling (Slivka et al. 2012). A more recent study of Ihsan et al. (2014) proved that even a short 15 min post-exercise cooling may enhance mitochondrial biogenesis and promote exercise adaptation via the enhanced PGC-1α expression in humans.

On the other hand, training induced mechanical and metabolic alterations (e.g. hypoxia, building of metabolic by-products and free-radicals, acidosis, and cell swelling), and even muscle damage with consequent inflammation are considered as essentially physiological, and necessary for repair (myofiber regeneration) and adaptive processes (myofiber hypertrophy and increased capillary supply) (Schönfeld 2010; Yamane et al. 2006). Also the activation of heat shock proteins, certain level of ROS production, and exercise-induced increase in blood flow following exercise may be important to training adaptation (Powers et al. 2007; Roberts 2014; Thompson et al. 2003). Thus, cold treatment potentially attenuates these temperature-dependent processes and could remove the contribution of muscular hyperthermia to the training effects. The next paragraphs briefly describe the role of heat in adaptive processes.

During hypertrophy, contractile elements enlarge as sarcomeres are added in series or in parallel, and the extracellular matrix becomes larger to support growth. Satellite cells, which reside between the basal lamina and sarcolemma, are thought to facilitate muscle hypertrophy in several ways, e.g. by donating extra nuclei to muscle fibers and by co-expressing various myogenic regulatory factors. The activation of mTOR (mechanistic target of rapamycin) signalling pathway is the most important regulator of muscle protein synthesis. (Schönfeld 2010.)

When activated in muscle, mTORC1 senses mechanical contraction through complex mechanisms, which stimulates protein synthesis to drive muscle hypertrophy (Drummond et al. 2009; Philp et al. 2011). Bentzinger et al. (2008) showed that mice lacking mTORC1 in muscles have reduced muscle mass and oxidative function, which may lead to early death. Also nutrition, especially some amino acids (e.g. leucine) and anabolic substances, may activate mTOR pathway and stimulate protein synthesis (Atherton et al. 2010). Other pathways involved in hypertrophy than mTOR include

such as mitogen-activated protein kinase (MAPK), and calcium-dependent pathways (calcineurin being a critical regulator in the Ca^{2+} signalling cascade) (Schönfeld 2010).

In animal studies, a period of acute heat exposure has caused increase in muscle mass, thus suggesting heat to be a potent stimulus for increased protein synthesis (Ohno et al. 2010; Uehara et al. 2004). More precisely, phospho-p70S6K expression, which is a component of mTOR pathway, has been associated with the increased mass of the rat muscles following heat exposure (Uehara et al. 2004; Yoshihara et al. 2013). Additionally, heat stress may stimulate muscle growth via a calcineurin-dependent signaling pathway. Kobayashi et al. (2005) found up-regulation of calcineurin expression with the increased mass of the rat soleus muscle 7 days after heat exposure. They suggested heat stress to modify the intracellular Ca^{2+} level in skeletal muscles, which affects calcineurin expression and hypertrophy.

Heat stress has also enhanced mTOR signalling in human skeletal muscles. A study of Kakigi et al. (2011) measured the phosphorylation of molecules involved in mTOR signalling after resistance exercise (4E-BP1, Akt/PKB, mTOR, and ribosomal protein S6), which all were positively affected by heat exposure, suggesting heat stress to promote protein synthesis. Moreover, Roberts (2014) demonstrated that cold treatment attenuated these key mTOR pathway protein responses, such as p70S6K activity and satellite cell accretion, following exercise.

It was earlier suggested that decreasing ROS production by applying cold might be beneficial as it has a potential to decrease the amount of muscle damage following exercise (Carvalho et al. 2010). However, elevated ROS generation affects the redox status of the muscle fibre, which may contribute to muscle adaptation to exercise, as many of the muscle re-modelling pathways are redox sensitive. Thus, it seems that a balance between ROS production and antioxidant defence system has a central role. Powers et al. (2007) suggest that there is an ideal level of ROS production resulting in hypertrophy and adaptation to training, but high levels of ROS modulating signalling pathways that possibly lead to cell death.

5 SUMMARY OF LITERATURE

With intense dynamic exercise the glycolytic pathway with formation of pyruvate and ATP is used to meet the energy demand of the cross-bridge cycle and muscle ion pumps. Once mitochondria cannot oxidize all of the formed hydrogens, pyruvate is converted to lactate as it accepts the excess hydrogens. Fundamentally, production of lactate has been suggested to contribute to acidosis together with carbon dioxide created during respiration. However, lactate production may actually help to curb the development of acidosis as it allows glycolysis to continue and moreover, lactate is used as an energy source. Only when lactate production exceeds its removal, lactate starts to accumulate and may increase H^+ concentration. For example, Steward's approach describes these acid-base changes via changes in strong ions (such as lactate), weak acids, and carbon dioxide concentration. Robergs et al. (2004), in turn, suggests that part of the H^+ is produced in ATP hydrolysis as the rate of proton production exceeds the rate of proton transportation into the mitochondria. However, this model has faced a lot of criticism.

The general agreement has long been that building up of H^+ in working muscles induces fatigue and limits performance during high-intensity exercise. The pH dependent processes contributing to fatigue include such as change in myosin ATPase activity, reduced activity of the key glycolytic enzymes phosphorylase and phosphofructokinase, and disturbance in membrane excitability. However, the recent whole-body studies in physiological temperatures have questioned the results gained in studies with isolated muscle fibers. As a result, also other fatigue factors than pH have been proposed. For example, it has been hypothesised that inorganic phosphate (P_i) would have a significant role in muscle fatigue as it disturbs muscle force production by altering SR and myofibrillar function.

Muscle injury also causes a decline in performance that reverses only very slowly. The initial events of exercise-induced muscle damage (EIMD) are caused by mechanical stress induced on muscle, which increases permeability in sarcolemma and alters contraction kinetics. During the days after the intense exercise, the inflammation

process is activated, which promotes oedema formation and causes muscle stiffness and soreness called delayed onset muscle soreness (DOMS). Recovery from the most serious injuries involves activation of satellite cells and regeneration of damaged fibers.

Different recovery strategies are used to treat the exercise-induced stress on the body. Immediately following the exercise it is common to use active recovery to improve circulation, which promotes nutrient transport and clearance of metabolic by-products. Consumption of protein and carbohydrates ensures the activation of protein synthesis and restoring of muscle glycogen stores. During the days after an intense exercise the balance between rest, proper nutrition and different recovery strategies, such as massage and stretching, is of importance. In this study cold treatment (cryotherapy) was used to prevent and treat EIMD.

The mechanisms of cold treatment are speculated to be related to temperature-induced changes in blood flow and cell metabolism, subsequently reducing post-exercise inflammation. However, it remains controversial whether cold treatment is effective in treatment of exercise-induced muscle damage following a single exercise, or not. In acute setting, cold treatment has been studied to prevent muscle soreness successfully, but the effect against decrements in neuromuscular function is unclear. Also the appearance of intracellular proteins, which are used as markers of muscle damage, in plasma during recovery is a matter of controversy. Although there is a lack of evidence-based guidelines for cold treatment, cooling has not resulted in negative outcomes and it is popular when the aim is optimal performance and recovery.

The effect of cold treatment over a longer time period is less studied. Since temperature dependent processes are vital to training adaptation cold treatment has been proposed to impair performance and adaptation when applied continuously. On the other hand, cold treatment may enhance recovery between training sessions, and consequently promote performance within subsequent sessions. Thus, it should be carefully considered when exercised-induced stress for the promotion of adaptation is desired and if applying cold treatment can be harmful. Moreover, it should be taken into account that cold water immersion (CWI) is used in most of the studies, and the outcome of other cooling strategies may be different.

Overall the influence of cold treatment in acute settings is rather well studied, but lack of guidelines for the use of cooling post-exercise still exists, whereas the effects of continuous application of cold treatment are rather unknown. There is also a demand for new cooling strategies, which could be used as an alternative for CWI.

6 PURPOSE OF THE STUDY

From the athlete's perspective, knowing the potential mechanisms and implications of intensive anaerobic work and EIMD on performance and being aware of the treatment of muscle damage and the accompanied loss of muscle function are vital for optimal performance. Thereby it is possible to e.g. develop correctly periodized training programme, maximize the training gains and avoid under-performance and injuries. The purpose of the recent study was to investigate the efficacy of a recently developed cold treatment modality on biochemical, neuromuscular and perceptual markers of EIMD following both an intensive anaerobic running exercise session and following a 7-day training microcycle including anaerobic interval running and hypertrophic strength training.

Research problem 1. Does cold treatment effect on the rate of muscle damage, muscle soreness, or on the recovery of physical performance following an anaerobic running exercise?

Hypothesis 1. Cold treatment can limit the extent of muscle damage (*creatine kinase, myoglobin, C-reactive protein, testosterone, cortisol*) and pain, and improve the functional recovery *(isometric force, countermovement jump, sprint ability*) after anaerobic exercise.

