ACUTE INFLAMMATORY RESPONSES TO THREE DIFFERENT ISOKINETIC BENCH PRESS LOADING PROTOCOLS

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ABSTRACT

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Introduction: Exercise disturbs the homeostasis in the body and induces a transient inflammatory response leading to an acute increase in acute phase proteins, cytokines and enzymes, which can be classified as an acute inflammatory response to exercising. The purpose of this study was to evaluate the effects of an isokinetic bench press protocols consisting of eccentric-only (ECC), concentric-only (CON) and combined (COMB) concentric-eccentric muscle actions on the acute inflammatory markers interleukin-6 (IL-6), C-reactive protein (CRP) and muscle damage marker creatine-kinase (CK).

Methods: Twelve healthy resistance-trained males completed the study. Subjects completed three different maximal isokinetic loadings: CON, ECC and COMB with each consisting of 5 sets of 10 maximal repetitions separated by 14 days in a randomized order. Maximal isometric force (ISOM) was measured before, immediately after (post-45s) and 24-hours after the protocol (post-24h). IL-6, CRP, CK and Blood lactate (LAC) was measured before (pre), 5-minutes (post-5min) and 24-hours after each protocol.

Results: ISOM force decreased significantly at post-45s and remained significantly decreased at post-24h after all protocols. No significant increases were found in IL-6 at any measured time-point. A significant increase was found in CRP at post-5min after the ECC protocol. No significant increases were found in CRP at any other time-point or protocol. CK increased significantly at post-5min in all protocols but no significant differences were found at any other time-point or protocol. A significant increase was found in blood lactate after all protocols at post-5min and a significant difference at post-5min between the ECC and COMB protocols.

Conclusions: Five sets of ten repetitions of maximal isokinetic bench press was sufficient to decrease maximal isometric force production to a moderate degree. However, no increases of acute inflammatory markers or markers of muscle damage was present 24 hours after the completion of the protocols, despite the difference in force loss at the post-45s between protocols and the lower metabolic cost of the ECC protocol.

Key words: Bench press, Blood Lactate, Creatine Kinase, C-reactive protein, Inflammation, Interleukin-6, Resistance training.

ABBREVIATIONS

% RM	% of the one repetition maximum
1RM	one repetition maximum
ANOVA	analysis of variance
ANCOVA	analysis of covariance
CRP	c-reactive protein
CVD	cardiovascular disease
DOMS	delayed onset muscle of soreness
HDL	high density lipoprotein
IL	interleukin
INF-γ	interferon gamma
LDL	low density lipoprotein
Mb	myoglobin
MRI	magnetic resonance imaging
mRNA	messenger RNA
MVC	maximal voluntary contraction
TFN-α	tumor necrosis factor alpha
RT	resistance training

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1 INTRODUCTION

Decreasing amounts of physical activity leading to a lower exercise capacity and energy expenditure, has been shown to be a bigger risk of premature death compared to predictors such as smoking, hypertension, diabetes, previous myocardial infarction or a history of heart failure (Mathur and Pedersen, 2008; Myers et al., 2004). Physical activity enhances the immune response, reinforces antioxidative capacity, reduces oxidative stress and increases the efficiency of energy generation. Therefore, exercise offers protection against various diseases such as, cardiovascular diseases, type-2 diabetes and cancer, physical activity offers an effective drug-free strategy as a preventative method and treatment for several diseases. Such findings have increased the interest in the effects of the inflammatory responses in relation to exercise (Mathur and Pedersen, 2008; Scheffer and Latini, 2020).

The inflammatory response is a complex adaptive component in the human body reacting to various stimuli and resulting in an acute phase response with the goal of eliminating the initial cause of infection and return to homeostasis. Meanwhile, long-term sustained chronic low-grade inflammation in the body, usually characterized by increased amounts of acute-phase proteins and pro-inflammatory cytokines in the circulation, has been linked to various degenerative diseases and ultimately premature death (Baizabal-Aguirre et al., 2016; Calder et al., 2011; Ferrero-Miliani et al., 2006; Germolec et al., 2018; Medzhitov, 2008; Scheffer and Latini, 2020).

Exercise disturbs the homeostasis in the body and induces a transient inflammatory response leading to an acute increase in acute phase proteins, cytokines and enzymes, which can be classified as an acute inflammatory response to exercising. These increases have been shown to happen both after endurance-type and resistance-type of exercising, however, the magnitude and specific effects on various markers vary (Peake et al., 2017; Ostrowski et al., 1998). The different muscle actions involved in various exercise settings may give insights to the responses and adaptations acquired from exercising. Eccentric muscle actions both in endurance-type and resistance-type exercise have shown to influence the inflammatory responses to a higher degree

than concentric muscle actions. However, during recent years, human muscle cells have been shown to be capable of producing acute phase cytokines, without clear signs of muscle-damage (Pedersen et al., 2003).

A lot of previous research have focused mainly on the effects of endurance-type of training and its effect on inflammation, immune function, acute phase proteins and cytokines. However, as resistance training has proven to be an effective method in the prevention of various diseases, such as, age related sarcopenia, osteoporosis, low grade inflammation related diseases and cardiovascular diseases (Calle and Fernandez, 2010), and triggers an inflammatory response in a similar extent as a bout of endurance training (Ihalainen et al.,2014), and it is of great interest to investigate further the acute responses to different muscle actions and loading protocols used in resistance exercising. The purpose of this study is to investigate the acute effects of concentric and eccentric muscle actions on the acute responses of interleukin-6, C-reactive protein and creatine kinase after a traditional repetition and set structure commonly utilized in resistance training.

2 LITERATURE REVIEW

2.1 Resistance training

Resistance training consists of lifting external weights and includes concentric, eccentric and isometric muscle actions. Resistance training aims to increase muscular strength, power and speed, muscle size, muscle endurance, motor performance, balance and coordination (Kraemer and Ratamess, 2004). To achieve the aforementioned adaptations, progressive overload (increasing intensity and volume over a period of time), is needed. Resistance training can be categorized into heavy resistance exercising, where higher intensities and fewer repetitions are used (1-6 repetitions, 85-100% of one repetition maximum, 1RM), which primarily aims to improve neural adaptations and maximal strength or moderate intensity resistance training with lower, more repetitions and higher metabolic demand seeking to increase muscle size intensities (6-12 repetitions, 60-85%RM) or resistance training focusing on muscle endurance capabilities (15+ repetitions, < 50%RM).

2.1.1 Resistance training variables

Most important variables in resistance training are muscle actions used, exercise selection, exercise order and structure, intensity of the performed exercise, volume of the exercise or training session, rest period length and the frequency of training. Most often, a specific physiological adaptation is targeted as an outcome of a resistance training program, commonly varying between muscle hypertrophy, maximal strength or maximal power (Kraemer and Ratamess, 2004).

Exercise selection during resistance training can vary from free weights to machines, with a training session usually combining both. Free weight exercises involve usually multiple joints and targets multiple muscles simultaneously during the movement, while machines are more commonly capable of targeting only a single muscle and a joint at a time (Kraemer and Ratamess, 2004).

Volume can be used to describe the total amount of work performed during a set, exercise or a workout session. Volume is calculated as follows: sets x repetitions x weight x distance (Haff, 2010), however for practical reasons, distance is seldomly involved in the equation. Exercise volume varies between the focus outcomes of the training program. Generally higher volumes are used during hypertrophic type of training, as repetition ranges vary between 6-12 repetitions and lower volumes are more common during maximal strength programs as repetition ranges commonly vary between 1-6 repetitions (Kraemer and Ratamess, 2004).

Resting periods between sets affect the amount of repetitions and the weight that can be lifted, due to the time to recover anaerobic energy stores (ATP and phosphocreatine) and to decrease muscle and blood acidity between sets. Shorter resting periods (30-90 seconds) allow little time for physiological recovery and generally lead to greater fatigue during training sessions, and lower volumes due to less repetitions and weight being lifted. However, short rest periods may lead to higher acute hormonal responses during lifting, such as increased serum growth hormone levels. Longer rest periods (90-360 seconds) allow for almost maximal recovery between sets, which leads to a capability to lift higher loads, do more repetitions and ultimately leads to better increases in maximal strength and muscle hypertrophy (Kraemer et al., 2012, 367-368).

Intensity of an exercise is often reported as a percentage of 1RM. Which presents the amount of weight that can be lifted a single time with a good technique. The higher the percentage of 1RM, the fewer repetitions can be performed. A set of repetitions can also be performed to the point at which no more repetitions are possible, which is considered to be momentary “failure” exercising (Kraemer et al., 2012, 370).

During activation of muscle fibers, they have the capability to produce tension and produce movement of a joint. The muscle fibers can shorten, remain the same length or extend. The actions are referred to as concentric, isometric and eccentric muscle actions and can produce force against an external object acting against the muscles (Kraemer et al., 2012, 87).

During a concentric muscle action, the force produced by the muscle is transmitted by the tendon through the skeleton, exceeds that imposed of the weight or resistance, and the muscle shortens inducing movement to the joint it is connected to. During isometric muscle actions, force in the muscle is produced and the fascicles in the muscle shorten, and the tendons lengthen, but no visible movement can be seen, and the joint angle remains constant. During eccentric muscle actions, the external resistance is greater than the force produced, leading to lengthening of the muscle and enlargement of the joint angle (Kraemer et al., 2012, 87).

Isotonic muscle actions are a common term used for describing traditional resistance training movements utilizing free weights or machines, involving both concentric and eccentric muscle actions. However, isotonic means that the force produced by the muscle remains the same during the whole movement, so instead isoinertial describes traditional resistance training in a better way. The term isoinertial is used to describe movements with a constant external resistance, but a variable velocity during the performed movement (Kraemer et al., 2012, 87).

Isokinetic training describes a movement during which the velocity of the movement is kept constant and controlled throughout the full range of motion of the specified movement pattern. Isokinetic machines are more commonly used in laboratory settings compared to traditional training, due to the need for specialized equipment and the expenses, but they work effectively as a testing tool for specific muscles and movement speeds (Kraemer et al., 2012, 88).

The capacity of the muscles to produce force during concentric, isometric and eccentric muscle actions are not equal. The force produced concentrically is always less than the force produced isometrically and when the velocity of the concentric muscle action increases, the capacity to produce force declines further. During eccentric movements, increasing the velocity increases the capability of the muscle to produce force up to a point. Force generated during eccentric muscle actions always exceeds the force produced with isometric- and concentric muscle actions. This phenomenon is known as the force-velocity curve and explains the relationship between velocity, muscle action and muscle force production capabilities (Kraemer et al., 2012, 88-90).

2.2 Muscle damage

Unaccustomed and high intensity exercise, particularly exercise that involves eccentric muscle actions, disrupts homeostasis in the contracting muscle fibers and may lead to subcellular disturbances and Z-line streaming in the contracting sarcomeres and causing damage to the muscle fibers, referred to as muscle damage (Clarkson and Hubal, 2002).