Cold treatment following exercise has been studied to e.g. prevent oedema formation, and decrease the extent of muscle cell damage as mechanical stress on cells is decreased, oxygen delivery is improved, and oxygen supply matches better to its demand (Ihsan et al. 2013; Vaile et al. 2011; Yanagisawa et al. 2003a). As less breakdown products of dying cells are released, the inflammatory response and secondary damage are weakened (Lamb et al. 1995; Verburg et al. 2005; Zhang et al. 2008), which can be seen in the amount of indirect muscle damage and inflammatory markers (creatine kinase, myoglobin, C-reactive protein). Additionally, cold treatment has been studied to reduce membrane permeability, and consequently less muscle damage and inflammatory markers are released from the muscle cells into the blood

stream (Eston & Peters 1999). The effect of cold treatment on endocrine response is studied less, Nemet et al. (2009) suggesting cooling to have no effect on testosterone and cortisol levels following an intermittent running exercise.

Cold treatment has been studied to be effective in restoring strength if applied post-exercise (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009). Also running performance has recovered better in some studies (Brophy-Williams et al. 2011; Ingram et al. 2009) but there are also studies showing no benefit of cold treatment for sprint performance (Ascensão et al. 2011). Cold treatment reduces the acute (1–3 h) pain perception following exercise via analgesic effect (Ascensão et al. 2011; Bailey et al. 2007) and the delayed soreness is reduced e.g. as less breakdown product sensitise nociceptors and there is less pressure on pain receptors (Enwemeka et al. 2001; Merrick et al. 1999; Proske & Morgan 2001).

Research problem 2. Does cold treatment effect on the adaptation to training?

Hypothesis 2. Long-term use of cryotherapy may impair the adaptation to training.

The results from the acute cold treatment studies suggest cooling to result in an enhanced stimulus for adaptation and improvements in performance (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009). However, when studying the effects of a long-term cold treatment on muscle aerobic performance, the demonstrated results are contradictory (Ihsan et al., 2014; Slivka et al., 2012; Yamane et al., 2006). For example, Yamane et al. (2006) suggested that micro-damages and associated metabolic alterations following exercise are essentially physiological, and necessary for repair and adaptive processes. Also decreasing ROS production and activation of heat shock proteins via cooling may be harmful, as they have a role in the training adaptation process (Powers et al. 2007; Thompson et al. 2003).

7 METHODS

7.1 Participants

Eight recreationally active males volunteered to participate in this study. Mean (± standard deviation) for age, height, body mass and fat percentage were 22.9 ± 1.4 years, 177.3 ± 5.8 cm, 79.1 ± 7.6 kg and 13.3 ± 2.9 %, respectively. The participants were free from acute and chronic illness, disease, injury or use of medications that would contraindicate participation. Participants were informed of the risks and benefits associated with the study and that they could withdraw from it at any time. Participants completed a mandatory health questionnaire (Appendix 1) and signed an informed consent document (Appendix 2). The study was approved by the local University Ethical Committee.

Height was measured using a stadiometer with an accuracy of 0.1 cm. Participants faced directly ahead and had feet together, arms by the sides, and heels, buttocks and upper back in contact with the wall when the measurement was made. The body mass and fat percentage were analyzed using bioimpedance (InBody 720 body composition analyzer, Biospace CO. Ltd., Seoul, South Korea). InBody 720 uses eight polar tactile electrodes where a different electrical voltage and current are sent through the body. It completes 30 impedance measurements by using six different frequencies (1, 5, 50, 250, 500 and 1000 kHz) and records 15 reactance measurements by using three different frequencies (5, 50 and 250 kHz) at each segment of the body. InBody 720 provides data of e.g. weight, fat and muscle mass, intra and extracellular fluid, mineral content, and it has a high correlation coefficient of 0.984 with DEXA. (Biospace Co. 2014.) Participants were advised to have a meal at least two hours before the body composition analyze and they went to a toilet before the measurement. During the analysis, participants stood in an upright position wearing minimal clothing and no socks. No jewelry was worn as they may interfere with the electrical conductivity. The electrodes were cleaned and left a bit wet to improve the electrical conductivity.

7.2 Experimental design

The study was carried out in the spring 2013 in the laboratories of the Department of Biology of Physical Activity, University of Jyväskylä. All participants performed a full familiarisation of the performance tests and exercise protocol two weeks prior to participation. During the familiarisation session, maximal sprint running and strength tests were carried to determine suitable exercise intensities for subsequent training sessions. All running tests (60 and 100 m) and training sessions during the training periods were performed at Hipposhalli indoor track and field hall (Novotan surfaced track, temperature of 17–19°C, and relative humidity of 30 %), whereas strength tests and training sessions were conducted at the gym of the University of Jyväskylä (temperature of 18–20°C, and relative humidity of 30 %). Maximal strength tests consisted of bench press, leg press, biceps curl, knee extension, and knee flexion.

Participants served as their own controls and received each treatment, cold (COLD) or control (CONTROL), in a random order. The two trials, each lasting all in all two weeks, were separated by at least two weeks to ensure full recovery and to minimize the training adaptations to the exercise protocol. Also abstaining from therapeutic treatments including massage, stretching and anti-inflammatory drugs for the duration of the investigation was advised. Participants were asked to refrain from alcohol and caffeine for 24 h, and strenuous exercise for 48 h before each testing session. Biochemical and perceptual markers of muscle damage were obtained at baseline, immediately after, and 30 min, 1, 24 and 48 h after the acute exercise. In addition, biochemical and perceptual markers of muscle damage were obtained after 4 d recovery from a 7 d training period. For the 24, 48 h and post-4d blood samples, participants returned to the laboratory after an overnight fast at approximately the same time of the morning. Neuromuscular markers of muscle damage were obtained at baseline, 48 h after the acute exercise, and 4 d after the training period.

Acute exercise bout. In the first morning (between 7 and 9 AM) of testing participants arrived at the laboratory in a fastened state (10 h) and a venous blood sample was taken from a vein in the antecubital fossa (baseline). Next, participants consumed a light breakfast (Appendix 3) and perceived muscle soreness was recorded. Baseline jump and

sprint abilities and quadriceps strength were assessed during the 1 h between the breakfast and the start of the acute exercise bout. Approximately 40 min after the breakfast participants did a 20 min warm-up, after which they completed the performance tests as outlined below. Subsequently, participants did a 10 x 20 m shuttle running test twice, separated by a 3 min break. Participants were required to run with the maximum pace and they received verbal encouragement during the test. Participants were advised to drink only a little or no water following the acute exercise to avoid haemodilution.

The 10 x 20 m shuttle running test was chosen, since high-intensity intermittent sprints employ high force actions (both eccentric and concentric) by a relatively large leg muscles and can be expected to produce mechanical and metabolic stress. Turns and changes of direction induce even more stress on working muscles, thus a shuttle run was chosen instead of track running. A work period of approximately 45 s can be expected to deplete phosphocreatine stores (roughly 10 seconds) and to utilize anaerobic glycolysis for most of the energy supply. Only a small quantity of energy is from aerobic processes. A rest period of 3 min was chosen to allow replenishment of phosphocreatine stores between the two sets. Immediately after (< 2 min) the second shuttle running set a blood sample was taken, after which the cold treatment was applied for 2 min. There was max 10 min in between the last shuttle running set and the cold treatment. The cold treatment (2 min) was repeated 12, 24 and 36 h after the exercise. A schematic representation of the protocol is provided in Figure 15.

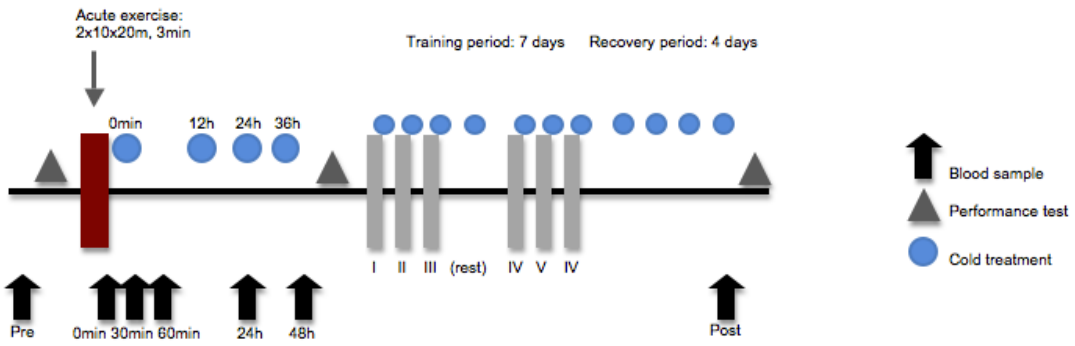

Figure 15. Study design. The first 48 h illustrate the acute phase, after which the training period starts.

Training period. After the acute part of the study the participants did a week lasting training period. The one week training period consisted of three hypertrophic strength training sessions and three anaerobic interval running sessions on alternating days, the 4th day being a rest day. Each of the training sessions lasted approximately an hour. The training loads for each session were determined from the maximal tests performed during the familiarization session.