Exercise induced muscle damage is often characterized by structural myofibrillar disruption, loss of muscle strength and power, delayed onset of muscle soreness (DOMS), swelling, reduced range of motion and an increased amount of circulating myocellular enzymes and proteins such as creatine kinase (CK) and myoglobin (Peake et al., 2017). Eccentric muscle actions have been shown to induce a higher amount of muscle damage after exercising, compared with other muscle actions. The amount of muscle damage present after cessation of exercising is dependent of the intensity and magnitude of stress opposed on the working muscle. However, the stress caused by mechanical loading and metabolic stress inducing muscle damage and inflammation, stimulate various cells to initiate tissue repair, remodeling and recovery muscles used (Clarkson and Hubal, 2002; Peake et al., 2017).

2.2.1 Muscle damage during exercise

As stated previously, high intensity eccentric muscle actions are prominent of producing a higher incidence of muscle damage and soreness. Concentric muscle contractions seem to not cause exercise-induced muscle damage and the recovery of loss of muscle strength and power takes a shorter period to recover, compared to eccentric muscle actions. Muscle damaging exercise often activates the acute inflammatory response to clear cellular debris from the injured area and to initiate repair and recovery. Interestingly, muscle damage has also been identified after only low intensity eccentric contractions (Peake et al., 2017).

The precise assessment of muscle damage is difficult in humans, as it requires taking muscle biopsies or magnetic resonance imaging. Muscle biopsies tend to over- or underestimate the amount of occurred muscle damage, as muscle damage is not prevalent and distributed evenly

throughout the muscle and occurs in more concentrated regions. Also, the procedure of taking a muscle biopsy, involve inserting a biopsy needle into the muscle, which may in itself cause muscle damage and affect the biopsy sample. Magnetic resonance imaging (MRI) only show the appeared edema in the muscles and problems in the interpretation of the images occur often (Peake et al., 2017). Due to the invasive nature of biopsies and the difficulties on analyzing MRI images, noninvasive methods such as blood protein analysis, maximal force production capability, cytokine analysis and subjective feelings of DOMS have been widely utilized (Clarkson and Hubal, 2002).

The loss of muscle strength after muscle damaging exercise has been widely used as a precise measurement of muscle damage. Force loss after concentric muscle actions generally show to recover within 1-4 hours after the cessation of exercising, demonstrating only metabolic or neural fatigue. Protocols consisting of only concentric muscle actions generally show force reductions of 10-30% immediately post-exercise. Protocols involving eccentric muscle actions tend to generally show larger decreases in force production capabilities and take a greater time to fully recover (Clarkson and Hubal, 2002; Douglas et al., 2017; Paulsen et al., 2012; Peake et al., 2017).

The amount of force loss after eccentric exercise makes it possible to categorize the amount of muscle damage to mild, moderate and high. Decreases of $\leq 20\%$ immediately after exercise can be categorized as mild exercise-induced muscle damage, with no significant increase in CK activity (< 1000 IU/L) and recovery of muscle function within 48 hours. Decreases between 20-50% of force loss immediately after exercising can be classified as moderate exercise-induced muscle damage. Recovery of muscle function range from two to seven days and subjects generally show increased levels of CK activity (1000-10 000 IU/L) and leukocytosis. Decreases of force production of $\geq 50\%$ can be classified as severe exercise-induced muscle damage. Recovery of muscle function usually takes over a week, subjects show high CK activity (up to > 10 000 IU/L) and increased amount of leukocytosis (Paulsen et al., 2012).

The mechanical stress during eccentric contractions leads to structural damage in the muscle fibers, causing damage to the contractile proteins and extracellular matrix. Increases in high

eccentric torque exercises and an increased amount of repetitions, causes a greater mechanical stress to the muscle fibers resulting in more damage to the contractile proteins and extra-cellular matrix, causing a larger amount of muscle damage. Longer muscle lengths may induce more damage due to a greater nonuniformity sarcomere length and by stretching the weaker sarcomeres more. Smaller and weaker muscle groups during single joint exercises may be more vulnerable to muscle damage due to a higher degree of overstretching of the sarcomeres in the muscle. Greater amounts of muscle damage have been shown in arm exercises compared to lower extremity exercises, which is probably due to the differences in the mechanical loading in these settings. Faster muscle contractions and fast twitch fibers show a higher amount of muscle damage compared to slow contractions, which might be due to the lower amount of activated cross-bridges, which leads to a higher strain on a single individual filament. Recovery seems not to be affected by the configuration of repetitions and sets, rest intervals or exercising when muscle damage is already present (Peake et al.,2017).

The occurrence of DOMS is a common symptom after muscle damaging strenuous exercise, however the precise mechanisms behind it remain somewhat unknown. It is widely believed that DOMS seem to be caused by tissue injury and microtears occurring in the muscle fibers. The structural damage to the sarcomere triggers an inflammatory response releasing histamines, prostaglandins and edema which causes a sensation of pain in the muscles (Clarkson and Hubal, 2002). Abnormally increased sensitivity to pain (hyperalgesia) is a common symptom following the days after muscle damaging exercise. Interestingly, studies have reported hyperalgesia after muscle damaging exercise without any signs of microscopic muscle damage or inflammation, which would indicate that DOMS is more associated with inflammation in the extra-cellular matrix than muscle myofiber damage and inflammation (Peake et al., 2017). Highest sensations of DOMS are usually shown between 24-48 hours after muscle damaging exercise compared to immediately after exercise (Clarkson and Hubal, 2002).

2.3 Markers of muscle damage

The severity of muscle damage after exercise can be measured by taking muscle biopsies and examining the Z-line streaming and differences in myofilaments of the sarcomeres. Magnetic

resonance imaging can be used to investigate the signal intensity and edema occurring after training. Force production can be measured to evaluate the structural damage on the muscle fibers resulting in lesser force production of the muscle.

The bloodstream provides another interesting indirect measurement path of muscle damage. After heavy muscle damaging exercise, muscle proteins and enzymes can be measured from the bloodstream. Enzymes such as lactate, aspartate dehydrogenase, aminotransferase, carbonic anhydrase isoenzyme II, CK and other muscle proteins such as myoglobin, troponin, and myosin heavy chain has been used to evaluate muscle damage from the blood stream after resistance training (Clarkson and Hubal, 2002). All the beforementioned have been shown to increase in the bloodstream after exercise, but CK is probably most investigated due to the generally high increases after exercise. However, these markers however only work as qualitative indicators of damage, as the proteins found in the bloodstream are a product of what is produced in the muscles and what are constantly being cleared out via the bloodstream, and do not give a perfectly clear picture about what is occurring in the muscle itself.

2.3.1 Creatine kinase

Creatine kinase is an enzyme which is often discussed in relation to muscle damage, DOMS and activation of inflammatory mechanisms caused by some form of damage to muscle tissues (Franklin et al. 1991; Sayers & Clarkson 2003). CK is found in the cytosol and mitochondria of tissues where energy demands are high and can be differentiated into type M (muscle type) and B (brain type) (Baird et al., 2012). The cytoplasmic isoforms of CK can further be classified into different subtypes: CK-MM (skeletal muscle), CK-MB (cardiac muscle) and CK-BB (brain), whereof CK-MM is located in the muscle fibers bound to the myofibrillar M-line, but found also in the I-band of sarcomeres (Baird et al., 2012; Brancaccio et al. 2008; Koch et al. 2014). The CK isoforms can be found in various places depending of the site of tissue damage. Elevated levels of CK-MB can be seen after myocardial infraction, elevated levels of CK-BB in turn after damage in brain tissues (Koch et al. 2014). In normal serum, the total CK is most commonly the CK-MM isoform and is mainly from skeletal muscles with values ranging from

40-400 UL, with variation due to sex, race, body mass, physical activity and training status (Brancaccio et al. 2008).

Until 1995, CK was used as a key tool in the diagnosis of myocardial infarction in patients representing severe chest pain and related symptoms in emergency departments. However, the diagnostic role has been replaced to a certain extent with measurements of the muscle protein troponin. Elevated CK levels, on the other hand, are still associated with cell damage, muscle disruption and disease, which cause the CK to leak into blood serum (Baird et al., 2012).

The mechanical loading during exercise damages the sarcolemma and Z-disks and the metabolically exhausted muscle fibers show an increased membrane permeability, that allows CK to leak into the interstitial fluid and the lymphatic system (Koch et al. 2014). The amount of CK seen in the bloodstream is affected by lymph flow, which is affected by muscular activity (Sayers & Clarkson 2003). Restrictions in lymph flow can delay the CK response seen in the blood (Hsu & Watanabe 1983) and immobilization after exercise has been shown to diminish this response after exercise (Havas et al. 1997; Sayers et al. 2000).

2.3.2 Individual responses of creatine kinase

An inconsistency exists in the CK response in relation to exercising between individuals. Studies have reported the CK response to range from 96 to 30 810 IU/L after various forms of resistance training (Nosaka & Clarkson 1996). Various researchers have classified people into low responders (LR), normal responders (NR) and high responders (HR), depending on the individual increase in the amount of serum CK after a bout of heavy resistance training (Hody et al. 2011). However, no distinct values or increases for these categories exist and researchers have been categorizing subjects regarding their sample group, increases under 500 IU/L as LR, NR with individuals between 500-200 IU/L and HR as individuals who reach over 2000 IU/L. Even a group of higher responders is identified for individuals reaching values over 10 000 IU/L (Koch et al. 2014).

2.3.3 Time course of muscle damage

Disruption of normal myofibrillar banding of muscle fibers is increased immediately after exercise and disruption of Z-disks and sarcomeres appear to peak between 1-3 days and may remain elevated for 6-8 days after cessation of muscle damaging exercise (Peake et al., 2017).

The amount of muscle strength loss affects the time to restore muscle strength back to normal ranges. Losses of < 20% muscle strength after cessation of exercise return normally back to baseline within 48 hours. Decreases in muscle force ranging from 20% to 50% of muscle after cessation of exercise may need a time course of two to seven days to recover. Decreases of force production after exercise of over > 50% requires over seven days to recover and symptoms of muscle damage might still be apparent after three weeks. The incidence of other variables such as DOMS and other muscle damage related factors varies. Delayed onset of muscle soreness is usually resolved within four days of recovery, even when muscle strength levels have not returned to baseline. Muscle swelling peaks at around 4-5 days and circulating CK activity can remain elevated after 8 days of recovery (Baird et al., 2012; Paulsen et al., 2012; Peake et al., 2017).

2.3.4 The repeated bout effect

A single bout of heavy unfamiliar muscle damaging eccentric exercise causes muscle damage, resulting in loss of strength, pain, muscle tenderness, swelling and an inflammatory response. However, performing the same muscle damaging eccentric exercise session again results in considerably milder symptoms of damage than the initial bout, this is referred to as the repeated bout effect (Nosaka and Clarkson, 1995). This repeated bout effect has been shown to last several weeks and up to six months, even if the initial bout did not result in any serious damage in the muscle tissues. It has been reported that as little as two to ten repetitions may lead to these protective adaptations in the muscles, however, these eccentric repetitions must be done close to maximal effort for this to happen (McHugh, 2003).

Interestingly, previous exposure to damage in the muscle cells is a key determinant for the duration of recovery from muscle damaging exercise. The shielding effect for muscle damage from the repeated bout effect extends to the contralateral muscles and limbs and results in a lesser amount of muscle damage than the initial bout of eccentric exercise (Peake et al., 2017).