Strength session (Appendix 4a). Participants started with a standardized warm-up consisting of 7 min of cycling with an increasing resistance. Additionally, participants performed a self-selected upper body warm-up (e.g. arm circles, push ups). After the warm-up, participants performed a resistance training program consisting of leg press, bench press, knee extension, biceps curls and knee flexion. The exercise order was kept same in all sessions. Participants performed 4 x 10 repetitions, the rest between the sets and exercises being 2 min. The load was varied from session to session so that the final repetition of each set was performed near failure and at the sudden onset of failure assisted repetitions were used to achieve minimum of eight repetitions. At the end of the strength session participants did 4 x 25 sit ups and 2 x 25 back extensions with a 1 min rest between the sets. The cool down consisted of 5 min cycling and short stretches for hamstrings and quads. The duration of resistance exercise session was approximately 75 min.

Anaerobic interval running session (Appendix 4b). The running session started with a warm-up consisting of jogging for duration of 7 minutes with a self-selected pace, static stretching (duration of < 20 s each: hamstrings, quadriceps, buttocks, hip flexors, calves), dynamic activities and sprinting. After the warm-up, the participants performed 5 x 100 m at 70 % of maximum running velocity with a 1 min rest in-between. After the last sprint there was a 3 min rest before the second part of the session. The second part consisted of 4 sets of 5 repetitions of a 60 m distance (4 x 5 x 60 m) at 80 % of maximum running velocity. The rest between the repetitions was 2 min and between the sets 4 min. The cool down consisted of 5 minutes of jogging and short stretches for hamstrings and quadriceps. The duration of running session was approximately 70 min.

7.3 Nutritional control

Prior the study, participants were given dietary instructions, which were in line with the national nutrition recommendations (Valtion ravitsemusneuvottelukunta 2005). For three days before data collection and during the protocol, they were instructed not to change their normal eating habits and to abstain from additional dietary supplementation. Participants were required to complete a food diary for five days during both trials, including both weekdays and weekend days. They were asked to write down all foods consumed, estimate the portion sizes as accurately as possibly, try not to alter the normal intake behaviour, and to replicate dietary intake as closely as possible. Food diaries were analysed for energy (kcal), protein, fat, carbohydrate and alcohol by using Nutri-Flow programme (Flow-Team Oy, Oulu, Finland, 2012).

7.4 Cold treatment

Wearing minimal clothing, the subjects were exposed to water mist shower (2 min at 10–15°C; Amandan®, Amandan Healthcare Oy Ltd., www.amandan.fi, Finland, 2015). This treatment was applied immediately after the 10 x 20 m acute exercise bout and at 12, 24 and 36 h thereafter. During the training period, the cold treatment was repeated after each training session and once a day during the 4-day recovery period. The shower room ambient temperature varied between 18 and 20°C and the relative humidity was approximately 30 % before starting the cold treatment. The shower was placed at the shoulder height, the mist targeting the upper body. In this study cooling was used repeatedly, although there are both studies using a single session of cryotherapy immediately following exercise and studies using cooling repeatedly (Ascensão et al. 2011; Bailey et al. 2007; Eston & Peters 1999; Ingram et al. 2009).

The earlier study of the low-energy water mist shower (Heikkinen et al. 2013) showed that the skin temperature falls 8.8–6.8°C as a result of 2 min shower (Table 1). This study was conducted with 14 thermographic sensors and the subjects were imaged prior the treatment, at 1 min and 2 min post-treatment to examine the temperature changes. According to Vatanen et al. (2012), the used cooling process is not dependent on the water temperature at water temperatures below 15°C and if the relative humidity of the

shower room is low. The normal pressure on water tap from 4 to 6 bar is enough for the use of mist shower and the pressure does not effect on the cooling rate.

Table 1. Skin temperatures following a 2 min water mist shower measured using 14 thermographic sensors (Heikkinen et al. 2013, 46–47).

	Pre	**Post 1 min**	**Post 2 min**
Sternum	31.8	24.2	25.1
Diaphragm	30.9	24.1	24.7
Upper arm left	30.3	22.2	23.3
Upper arm right	30.7	21.9	23.1

6.5 Blood analysis

Venous blood samples (5ml) were collected using sterile needles into serum tubes (Venosafe, Terumo Mediacl Co., Leuven, Belgium) by a qualified lab technician. Whole blood mode was measured using Sysmex KX-21N automated hematology analyzer (Sysmex Co., Kobe, Japan), and haemoglobin and haematocrit were used to calculate plasma volume changes. The inter-assay coefficients of variation were for haemoglobin 1.5% and for haematocrit 2.0%. Subsequently, the whole blood was prepared for the later analysis. It was centrifuged at 3.500 rpm (Megafuge 1.0R, Heraeus, Germany) for 10 min after which serum was removed and the sample was stored at -80°C until further analysis. The changes in plasma volume were calculated using following formula (Harrison 1985):

$$\%\,\Delta\,PV = 100\left(\frac{(Hb^1)\,(1 - Hcr^2)}{(Hb^2)(1 - Hcr^1)} - 1\right)$$

Hb^1 = haemoglobin pre, Hb^2 =haemoglobin 0min, Hcr^1 = haematocrit pre, Hcr^2 =haematocrit 0min

Muscle damage and inflammation. In this study myoglobin and creatine kinase concentrations were measured to evaluate the amount of EIMD, and C-reactive protein to evaluate the inflammatory response. Myoglobin and hsCRP (high sensitivity CRP) were analyzed using chemical luminescence techniques (Immulite 1000, DPC, Los Angeles, USA). The sensitivity for serum myoglobin was 0.5 ng/ml and for hsCRP 0.1 mg/l. The inter-assay coefficients of variation were: myoglobin 7.2% and hsCRP 6.1%. Creatine kinase analyze was performed using KoneLab 20 XTi -analyzer (Thermo

Fisher Scietific,Vantaa, Finland) with Thermo reagents. The sensitivity for serum CK was 10 U/l. The inter-assay coefficient of variation was for CK 2.8%.

Serum hormones. Serum testosterone (TT), cortisol (C) and sex hormone binding globulin (SHBG) were measured to describe the anabolic/catabolic state following the exercise. The analyses were performed using chemical luminescence techniques (Immunlite 1000, DPC, Los Angeles, USA) and hormone specific immunoassay kits (Siemens, New York, NY, USA). The sensitivities for serum hormones were for TT 0.5 nmol/l, for C 5.5 nmol/l and for SHBG 0.2 nmol/l. The intra-assay coefficients of variation were for TT 8.7%, for C 7.1% and for SHBG 6.5%.

Anaerobic metabolism. Lactate was measured to evaluate anaerobic metabolism. The analyses were performed using Biosen c_Line Sport -analyzer (EKF Diagnostic, Magdeburg, Germany) with EKF reagents. The whole blood samples were taken from the fingertip. The sensitivity for the whole blood lactate was 0.5 mmol/l. The inter-assay coefficient of variation was 6.2 %.

7.6 Performance tests

Performance test order was kept the same throughout all testing. The tests were performed prior to the 10 x 20 m shuttle run, and again 48 h post-exercise and 4 d after the training period. Before each physical testing session the participants did a standardized 20 min warm-up including jogging, static stretching (duration of < 30s each: hamstrings, quadriceps, buttocks, hip flexors, calves), dynamic activities and sprinting.

Countermovement jump (CMJ). Maximum CMJ was determined using the flight time of the jump, measured using a contact mat (Department of Biology of Physical Activity, Jyväskylä, Finland). One-minute rest separated the three trials and participants were instructed to jump for maximum height. The depth of the countermovement was self-selected and represented each participant's optimal depth for maximal jump. The highest jump was recorded. Height (h) was calculated using following formula (Keskinen et al. 2007, 153).

$$h = \frac{1}{8} g t_n^2 \qquad g = 9{,}81\ m/s^2,\ t_n = flight\ time$$

Isometric strength. The maximal voluntary isometric contraction of the leg extensors was measured using an isometric leg press (Department of Biology of Physical Activity, University of Jyväskylä, Finland). After a warm-up set of two sub-maximal repetitions of leg extension at a knee angle of 107°, participants completed two to three maximal repetitions separated by 2 min rest. The referred angle was measured using a goniometer during the familiarisation; the greater trochanter and the lateral malleolus of the ankle were used as anatomical markers. The leg press settings were kept same throughout the study. Participants received verbal encouragement and the best performance was recorded.

Sprint test. The repeated sprint test comprised 10 x 20 m maximal sprints conducted in an indoor track and field hall (Novotan surfaced track, temperature of 17–19°C, and relative humidity of 30 %). The sprinting times were recorded using electronic timing gates (Newtest Oy, Finland) positioned at the start and at 20 m. The participants stood 0.70 m behind the starting line and began running upon a verbal signal. Timing began when the participants crossed the first pair of photocells. There was one-minute rest between the sprints. The best single 20 m sprint time and the total time for 10 sprints were calculated.

7.7 Perception of muscle soreness

Ratings of perceived muscle soreness were evaluated using a scale ranging from 0 (no soreness) to 10 (very intense soreness) before, immediately after, and 30 min, 1, 24 and 48 h after the acute exercise bout. In addition, participants rated the muscle soreness 4 d after the training period. Participants rated soreness for quadriceps and hamstrings while standing and they were encouraged to palpate muscles during assessment.

7.8 Statistical analysis

Data was analysed and graphed using Microsoft Excel 2010 and IBM SPSS Statistics v.20 computer software. Within group differences were analysed using repeated

measures ANOVA with seven levels (Pre, 0min, 30min, 60min, 24h, 48h and Post). Between group differences were analysed with an independent samples t-test. Additionally, inter- and intra-individual variations were calculated using the coefficient of variation (CV %). Significances were set at * $P < 0.05$.