2.4 Inflammation

Inflammation is a complex adaptive component of an organism's biological response against pathogens, damaged cells, or irritants and trauma. The most common signs of inflammation include increased blood flow, vasodilation, elevated cellular metabolism, release of cytokines, cellular influx, extravasation of fluids. Followed by the delivery of immune cells to the site of injury and in a carefully coordinated manner mediated by cytokines and acute phase proteins to remove the inflammatory stimulus, damaged cells and initiate cell repair (Ferrero-Miliani et al., 2006; Germolec et al., 2018; Medzhitov, 2008; Scheffer and Latini, 2020).

Inflammation is a natural beneficial and necessary response to an acute infectious episode triggered by infection or tissue injury, inflammation can be classified either as acute or chronic, and there can be an overlap of these two phases (Germolec et al., 2018). If the self-regulated mechanisms of the body fail to resolve the inflammatory process and regain homeostasis for a prolonged time, it can lead to a chronic state of inflammation, which may contribute to the development of various degenerative diseases such as, arthritis, asthma, atherosclerosis, diabetes, cardiovascular diseases, autoimmune diseases and cancer (Baizabal-Aguirre et al., 2016; Germolec et al., 2018).

As the acute inflammatory response is a natural and necessary response to regain homeostasis in the body, chronic inflammation has been characterized as a sign of chronic infection. Interestingly, the chronic and prolonged inflammation-state does not seem to be caused directly by the main initiators of inflammation such as infection and injury, but instead seem to be more associated with malfunction and homeostatic imbalance of one or several physiological systems in the body (i.e. the secretion of anti-inflammatory cytokines, inhibition of pro-inflammatory signaling cascades and activation of regulatory cells), and serves as a key component underlying

in diseases such as type-2 diabetes, atherosclerosis, cardiovascular diseases, cancer, asthma, dementia and neurodegenerative diseases (Calder et al., 2011; Medzhitov, 2008; Scheffer and Latini, 2020).

The activation of the immune system results in the release of both anti-inflammatory and pro inflammatory cytokines. Anti-inflammatory cytokines such as interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), and interleukin-13 (IL-10) and pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ) and many others coordinate and control the immune system to repair and regain homeostasis in the body (Scheffer and Latini, 2020).

2.4.1 The acute inflammatory response

The acute inflammatory response is very complex process coordinated by a large range of mediators such as vasoactive amines, vasoactive peptides, fragments of complement components, lipid mediators, cytokines and proteolytic enzymes, which alter the functional states of tissues and organs to adapt to the conditions indicated by the particular initiator of inflammation. The stimuli of inflammation can be divided into exogenous (microbial and non-microbial), and endogenous (allergens, irritants, foreign bodies and toxic compounds) stimuli. The endogenous stimuli of inflammation are commonly viewed as signals from stressed, damaged or otherwise malfunctioning tissues (i.e. damaged muscle cells) (Medzhitov, 2008). The detailed specific explanation of the inflammatory response is beyond the scope of this paper, and a basic overview follows.

The acute inflammatory response is triggered by a stimulus (infection or tissue injury) and a coordinated delivery of blood components to the site of infection follows. The acute inflammation response is mediated by tissue resident macrophages and mast cells leading to a production of a variety of inflammation mediators such as cytokines and chemokines which initiate a coordinated delivery of leukocytes to the site of infection or trauma and control the immune response (Medzhitov, 2008). The acute phase proteins and cytokines alleviate the arrival of neutrophils, monocytes and lymphocytes to the inflamed site (Calle and Fernandez,

2010). Numerous different cytokines work as messengers and coordinate the interplay between various cell types by amplifying and regulating the inflammatory response. Interleukin-1β for example, has the capability of inducing fewer, hypotension, the release of adrenocorticotrophic hormone and the production of another interleukin, IL-6. Interleukin-6 can induce the hepatic production of CRP, which thereafter stimulates the synthesis of leukocytes, that induce an increase in the circulating leukocyte (Leukocytosis) and thrombocyte amount (Thrombocytosis) (Ferrero-Miliani et al., 2006; Germolec et al., 2018).

These changes lead to the careful coordination and interplay between the acute phase proteins and cytokines lead to the activation of various white blood cells, that initiate tissue repair and removal of the stressor, adaptation to the abnormal conditions and ultimately restore functionality and homeostasis to the affected tissue (Medzhitov, 2008).

2.4.2 Chronic low-grade inflammation

A prolonged systemic activation of the immune system without a clear cause (i.e. disease or injury) and a release of acute-phase proteins, pro inflammatory cytokines and chemokines to the circulation, accompanied with increased amounts of leukocytes, causes a state in the body classified as chronic-low grade inflammation (Calder et al., 2011).

Low grade chronic inflammation is often characterized by increased systemic levels of circulating acute phase proteins (such as CRP) in the bloodstream and active inflammatory cytokines such as, TNF-α, IL-1β, IL-6 and IL-17 in association with occurrence of degenerative diseases such as atherosclerosis and type-2 diabetes, without any structural changes or loss of primary functions in tissues (León-Pedroza et al., 2015; Mathur and Pedersen, 2008).

High amounts of excess adipose tissue are a leading cause for development of low-grade inflammation. Among the first mechanisms in low grade inflammation is the inflammation of white or visceral adipose tissue. Due to high energy input and low energy outputs adipocytes accumulate large amounts of fatty acids which lead to expansion in the adipose tissue. The excess adipose tissue is often exposed to hypoxia due to the lack of blood vessels which lead to

necrosis. This leads to activation of phagocytic cells and an inflammatory response with an attempt to remove these cells. The expansion and hyperplasia of adipose tissue may further increase lipid peroxidation which causes an increase in reactive oxygen species that leads to an increase of numerous immunological cells such as TNF-a and leptin, and reductions in IL-10 and adiponectin levels. Both dyslipidemia and hyperglycemia have also shown to participate in the initiation of systemic low-grade inflammation by polarizing macrophages more towards pro-inflammatory phenotype by activating the inflammation through toll-like-receptors which recruit immune the immune cells (León-Pedroza et al., 2015).

Increases in low grade-inflammation and dyslipidemia may consequently lead to increased atherogenesis which together may be active and be one of the causes for type-2 diabetes, characterized by chronic hyperglycemia, which further increases the release of local and systemic inflammatory factors (León-Pedroza et al., 2015). Two of the main markers of immune system function that are linked to the development of cardiovascular diseases and type-2 diabetes are CRP and predecessor IL-6. Indeed, it has been shown that increased basal CRP values of > 3,0 mg/L are associated with an increased risk of first ever cardiovascular disease (CVD) event, ischemic stroke and transient ischemic attack, hypertension, peripheral artery disease, cardiovascular diseases and elevated fasting glucose and fasting insulin levels which are associated with development of type-2 diabetes (Donges et al., 2010; Lakka et al., 2005). Interestingly, it has also been demonstrated that CRP is a better predictor of CVD than IL-6, total cholesterol, LDL cholesterol, and the rate of total cholesterol to HDL cholesterol (Kelley and Kelley, 2006). Interleukin-6 and TNF-α are expressed and released by adipose tissue. Visceral adipose tissue has the capacity of secreting three times the amount of IL-6 compared to subcutaneous adipose tissue. As CRP synthesis in the liver predominantly is regulated by IL-6, it is plausible that IL-6 originating from adipose tissue may systematically elevate the circulating CRP levels (Aronson et al., 2004; Donges et al., 2010).

Good physical condition has shown to reduce the risk of coronary heart disease, ischaemic stroke, and premature cardiovascular and total mortality, due to the effect of exercising on immune function response, anti-oxidative capacity and reduced oxidative stress (Lakka et al., 2005; Scheffer and Latini, 2020). Low systematic levels of circulating CRP have also been shown in subjects with a high fitness level. Aronson et al. 2004 demonstrated an inverse

relationship with circulating CRP levels, metabolic profile and aerobic fitness capacity in middle aged subjects. With every metabolic equivalent achieved during the Bruce treadmill test demonstrated a systematic decrease in the CRP concentration (Aronson et al., 2004).

2.4.3 Cytokines

Cytokines are a broad category of small proteins that include chemokines, interferons, interleukins, lymphokines and tumor necrosis factors, which all are essential in signaling and interplay among different cells. Various cell types have the capability to produce of a given cytokine. Cytokines play a central role in the immune system and especially in the initiation, coordination and control of an acute inflammatory response. Cytokines act as molecular messengers in the coordination, and control of different cell types involved in the amplification and regulation of immune and inflammatory responses, regardless of the cause. For example, cytokines can be secreted by phagocytic cells and NK cells during innate immune system activation but during adaptive immune responses the secretion is mainly from antigen-presenting cells and lymphocytes (Germolec et al., 2018).

An effective immune response and damage to the tissues is dependent on a careful regulation of the cytokine network and cytokines, which generally have a short lifespan and therefore are rapidly eliminated during normal conditions. However, during acute and chronic inflammation, cytokines may be released so frequently, that they appear in a systemic manner when measured from the bloodstream (Germolec et al., 2018).

2.5 Exercise and the inflammatory response

Strenuous exercising disrupts the homeostasis in the body and induces numerous different changes in the immune system, such as muscle soreness and swelling, prolonged loss of muscle function and leakage of muscle proteins such as CK and Myoglobin (Mb) into the circulation and an increase in circulating immune markers (Hirose et al., 2004). These changes are similar to that which occurs during trauma, sepsis and burns, and can be classified as an inflammatory response to exercising. The cytokines released at the site of inflammation in the muscle

facilitate an influx of lymphocytes, monocytes and initiate the clearance of damaged cells and return towards homeostasis (Pedersen et al., 2001).

During the initiation of exercise, leukocytes may start to accumulate in the exercising muscles immediately after exercise and have consistently been observed in muscle biopsies after high-intensity and volume resistance training, downhill running and long-distance running involving both concentric and eccentric muscle actions (Peake et al., 2017; Ostrowski et al., 1998). This change is led by the release of different cytokines such as TNF-α, IL-1β, IL-6, IL-1 receptor antagonist (IL-1ra) and IL-10 to the site of inflammation, which thereafter activate an influx of lymphocytes, neutrophils, monocytes and other inflammatory cells to heal the inflamed tissues (Pedersen et al., 2001; Steensberg et al. 2000). The increased amounts of circulating monocytes infiltrate into muscle tissue and differentiate to macrophages, which are essential for muscle repair. Macrophages help repair damaged muscle tissue by aiding satellite cells to recruit more monocytes, aid satellite cells to proliferation and differentiation and by mediating extracellular matrix repairs. Neutrophils help macrophages in this repair process by inducing oxidative damage to muscle cell membranes and by removing cellular debris with macrophages through phagocytosis (Friedenreich and Volek, 2012).

Exercising in general, depending on intensity, duration and mode, produces a significant anti-inflammatory cytokine response, with most marked increases in the circulating amounts of IL-6 (Pedersen et al., 2003). The magnitude of the changes in plasma cytokine concentrations are dependent of the mode of exercise, intensity and duration of exercise and muscle contractions used during the exercise bout (Hirose et al., 2004). This inflammatory response, in relation to exercise, has not always been considered to be a “good” response, but a detrimental process, which is associated with tissue damage, pain and a delayed recovery. However, inflammation after exercise is a key process underlying muscular repair, adaptive remodeling and return to homeostasis (Peake et al., 2017).