8 RESULTS

8.1 Dietary intake

There were no significant differences ($P > 0.05$) in the study participants' nutrition between the control and cold experiments (Table 2). During the both trials, food diaries were kept for five days including both weekdays and weekend days. The daily intake of carbohydrates accounted for 39.9 % and 40.7 % of the total energy intake in CONTROL and COLD, respectively. The daily fat intake was 32.0 % (CONTROL) and 31.3 % (COLD), whereas the daily protein intake accounted for 25.1 % and 23.4 % of the total energy intake. Alcohol accounted for the rest of the energy intake, 3.0 % in CONTROL and 4.6 % in COLD. The protein intake in grams per kilogram of body weight was 1.86 ± 0.38 g/kg in CONTROL and 1.80 ± 0.63 g/kg in COLD.

Table 2. Dietary intake during control and cold trials. There are no significant ($P > 0.05$) differences between the groups.

	CONTROL	**COLD**
Energy (kcal)	2390 ± 282	2474 ± 404
Carbohydrates (g)	233 ± 60	246 ± 62
Carbohydrates (kcal)	954 ± 244	1008 ± 252
Protein (g)	146 ± 28	141 ± 48
Protein (kcal)	599 ± 114	579 ± 196
Fat (g)	87 ± 20	88 ± 17
Fat (kcal)	767 ± 178	775 ± 150
Fiber (g)	26 ± 6	28 ± 17
Alcohol (g)	2 ± 3	6 ± 15

8.2 Acute exercise and anaerobic metabolism

The acute exercise consisted of two sets of 10 x 20 m shuttle running, which were separated by a 3 min break. The time of the 1st set was 44.3 ± 2.7 sec in CONTROL group, whereas in COLD group the time was 44.2 ± 2.5 sec. The time of the 2nd set was

slightly but not significantly (P > 0.05) slower in both groups (CONTROL 47.8 ± 4.0 sec vs. COLD 47.6 ± 2.3 sec). There were no differences between the groups (P > 0.05).

Lactate. Lactate (figure 16) reached its peak immediately (0 min) following the anaerobic exercise in both groups and there is no statistical difference between the groups (CONTROL 16.3 ± 3.6 vs. COLD 16.2 ± 3.4). Lactate levels return near to baseline an hour following the anaerobic exercise bout in both groups (CONTROL 3.1 ± 1.0; COLD 2.6 ± 1.4), however, the difference compared to the pre-level remains significant (P < 0.05) only in CONTROL. The absolute difference of the 0 min–sample and the 30 min–sample is smaller in CONTROL compared to COLD (CONTROL 8.4 ± 2.0 vs. COLD 10.7 ± 2.5) and the difference between the groups is significant (P < 0.05). However, the absolute difference of the 0 min–sample and 60 min–sample is similar (P > 0.05) in both groups. The inter-individual coefficients of variation were 20.4% (CONTROL) and 19.4 % (COLD) at 0 min, 40.3 % and 44.4 % at 30 min, and 28.9 % and 50.1 % at 60 min.

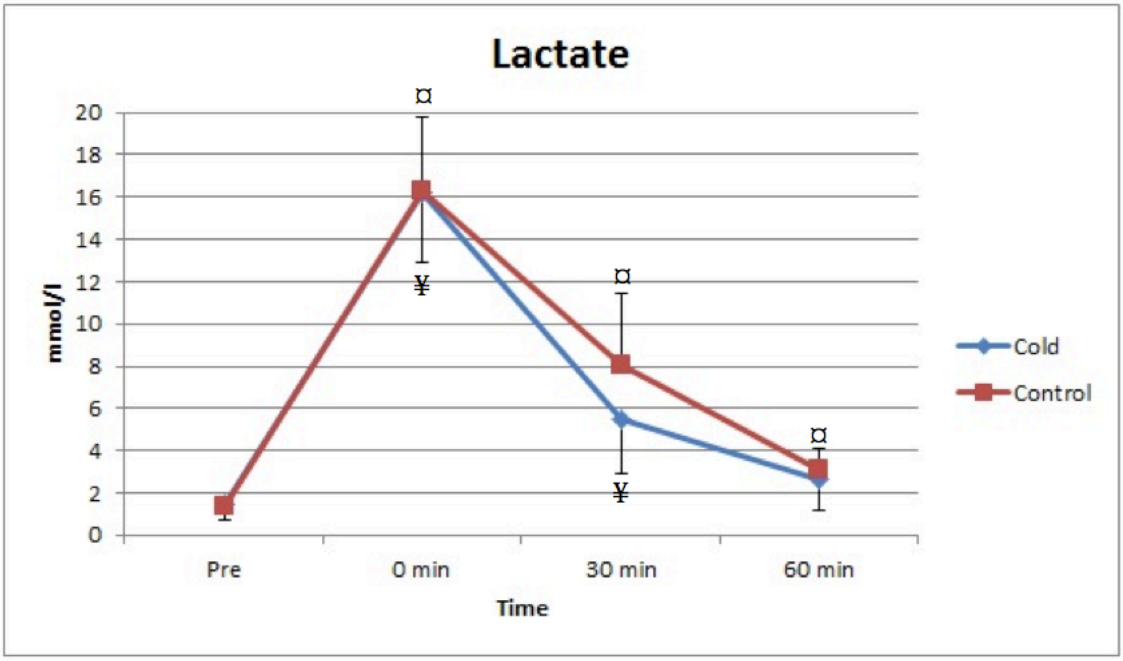

Figure 16. Changes in lactate concentrations at different time points in control (red) and cold (blue) trial. ¥ significant difference (P < 0.05) compared to pre-sample in cold group, ¤ significant difference (P < 0.05) compared to pre-sample in control group.

8.3 Blood variables

Haemoglobin and haematocrit. There is no difference between the groups in the pre-level of haemoglobin (CONTROL 157 ± 10 g/l; COLD 157 ± 12 g/l). In both groups, there are no significant ($P > 0.05$) changes compared to the pre-level at any time point. Similarly, there is no difference between the groups in the pre-level of haematocrit (CONTROL 45.6 ± 2.5 %; COLD 45.8 ± 3.0 %). However, the increase in haematocrit at 0 min compared to the pre-level reaches significance ($P < 0.05$) in both groups (CONTROL 48.6 ± 2.6 %; COLD 48.6 ± 3.2 %). There are no differences ($P > 0.05$) in haemoglobin or haematocrit between the groups at any time point. Plasma volume change was calculated using haemoglobin and haematocrit (Harrison 1985). The change was largest from the baseline to 0 min (CONTROL -9.9 ± 5.0 %; COLD -9.0 ± 3.2 %), the difference between the groups not being significant ($P > 0.05$).

Myoglobin. Following the anaerobic interval running, myoglobin values increase in both groups (Figure 17). The peak is reached 60 min after the exercise (CONTROL 107.36 ± 40.40 ng/ml; COLD 174.48 ± 98.94 ng/ml) and it is significantly higher ($P < 0.05$) compared to pre-level. The post-sample myoglobin concentration is significantly lower ($P < 0.05$) in COLD (24.64 ± 5.63 ng/ml) compared to CONTROL (36.35 ± 14.02 ng/ml). Additionally, the post-level in COLD is significantly ($P < 0.05$) lower compared to the pre-level (Pre 29,95 ± 6,90 ng/ml vs. Post 24,64 ± 5,63 ng/ml). The absolute difference of the 60 min-sample and the 48 h-sample is 79.65 ± 37.76 ng/ml in CONTROL and 145.74 ± 95.75 ng/ml in COLD. There is almost a statistically significant difference between the groups ($P = 0.051$) when comparing this difference. The average value of the intra-individual coefficient of variation is 6.7 ± 5.7 % in the pre-sample and 24.9 ± 19.9 % in the post-sample. The inter-individual coefficients of variations are presented in Table 3.