2.5.1 Acute endurance exercise

A lot of previous research have focused mainly on the effects of endurance-type of exercise and its effect on inflammation, immune function, acute phase proteins and cytokines. This might be a result due to a lot of previous research presenting evidence of the benefits of endurance-type of training as a preventive method for various degenerative diseases by improving the lipid profile, elevating insulin sensitivity and lowering the blood pressure (Pedersen et al., 2003; Lakka et al., 2005).

During the 1990s, studies investigating cytokines in relation to exercising started to gain more interest. Ostrowski et al. (1998) investigated the effects of marathon running on inflammatory response and markers. A group of subjects completed the Copenhagen marathon in 1996 and blood samples and muscle-biopsies were drawn a week before, immediately after and two hours after the completion of the marathon race. It was discovered that after the completion of the marathon, mRNA levels for IL-6 were detectable in muscle cells, but not in the circulating blood mononuclear cells. The circulating levels of IL-6 increased to over 100-fold immediately after the competition and started to decline towards baseline values two hours after. Increases in circulating IL-1ra and IL-1β were observed two hours after the marathon. Blood CK levels increased over 10-fold the day after the marathon indicating the presence of muscle damage (Ostrowski et al., 1998).

Following the findings of Ostrowski et al. (1998) study, it was hypothesized that the increase in circulating IL-6 was produced locally in the contracting muscle fibers instead of white blood cells due to the increased mRNA expression in the muscle cells, but not in the white blood cells. Previously, Brunsgaard et al. (1997) had demonstrated, increased levels of IL-6 present two hours after 30-minutes of eccentric cycling, without increases after 30-minutes of concentric cycling with no differences in the concentration of white blood cells (Brunsgaard et al., 1997). It was hypothesized to be a result of the eccentric muscle actions during the eccentric actions leading to damaged muscle cells and an increase in circulating cytokines and inflammation markers.

It was later demonstrated by Steensberg et al. (2000) that the increase in circulating IL-6 during exercise is in fact originated from the contracting muscle fibers. Subjects in this remarkable study had cannulas inserted in their arteries and veins in both the exercising limb and non-exercising limb. The method was able to demonstrate that the increase in circulating IL-6 during the 5-hour long leg extension protocol originated from the contracting muscle fibers in the exercising leg (Steensberg et al., 2000). Due to its anti-inflammatory nature and the possibility for muscle cells to produce IL-6, it was proposed that IL-6 should be characterized as a myokine instead of a cytokine (Pedersen et al., 2004).

Other studies investigating endurance type-exercise has constantly reported findings of increased amounts of IL-1ra, IL1-β, TNF-α, IL-6, IL-8 and IL-10 in the circulation after exercising, which are classified as anti-inflammatory cytokines, however the increases in plasma concentrations of IL-6 show the most marked increases in relation to exercise (Hirose et al., 2004; Pedersen et al., 2003). Interestingly, IL-6 is generally classified as a pro-inflammatory cytokine when its secreted by macrophages and T-cells, it has anti-inflammatory and immunosuppressive effects when it is produced from the contracting skeletal muscles (Scheffer and Latini, 2020).

2.5.2 Acute resistance training

During the recent years, the field of research has shifted more to investigate the effects of resistance training and its benefits on health, immune system, and the prevention of various diseases, such as, age related sarcopenia, osteoporosis, low grade inflammation related diseases and cardiovascular diseases (Calle and Fernandez, 2010).

A bout of resistance training triggers a transient inflammatory response and stimulates both pro- and anti-inflammatory cytokine production a similar extent as a bout of endurance training (Ihalainen et al., 2014). The physiological stress on the contracting muscle fibers caused by lifting external weights acts as a stimulus, which results in muscular hypertrophy as well as in a coordinated inflammatory response and the repair of damaged muscle tissue (Hirose et al., 2004). The transient increase in circulating cytokines can be seen both after concentric and

eccentric resistance training and they are proposed to play a role in tissue remodeling after resistance training causing damage to muscle fibers (Ihalainen et al., 2014; Izquierdo et al., 2009). The increased cytokine production after resistance training has also been shown to be a regulator of satellite cell mediated muscle hypertrophy. Serrano et al. 2008 demonstrated that skeletal muscles induce the local transient expression and release of IL-6 which acts on proliferation of satellite cells in myofibers (Serrano et al., 2008).

However, even though the inflammatory response is similar to what happens during endurance-type of exercising, some differences have been identified when comparing to resistance training. Muscle damage measured by CK activity after heavy resistance training is generally higher compared with endurance-type training, and it would be hypothesized that the amount of circulation of cytokines would be higher, this is not the case, however. Decreases in TNF-α, and IL-8 have been shown, with increases in IL-10 and no significant effects on IL-1β, IL-1ra, and IL-6 after strenuous resistance training involving eccentric actions (Hirose et al., 2004). Others report decreases in IL-1β and significant increases in circulating amounts of IL-6 and IL-10, with no effect on TNF-α after resistance training involving heavy eccentric bench press actions (Smith et al., 2000). Factors other than muscle damage may explain the differences between the cytokine responses between resistance training and endurance training, which include muscle actions used, energy depletion, oxidative stress, metabolic and hormonal responses.

Cytokine responses after resistance training show a more consistent finding on the increase of IL-6 levels in the circulation. The timing of the peak amount of measured IL-6 however differs between studies. Expressions of other cytokines, such as IL-10 show varying results and effects on TNF-α seem to be unaffected after a single bout of resistance training (de Salles et al., 2010). The differences might be due to variations of muscle groups used, different intensities of the training protocols and the sampling point at which time the samples are taken. It is recommended to use multiple sampling points and also as close as possible to the cessation to the exercise regimen to allow the possibility for any clearance of the markers before sampling (Calle and Fernandez, 2010).