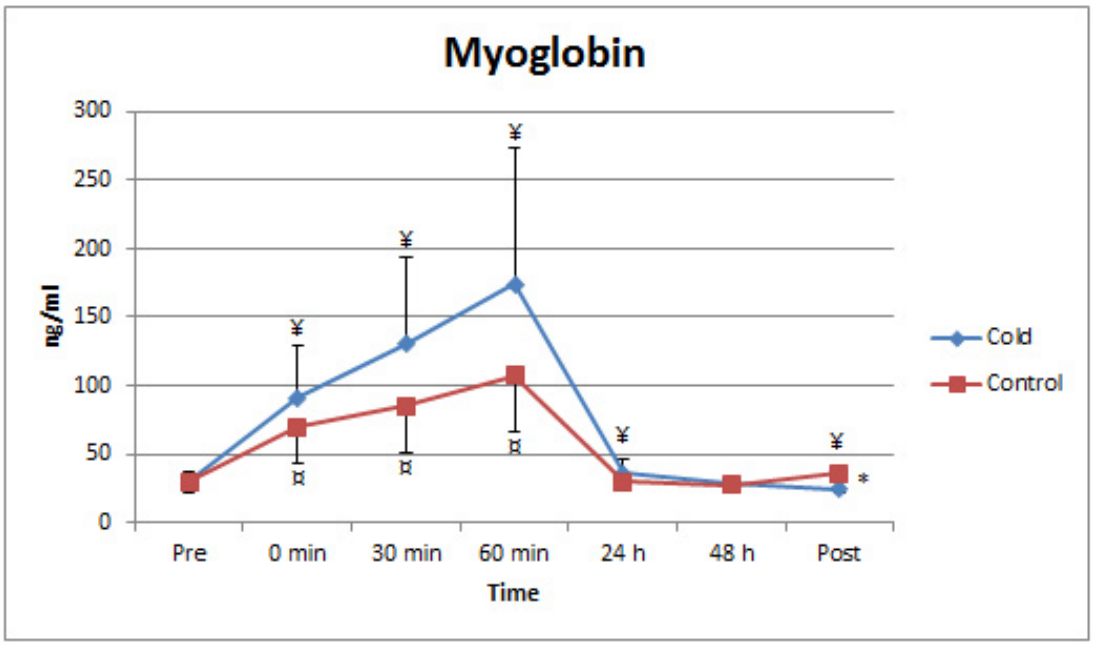

Figure 17. Changes in serum myoglobin concentrations at different time points in both control (red) and cold (blue) trial. ¥ significant difference ($P < 0.05$) compared to pre-sample in cold group, ¤ significant difference ($P < 0.05$) compared to pre-sample in control group, * significant difference ($P < 0.05$) between the groups at the corresponding time point.

Creatine kinase. Following the anaerobic interval running, CK values increase in both groups reaching the peak 24 h after the exercise (CONTROL 329.25 ± 156.46 U/l; COLD 542.00 ± 263.83 U/l). The 24 h–value is significantly higher ($P < 0.05$) compared to the pre-level in both groups (Figure 18). The post-sample value is lower compared to the pre-sample value in COLD (Pre 251.00 ± 143.83 U/l vs. Post 168.13 ± 91.90 U/l). There are no significant ($P > 0.05$) differences between the groups at any time point. Similarly, there is no significant difference ($P = 0.105$) between the groups when comparing the absolute difference of the 24 h–sample and the 48 h–sample (CONTROL 116.88 ± 72.79 U/l vs. COLD 193.75 ± 145.35 U/l). The average value of the intra-individual coefficient of variation is 47.3 ± 34.9 % in the pre-sample and 32.5 ± 16.0 % in the post-sample. The inter-individual coefficients of variations are presented in Table 3.

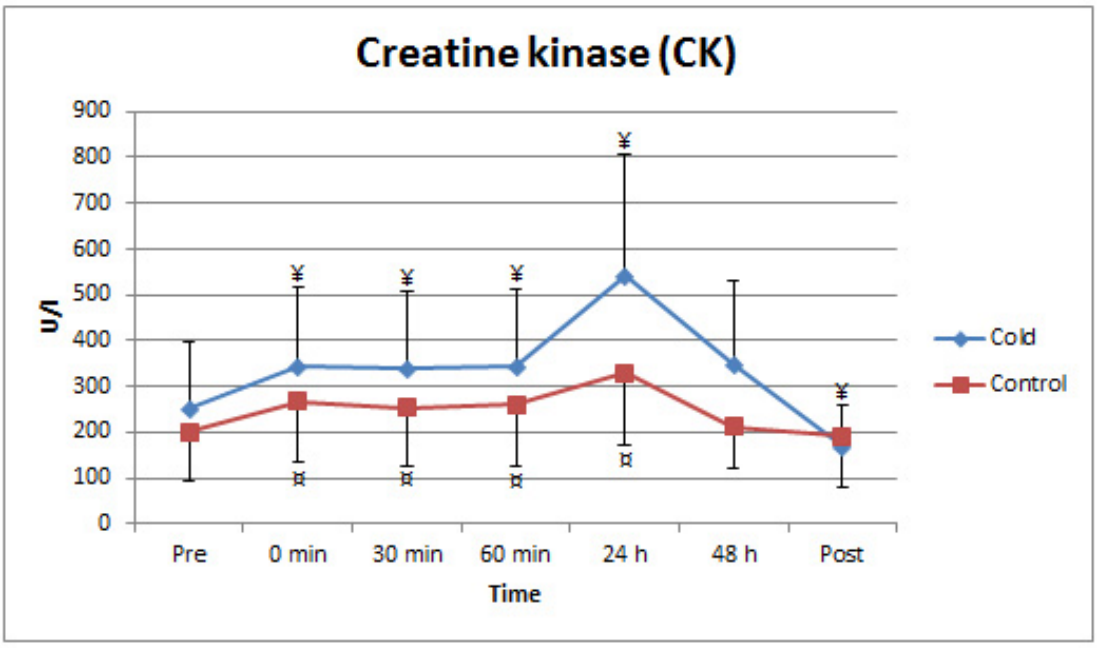

Figure 18. Changes in serum creatine kinase concentrations at different time points in both control (red) and cold (blue) trial. ¥ Significant difference ($P < 0.05$) compared to pre-sample in cold group, ¤ significant difference ($P < 0.05$) compared to pre-sample in control group.

Table 3. The inter-individual coefficients of variations (CV %) of myoglobin and creatine kinase.

		Pre	**0 min**	**30 min**	**60 min**	**24 h**	**48 h**	**Post**
Myoglobin (CV %)	Control	27.7	34.6	36.7	35.2	22.5	17.3	36.1
	Cold	21.5	39.4	45.8	53.0	27.1	18.3	21.4
Creatine kinase (CV %)	Control	50.7	46.7	47.0	48.1	44.5	40.7	54.0
	Cold	53.6	48.2	47.2	46.3	45.5	48.9	51.1

C-reactive protein, CRP. CRP concentration stays relatively stable during the study and there is no significant ($P > 0.05$) peak or difference between the groups at any time point. The pre-level is 0.86 ± 0.75 mg/l in CONTROL and 0.47 ± 0.31 mg/l in COLD.

Testosterone. There is a significant ($P < 0.05$) drop in testosterone at 30 min and 60 min compared to pre-level in both groups (Table 4). At 48 h, testosterone remains significantly ($P < 0.05$) lower in COLD compared to the pre-level, but there is no difference in CONTROL ($P > 0.05$). There are no significant differences ($P > 0.05$) between the groups in testosterone levels at any time point.

Table 4. Hormone and CRP values during the experiment. ¤ significant difference ($P < 0.05$) compared to pre-sample in control group, ¥ significant difference ($P < 0.05$) compared to pre-sample in cold group.

		Pre	0 min	30 min	60 min	24 h	48 h	Post
Testosterone	Control	17,66 ± 4,68	16,51 ± 5,65	14,73 ± 4,59 ¤	12,74 ± 3,86 ¤	18,43 ± 6,86	17,37 ± 7,11	20,11 ± 9,31
	Cold	19,65 ± 7,12	17,56 ± 5,91	15,21 ± 5,80 ¥	14,17 ± 4,46 ¥	17,84 ± 5,86	17,65 ± 5,79 ¥	18,11 ± 4,79
SHBG	Control	25,29 ± 5,82	27,84 ± 5,63 ¤	26,89 ± 5,75 ¤	25,74 ± 5,51	25,74 ± 6,39	25,76 ± 6,47	28,53 ± 5,36 ¤
	Cold	25,05 ± 5,55	27,35 ± 6,91 ¥	25,19 ± 7,77	23,95 ± 6,28 ¥	22,99 ± 9,17	26,88 ± 4,22	24,61 ± 8,11
Cortisol	Control	579,63 ± 120,89	435,75 ± 115,43 ¤	530,63 ± 120,23	447,00 ± 125,49 ¤	543,75 ± 62,05	519,75 ± 115,44	548,88 ± 68,46
	Cold	483,50 ± 83,57	458,63 ± 90,79	527,75 ± 116,51	436,38 ± 133,82	520,50 ± 79,58	510,38 ± 71,52	503,88 ± 64,37
CRP	Control	0,86 ± 0,75	0,87 ± 0,78	0,76 ± 0,82	0,77 ± 0,66 ¤	0,83 ± 0,54	0,60 ± 0,40	1,50 ± 3,08
	Cold	0,47 ± 0,31	0,42 ± 0,30	0,46 ± 0,30	0,43 ± 0,27	0,73 ± 0,44	0,99 ± 1,12	0,22 ± 0,10

SHBG. Following the acute exercise, there is immediately (0 min) a significant ($P > 0.05$) increase in both groups (Table 4). At 30 min, SHBG returns near the pre-level in COLD, but remains significantly ($P < 0.05$) higher in CONTROL. On the contrary, there is a significant ($P < 0.05$) drop compared to the pre-level in COLD at 60 min. Following the training week, SHBG rises significantly ($P < 0.05$) above the baseline in CONTROL, whereas there is no difference in COLD compared to the baseline. There are no differences ($P > 0.05$) between the groups in SHBG at any time point.

Cortisol. In the control group, there is a significant ($P < 0.05$) decline compared to pre-level at 0 min and at 60 min following the acute exercise (Table 4), whereas cortisol levels stay stable in COLD group throughout the study. There are no differences ($P > 0.05$) between the groups in cortisol at any time point.

8.4 Performance tests

All the performance test results are presented in Table 5. There were no within or between group differences ($P > 0.05$) in the mean sprint times recorded prior, 48 h after the acute exercise and following the training week. The best sprint times followed the same pattern reaching no significances ($P > 0.05$) between CONTROL and COLD or

within the groups. The absolute difference of the best 20 m time at pre and post measurement was 0.03 ± 0.09 s in CONTROL and -0.03 ± 0.05 s in COLD. In other words, COLD had improved the sprint time slightly, however, the difference between the groups not reaching significance (P = 0.086). Similarly there were no significant differences (P > 0.05) within the groups or between the recovery conditions for the isometric strength and CMJ measures.

Table 5. Performance tests resulted in no significant differences (P > 0.05) between the groups.

		Jump height (cm) n=7	**Isometric leg press** (kg) n=8	**The best 20m time** (sec) n=7	**The mean 20m time** (sec) n=7
Pre	Control	43,3 ± 7,8	398 ± 129	3,08 ± 0,18	3,13 ± 0,19
	Cold	41,4 ± 9,9	402 ± 110	3,09 ± 0,10	3,13 ± 0,11
48 h	Control	43,3 ± 7,0	390 ± 114	3,06 ± 0,13	3,12 ± 0,15
	Cold	41,9 ± 8,7	403 ± 101	3,09 ± 0,10	3,14 ± 0,11
Post	Control	41,2 ± 8,6	382 ± 130	3,10 ± 0,15	3,17 ± 0,18
	Cold	40,0 ± 7,7	405 ± 129	3,05 ± 0,09	3,13 ± 0,11

8.5 Perception of muscle soreness

DOMS following the exercise is biphasic, peaking first immediately following the exercise and for the second time 48 h after the exercise in both groups (figure 19). The difference compared to the baseline is significant (P < 0.05) at 0 min and at 30 min in all occasions. In COLD, the pain perception in hamstrings at 60 min does not significantly (P > 0.05) differ from the pre-level, whereas in quadriceps the difference is significant in both groups (P < 0.05). Following the training week, neither group perceives soreness and there is no difference compared to the pre-state (P > 0.05). There are no significant differences between the groups in the perceived muscle soreness (P > 0.05). The inter-individual variation is expressed as the range: the difference between the highest and lowest values (Table 6).

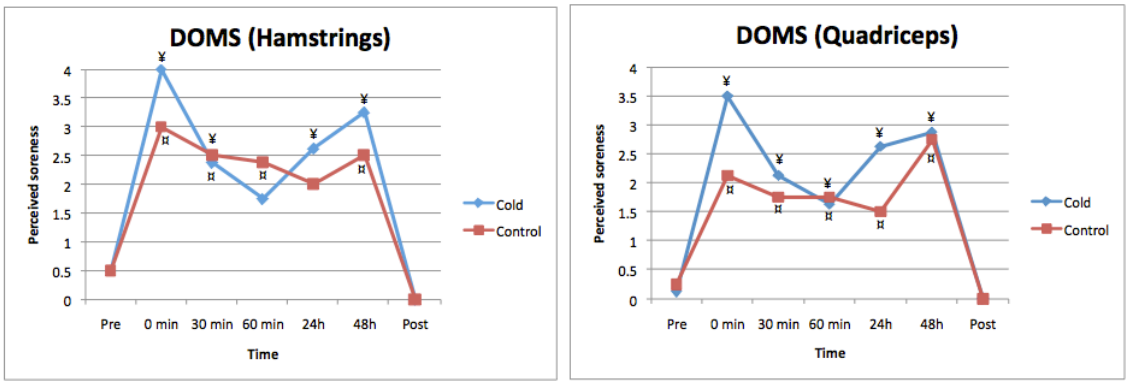

Figure 19. Delayed onset of muscle soreness (DOMS) during the experiment in hamstring muscles. ¥ significant difference ($P < 0.05$) compared to pre-sample in cold group, ¤ significant difference ($P < 0.05$) compared to pre-sample in control group.

Table 6. The range (highest–lowest, maximum 10) in perceived muscle soreness.

		Pre	**0 min**	**30 min**	**60 min**	**24 h**	**48 h**	**Post**
Hamstrings	Control	2	8	6	6	5	7	0
	Cold	2	9	7	5	5	8	0
Quadriceps	Control	2	4	4	4	3	7	0
	Cold	1	6	6	4	4	8	0

9 DISCUSSION

The present study investigated the effects of cold treatment on recovery of performance and indicators of EIMD following an anaerobic running exercise and a weeklong training period including anaerobic interval running and hypertrophic strength training. The main findings were:

(1) The anaerobic exercise induced modest muscle soreness and damage, as evidenced by a small increase in DOMS, and serum myoglobin and CK levels post-exercise, and it appears that cryotherapy does not affect these measures.

(2) Cryotherapy used during a short training period lowered myoglobin concentration, which suggests long-term use of cryotherapy to potentially decrease the amount of muscle damage and improve adaptation to training.

9.1 Recovery from the acute exercise

To make sure the participants dietary habits followed national nutritional recommendations (Valtion ravitsemusneuvottelukunta 2005), and that there were no differences between the groups, the food diaries were analysed. Since no differences were found, it can be assumed that nutrition does not cause the differences observed between the cold and control condition, and that there is no nutritional aspect restraining recovery.

To ensure participants were exerting the same level of effort in both trials, average running time and lactate concentration were compared between groups, and no significant differences were observed. This indicates that participants were exerting a similar effort during the exercise protocol prior to cold and control treatment, and they are assumed to experience an equal level of stress. Average lactate achieved in each trial is indicative of anaerobic rate of work, while average sprint time evaluates the power generated during the sprint-exercise. Both groups achieved a relatively high lactate concentration, which suggests that the rate of anaerobic metabolism has been dominant

during the acute exercise. Fatigue in these high-intensity exercises is mainly pH dependent, however, also other ionic changes affect the force production as well. Acidosis, caused predominantly by lactate and carbon dioxide production during exercise, reduces the activity of multiple key enzymes of energy metabolism and disturbs membrane excitability. Inorganic phosphate and ROS, in turn, disturb the function of E-C coupling. Together these metabolic disturbances occurring during exercise contribute to fatigue and may also disturb calcium homeostasis, which has a central role in the development of exercise-induced muscle damage (EIMD). (Allen et al. 2008; Cairns 2006; Maughan & Gleeson 2010, 91.)

Since lactate levels drop faster following the cryotherapy treatment, it is possible that cooling increases the clearance of lactate for example in a similar way as active recovery does. The temperature gradient between the core and skin likely causes increased blood flow during the first 30 min after applying cryotherapy when the body is trying to equalize the temperature difference between the two tissues (Enwemeka et al. 2001). This results in increased blood flow through the tissues that are able to oxidize lactate or synthesize glucose from it. In other words, as sufficient oxygen is available during recovery, the lactate-using tissues (liver, heart and muscles) use lactate as energy and facilitated blood flow brings more lactate available and recovery progresses faster. (McArdle et al. 2010, 163–164, 176.) Facilitating lactate removal may also decrease the post-exercise acidosis as lactate has a role in H^+ formation. If lactate is removed faster after exercise, acidosis is not as severe and force recovery may occur faster. (Cairns 2006; Lindinger et al. 2005.)

9.1.1 Muscle damage and inflammatory response

It was hypothesised that cryotherapy used following exercise would lower the blood levels of muscle damage markers (myoglobin, CK) like reported in earlier studies (Ascensão et al. 2011; Bailey et al. 2007; Eston & Peters 1999). Myoglobin peaked 60 min and CK 24 h after the anaerobic exercise in both groups, which are in line with the previous studies. However, the absolute values of both myoglobin and CK are not of the same magnitude as those reported elsewhere. (Ascensão et al. 2011; Bailey et al. 2007.) The pre-level is within the normal range in both groups for both myoglobin and CK

(Huslab 2014), and the differences in muscle damage and inflammatory markers between the groups are expected to be unrelated to changes in plasma volume, since the volume changes were similar in both groups.

Myoglobin and CK levels have been suggested raise as result of either post-exercise damage or due to increased post-exercise efflux from the muscle into the lymphatic system, or both (Eston & Peters 1999). However, Semark et al. (1999) proposed that injury to non-contractile tissue could explain the lack of an increase in post-exercise CK activity. The recent results suggest the amount of muscle damage following the anaerobic exercise to be rather small in the active muscle structures, since the level of muscle damage markers is lower compared to earlier studies (Ascensão et al. 2011; Bailey et al. 2007). This is probably due to the different exercise mode used in the present study. Most of the earlier studies using a dynamic whole-body exercise have used a prolonged intermittent protocol to elicit muscle damage (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009), whereas a maximal anaerobic shuttle running was used in this study. In addition to rather small changes compared to the baseline level, there was a large inter-individual variation in the recent study, thus only few between group significances were found.

Cryotherapy has been studied to induce vasoconstriction, which limits blood flow to the damaged muscles, decreases membrane damage, attenuates oedema formation and subsequently decreases the extent of secondary damage (Ihsan et al. 2013; Vaile et al. 2011; Yanagisawa et al. 2003a; Yanagisawa et al. 2004). However, in the recent study there are no significant differences in the absolute myoglobin and CK values between the groups following the anaerobic exercise. This suggests that cooling does not affect the amount of muscle damage and/or post-exercise efflux from the muscle into the lymphatic system. Although we hypothesised contrary results, there are studies reporting little or no effect of cooling on the biochemical muscle damage markers (Ingram et al. 2009; Yanagisawa et al. 2003b). When comparing the recent cryotherapy method to e.g. cold water immersion, it is not as radical and induces smaller temperature change in the body. This possibly explains why no differences in muscle damage markers between the control and cold conditions were observed.

It was hypothesised, that cryotherapy would lower the inflammatory response (Carvalho et al. 2010), and C-reactive protein (CRP) was used as an indicator of inflammation. Against expectations based on the study of Ascensão et al. (2011), CRP levels stayed relatively stable throughout the study in both groups, which suggests that the performed anaerobic running did not cause inflammation. However, it is controversial if the increase in CRP is physiologically remarkable in the study of Ascensão et al. (2011) either, since the peak CRP in their study was below 2.5 mg/l, whereas CRP concentration is above 10 mg/l when there is remarkable inflammation (Huslab 2014). Since the anaerobic exercise did not induce changes in CRP, it was not possible to evaluate the effect of cooling on it. Nonetheless, the weaker inflammatory response possibly explains the smaller increase in muscle damage markers, since post-exercise inflammation has been suggested to increase membrane permeability e.g. via ROS production and thus promote efflux of intramuscular substances into the blood (Nguyen & Tidball 2003a, Nguyen & Tidball 2003b).

9.1.2 Performance recovery

The hypothesis was that cryotherapy potentially reduces decrements in neuromuscular function following the anaerobic exercise. However, there was no change in muscle function measured using isometric leg extension strength, countermovement jump and sprint performance. As immediate and sustained loss of muscle function is a common indirect marker of EIMD (Warren et al. 1999), it can be suggested that the anaerobic exercise only caused minor muscle damage and thus did not impair muscle function. In addition, it has been suggested that if the neuromuscular function is not affected following a damaging exercise, the disruption may be of the non-contractile tissue (Semark et al. 1999). The effect of cryotherapy could not be tested due to the lack of impairment in performance. The possible benefits of cooling have been suggested to relate to restricted oedema and muscle damage, which are associated with muscle soreness and reduced muscle function (Byrne and Eston 2002a).

There are earlier studies showing both significant reductions in sprint performance following a damaging exercise (Ingram et al. 2009) and no evident effects of EIMD and/or DOMS on a single sprint performance (Ascensão et al. 2011; Semark et al.

1999). Additionally, Rowsell et al. (2009) did not observe any decrements in repeated sprint performance during a 4-day soccer tournament. Thus, the recent results are in accordance with those reported earlier. There was no effect of cooling on sprint performance at 48 h following the anaerobic exercise, which is in line with the study of Ascensão et al. (2011). Nonetheless, cold treatment resulted in slightly faster 20 m sprint time following the training week compared to the control group. Although the difference did not reach statistical significance, the 0.06 sec difference in 20 m sprint time would be remarkable in a competitive situation. Thus, the recent results slightly suggest that using cold treatment may be beneficial during a short training period.

Similarly, there is not always change in jump performance following EIMD. Byrne and Eston (2002a) investigated the effect of EIMD following eccentric exercise on vertical jumping performance with and without the use of the SSC. Vertical jump performance was particularly affected in the squat jump (no SSC) compared to the countermovement or drop jump (with SSC). Thus, the way in which strength is utilized appears to be an important determinant of performance and the SSC possibly attenuates the detrimental performance effects related to EIMD. Since there was no decline in the countermovement jump performance in this study, it can be suggested that at least the passive muscle structures remained undisrupted and compensated the possible damage of active components. The damage to active components of the neuromuscular system could be seen in squat jump performance.

However, EIMD has caused immediate and prolonged reduction in maximal strength in many earlier studies (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009). This has been explained by the specific sensitivity of a more contractile-dependent performance like strength to decrements in muscular function compared with sprint and jump performance (Bailey et al. 2007; Warren et al. 1999). Additionally, post-exercise cold treatment has been studied to be effective in restoring strength (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009). However, in the recent study there were no changes in muscle strength. In some earlier studies, strength has already recovered to baseline at 48 h although there has been a significant drop 24 h after the exercise (Ascensão et al. 2011; Bailey et al. 2007). Therefore, it is possible that the recovery time may have been too long in the present study, as the performance tests were repeated only 48 h after the anaerobic exercise.

9.1.3 Perception of muscle soreness

The perception of muscle soreness is biphasic, peaking first immediately and again 48 h following the exercise. The immediate muscle soreness is probably due to an acute increase in metabolic by-products, whereas the sub-acute soreness is related to muscle damage and inflammation. (Cheung et al. 2003.) A number of studies investigating the effect of cryotherapy on exercise-induced muscle soreness have proved cooling to be more effective than passive rest for decreasing perceptions of muscle soreness (Ascensão et al. 2011; Bailey et al. 2007; Ingram et al. 2009). According to these studies, it was hypothesised that cold treatment decreases the pain perception, e.g. by restricting the amount of secondary damage (Lamb et al. 1995; Verburg et al. 2005; Zhang et al. 2008) or by restricting oedema (Enwemeka et al. 2001).

However, in the present study the anaerobic exercise protocol did not induce high perceptions of muscle soreness as evidenced by relatively small increase in DOMS and only minor differences were observed between cold treatment and passive recovery. Additionally, there was large inter-individual variation (Table 5), which suggests that some of the subjects were possibly more familiar with intense exercise and did not find it unpleasant, whereas some subjects felt extremely uncomfortable following the anaerobic exercise. There was also some inter-individual variation in the lactate levels, suggesting that some subjects relied more on anaerobic energy production and possibly more metabolic by-products accumulated in their muscles and caused pain. Furthermore, the larger inter-individual variation in the cold group at 60 min suggests that some of the subjects responded better to cold treatment than the others by means of lactate removal. As the performance tests were not affected by the DOMS at 48 h, it can be suggested that the pain was caused by damage to the non-contractile tissue, like cytoskeleton (Zhang et al. 2008) and thus not affecting muscle force production.

At 60 min the perceived muscle soreness in hamstrings had already recovered to baseline in the cold group, whereas in control condition muscle soreness still remained elevated. Since the lactate concentration seemed to drop faster following the cold treatment, cooling may have promoted the clearance of metabolic by-products, which could reduce the acute pain perception following exercise. As the used cooling method

is not as radical as e.g. CWI, it may have restricted blood flow to the muscle only for a short period, but subsequently increase blood flow as the body tries to equalize the temperature gradient between skin and deeper tissues. This has a potential to facilitate the removal of accumulated fluid and metabolites from damaged muscles, and reduce pain and oedema (Enwemeka et al. 2001). Since the analgesic effect has been studied to occur only when skin is cooled to 10–15°C (Meeusen & Lievens 1986), it is an unlikely explanation in this study, because skin was cooled to approximately 22°C.

9.2 Potential benefits of cryotherapy over a training microcycle

There are many temperature-dependent processes, which contribute to training adaptation. Some studies have suggested the metabolic alterations following exercise to be essential for training adaptation (Thompson et al. 2003; Yamane et al. 2006), thus they indicate cooling to have negative effects on training adaptation in long-term. However, the recent study did not find cooling to be detrimental when used during a weeklong training period. On the contrary, myoglobin and CK levels decreased and it is likely that cooling could also affect the performance outcomes. This is in line with the study of Halson et al. (2014) who did not observe impairments in training adaptation over a 3 weeks training period with cryotherapy.

However, Yamane et al. (2006) found cooling to affect negatively. We suggest the negative effect to be due to an extreme study protocol they used. The water temperature in their study was rather low (5°C and 10°C) and time for immersion long (1–2 x 20 min), whereas a tolerably 2 min application was used in this study and the shower mist cooling is not as radical as water immersion. Their training period was also longer (4-6 weeks). Thus, it can be suggested that a modest cryotherapy applied not over two weeks, can potentially be beneficial and not disturb the training adaptation, whereas a continuous use of radical temperatures may be detrimental.

The possible way to improve recovery and adaptation to training via cooling include the adjustment of ROS production. Since ROS contribute to both training adaptation (Powers et al. 2007) and muscle damage (Carvalho et al. 2010), it is vital to find the ideal level of ROS production following exercise. Therefore, the modest cryotherapy

method used in the recent study could possibly decrease the amount of muscle damage, but simultaneously enable muscle adaptation to training by limiting ROS production to an optimal level. Another possible mechanism is via PGC-1α activation following exercise and cold treatment. Ihsan et al. (2014) proved that even a short 15 min post-exercise cooling might enhance mitochondrial biogenesis and promote exercise adaptation via the enhanced PGC-1α expression.

9.3 Limitations of the present study and future study proposals

The sample size was low (n=8) and inter-individual variation large in the present study, which made it difficult to show statistically significant differences between the two trials. Additionally, participants should be excluded from participation in the study if there are differences in the baseline measures between the trials to ensure they start each trial at the same level of muscle damage, inflammatory status, neuromuscular performance and perception of soreness. Due to small number of participants this was not done. Since participants were recreationally active, there is possibly learning effect in the performance tests when they became more familiar with the exercise protocol.

Additionally, evaluating jump height by using a contact mat is problematic, since incorrect jumping technique affects the result, i.e. bending the knees while in the air can alter the score by increasing the flight time. Although the participants were familiarized with the jump test, there were difficulties to perform the jumps correctly. Thus, a force platform would be more accurate tool to measure jump height, as the landing technique does not affect the result. Moreover, combining the recent results with those reported elsewhere (Byrne and Eston 2002a; Semark et al. 1999), it could be suggested that sprint and jump abilities may not be affected by muscle damage. Thus, such tests should be preferred in studies examining a specific sport characterised by such events (e.g. football). Especially, if studying the (recovery) treatment efficacy it could be better to choose performance tests so that there is little doubt a certain change takes place from the baseline, thus ensuring that there will be a clear recovery phase.

The level of muscle damage markers in blood reflects the release and clearance of proteins in circulation and the rates may vary independently. For example,

concentration of muscle damage markers may increase due to exercise-induced increase in lymph flow, not because of increased muscle damage. (Warren et al. 1999.) Especially, it should be taken into account that cooling changes blood flow to the muscles and potentially affects the clearance (Enwemeka et al. 2001). Additionally, special caution in the interpretation of CK activity as a marker of muscle damage has been advised, since large inter-individual variation has been noted and people can be classified either as a high-responder or as a low-responder depending on the peak CK values (Totsuka et al. 2002; Warren et al. 1999). The large variations in the recent study could be explained by the low- and high-responders to training among the participants.

However, there is also a great intra-individual variability in the pre CK levels, which makes it difficult to classify subjects into two groups according to the value of their peak CK post-exercise. According to Statland et al. (1976) the intra-individual coefficient of variation in CK is 25.7 %, whereas the average value of coefficient of variation in the recent study is 47.3 % (Pre-sample). Thus, it is possible that the advice to restrain from extraneous physical activity for 48 h before each testing session was not enough and some participants had exercised more prior one trial than another, causing differences in the pre CK-levels between the two trials. In fact, CK can be elevated up to 7 days following an extreme eccentric exercise (Brancaccio et al. 2007). This makes it difficult to classify the participants as high- and low-responders, since the pre-exercise status possibly affects the CK levels during the recovery as well.

Generally cryotherapy is not recommended prior exercising, as it can impair the neuromuscular function. However, in this study cooling possibly facilitated the clearance of lactate, and thus the recent cryotherapy method should be tested during a short (30–60 min) recovery period between two exercise bouts. If the method can improve the clearance of metabolic by-products, it could facilitate the immediate performance recovery. As analgesic effect has been studied to occur when skin temperature is 10–15°C (Meeusen & Lievens 1986) and motor neuron function is not affected until reaching even lower temperatures (Herrera et al. 2010), the recent method should not impair neuromuscular function. Additionally, it would be useful to test the cryotherapy method with an exercise, which would cause more severe muscle damage, DOMS and performance decrements. To get more information about the new

cryotherapy method, it should be compared to a more “traditional” cold treatment method, e.g. cold water immersion.

Cryotherapy may be potential in treatment of diseases. More studies are needed, but it is possible that the combining cryotherapy and physical activity is effective in treatment of diabetes, since both cryotherapy and exercising affect PGC-1α expression, which is a key pathway in diabetes as well. Understanding the effects and mechanisms of cryotherapy will be crucial in the development of temperature-related treatments, thus the molecular mechanisms should be studied more.

10 CONCLUSION

The recent study shows, that a single anaerobic exercise performed together with a thorough warm-up induces only a modest EIMD and the performance is already fully recovered at 48 h, although the subjects perceive muscle soreness. There was no effect of cryotherapy on the acute recovery. However, when applied during a training period, cryotherapy has a potential to decrease the amount of muscle damage and to improve performance. This data provides useful information for athletes and coaches of potential use of cold mist shower both in acute settings and during a short training microcycle.

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APPENDIX 1 – HEALTH QUESTIONNAIRE (IN FINNISH)

Terveyskysely

Lue kysymykset huolellisesti ja vastaa niihin rehellisesti kyllä tai ei.

1. Onko lääkäri suositellut Sinulle liikuntaa vain tietyn ohjeistuksen mukaan sydän-, verenkierto- tai hengityselimistön sairauden vuoksi?

❑ Kyllä

❑ Ei

2. Onko Sinulla ollut rintakipua liikunnan aikana?

❑ Kyllä

❑ Ei

3. Onko Sinulla ollut rintakipua viimeksi kuluneen kuukauden aikana levossa?

❑ Kyllä

❑ Ei

4. Oletko menettänyt tajuntasi tai oletko kaatunut huimauksen takia yhden tai useamman kerran?

❑ Kyllä

❑ Ei

5. Onko Sinulla luustossa tai nivelissä ongelmia, jotka saattaisivat pahentua liikunnan aikana?

❑ Kyllä

❑ Ei

6. Onko Sinulle koskaan suositeltu tai määrätty lääkitystä kohonneen verenpaineen tai sydänsairauden vuoksi?

❑ Kyllä

❑ Ei

7. Onko sinulla mielestäsi mitään sellaista terveydellistä ongelmaa, joka vaatisi lääkärin ohjeita liikuntaa varten?

❑ Kyllä

❑ Ei

8. Oletko viimeisen kahden viikon aikana ollut flunssassa/kuumeessa?

❑ Kyllä

❑ Ei

Allekirjoitus, nimenselvennys Jyväskylä, ___/___ 2013

APPENDIX 2 – INFORMED CONSENT DOCUMENT (IN FINNISH)

KYLMÄSUMUSUIHKUTUKSEN VAIKUTUKSET PALAUTUMISEEN AKUUTISTA FYYSISESTÄ KUORMITUKSESTA JA VIIKON HARJOITTELUJAKSOSTA NUORILLA KUNTOILIJAMIEHILLÄ

TUTKITTAVAN SUOSTUMUS TUTKIMUKSEEN OSALLISTUMISESTA

Olen perehtynyt tämän tutkimuksen tarkoitukseen ja sisältöön, tutkittaville aiheutuviin mahdollisiin haittoihin sekä tutkittavien oikeuksiin ja vakuutusturvaan. Suostun osallistumaan mittauksiin ja toimenpiteisiin annettujen ohjeiden mukaisesti. En osallistu mittauksiin flunssaisena, kuumeisena, toipilaana tai muuten huonovointisena. Voin halutessani peruuttaa tai keskeyttää osallistumiseni tai kieltäytyä mittauksista missä vaiheessa tahansa. Tutkimustuloksiani saa käyttää tieteelliseen raportointiin (esim. julkaisuihin) sellaisessa muodossa, jossa yksittäistä tutkittavaa ei voi tunnistaa.

NIMI: _____ Puh no:_____ Jyväskylässä _____ 2013.

Tutkittavan allekirjoitus:

_____

Tutkijan allekirjoitus:

_____

APPENDIX 3 – BREAKFAST

Rye bread 1 piece

Margarine 6 g

Cheese 15 g

Ham 12g

Cucumber 20 g

Yoghurt 150 g

Apple 150 g or mandarin 70 g

APPENDIX 4 – TRAINING PROGRAMMES

A. Gym programme

**** Warm-up:** 7 min cycling with increasing tempo + arm circles

1. Leg press
4 x 10, rest 2 min
1st training session ___/___ 2013, weights: _____
2nd training session ___/___ 2013, weights: _____
3rd training session ___/___ 2013, weights: _____

2. Bench press
4 x 10, rest 2 min
1st training session ___/___ 2013, weights: _____
2nd training session ___/___ 2013, weights: _____
3rd training session ___/___ 2013, weights: _____

3. Lever seated leg curl
4 x 10, rest 2 min
1st training session ___/___ 2013, weights: _____
2nd training session ___/___ 2013, weights: _____
3rd training session ___/___ 2013, weights: _____

4. Biceps curl with a barbell
4 x 10, rest 2 min
1st training session ___/___ 2013, weights: _____
2nd training session ___/___ 2013, weights: _____
3rd training session ___/___ 2013, weights: _____

5. Lever leg extension
4 x 10, rest 2 min
1st training session ___/___ 2013, weights: _____
2nd training session ___/___ 2013, weights: _____
3rd training session ___/___ 2013, weights: _____

Sit-ups 4x25 with fast tempo

Hyperextension 2x25 with slow tempo

**** Cool-down: cycling 5 min + short stretches < 20s**

B. Interval running programme

**** Warm-up:** Jogging 5-7 min, dynamic stretching, coordination drills (e.g. high knees), few sprints

5x100m, with 70%, rest 1 min

1st training session ___/___ 2013, time:

_____

2nd training session ___/___ 2013, time:

_____

3rd training session ___/___ 2013, time:

_____

**** 3min break ****

4x5x60m, with 80%, rest between the intervals 2min, between the sets 4min

1st training session ___/___ 2013, time:

_____

2nd training session ___/___ 2013, time:

_____

3rd training session ___/___ 2013, time:

_____

**** Cool-down: jogging 5min + short stretches < 20s**