2.6 Concentric and Eccentric training and the acute IL-6, CRP and CK responses

Cytokines, acute phase proteins and enzymes measured in the proximity of exercise give us interesting insights to what is occurring in the body. The most common variables measured in relation to exercise, inflammation and muscle damage include IL-6, CRP and CK.

IL-6 is both a pro- and anti-inflammatory cytokine associated with the coordination of immune responses and is often found increased in the circulation after a strenuous exercise bout. Previously, cytokines were usually considered as a part of an acute phase response to an infection or tissue injury, however this view changed as it was demonstrated that various cytokines can be detected in plasma after a bout of strenuous physical activity, especially IL-6, which is produced in larger amounts in relation to exercise than any other cytokine (Pedersen et al., 2001). Interleukin-6 has also been classified as a myokine, due to it being produced locally in the contracting muscle fibers (Pedersen et al., 2003; Steensberg et al., 2000).

C-reactive protein is an acute phase protein synthetized in the liver and often measured from blood serum, which increases following IL-6 secretion by macrophages and T-cells during normal pathogenic inflammation. Hepatic stimulation by IL-6 and TNF-α enhances the synthesis of CRP in the liver and the systemic release as part of the acute-phase inflammatory response (Donges et al., 2010). The cytokine-induced production of CRP has also been shown to occur locally in human coronary artery smoot muscle cells and might influence the development of atherosclerosis (Calabró et al., 2003). Interleukin-6 and TNF-α is expressed and released by adipose tissue. Visceral adipose tissue has the capacity of secreting three times the amount of IL-6 compared to subcutaneous adipose tissue. As CRP synthesis in the liver predominantly is regulated by IL-6, it is plausible that IL-6 originating from adipose tissue may systematically elevate the circulating CRP levels (Aronson et al., 2004; Donges et al., 2010).

As described before, CK is an enzyme linked to disruption in the contracting muscle fibers appearing in the blood stream after strenuous muscle damaging exercise and can aid in the measurement and evaluation of muscle damaging exercise.

2.6.1 Acute interleukin-6 responses to resistance training

A single bout of resistance training triggers a transient inflammatory response and stimulates both pro- and anti-inflammatory cytokine production that commonly shows an increase in the production of IL-6.

Peake et al. (2006) investigated the effects of a submaximal eccentric protocol compared to a maximal eccentric training protocol. The submaximal protocol consisted of 10 sets and 60 repetitions of bicep curls with 10% of MVC with one-minute rest and the maximal protocol of 10 sets of 3 maximal voluntary contractions (MVC) with 3-minutes rest in between the sets with opposing arms on an isokinetic arm dynamometer. Blood sampled before, immediately after, 1- and 3 hours after and 1-4 days after the protocols. Interestingly, after the submaximal protocol, serum IL-6 increased and was significantly increased three hours post-workout, but no significant differences occurred in the maximal protocol or during later sampling time points in either groups (Peake et al., 2006).

Hirose et al. (2004) investigated the effects of 6 sets and 5 eccentric repetitions with 40% of the maximal eccentric weight with dumbbell curls with a 2-minute inter-set rest. Blood samples were drawn before, immediately after, 1, 3, 6, 24, 48, 72 and 96 hours after completion of the protocol. Despite the apparent muscle damage induced due to the increase in CK activity after the protocol, no significant differences were found in circulating serum IL-6 on any of the measured time-points (Hirose et al., 2004).

In a study by Smith et al. (2000) subjects performed 4 sets of 12 eccentric repetitions with 100% of their concentric 1RM with 2-minute rest periods on bench press and leg curl. Blood was collected before, 1,5, 6, 12, 24, 48, 72, 96, 120 and 144 hours after completion of the protocol. Serum IL-6 was significantly increased over baseline values at 12, 24 and 72 hours after the protocol and peaked at the 24-hour point (Smith et al., 2000).

Phillips et al. (2003) reported increases in serum IL-6 after a protocol consisting of 3 sets and 10 repetitions at 80% of the eccentric 1RM on a bicep curl machine. A significant increase in

serum IL-6 was present 3 days after the protocol and returned to baseline 10 days after. However, a blinded group who performed the same protocol and received a vitamin E supplement, showed no increases in circulating IL-6 at the time-points (Phillips et al., 2003).

In a study by Croisier et al. (1999) subjects performed 3 sets of 30 eccentric repetitions on both leg extensor and leg flexor muscles with 1-minute rest periods in between the sets. Blood was sampled before, immediately after, 30 minutes after, 48, 72 and 96 hours after the protocol. Significantly increased values of serum IL-6 were observed immediately after the exercise and at the post 30-minutes time-point. The values returned to baseline 48-hours after and did not change during the remainder of the measurement points (Croisier et al., 1999).

Regarding traditional isotonic resistance training consisting both concentric and eccentric muscle actions Izquierdo et al. (2009) examined a traditional program consisting 5 sets of 10 repetitions with as high as possible load with 2-minute rest intervals on a leg press machine. Blood was drawn before, during (3rd set), immediately after, 15 and 45 minutes after the loadings. Subjects performed a seven-week long training period after the first loading, performed the same protocol again with the same weights as the previous protocol and with the new 10RM weights. Interestingly, serum IL-6 was significantly increased 45 minutes after the completion of the protocol both during the pre- and post-training measurements when the new 10RM was used, but not during the post-training measurement when the same load was used as before the training period (Izquierdo et al., 2009).

In a study by Uchida et al. (2009) subjects performed protocols consisting of various intensities ranging from 50%RM to 110%RM on a volume matched bench press protocol consisting both of concentric and eccentric contractions. The sets ranged between 4-10 and repetitions ranged between 4-20. The 110%RM group performed only eccentric muscle actions. However, no increases in any pro-inflammatory cytokines, including IL-6, were found after 24-48 hours in any of the investigated groups (Uchida et al., 2009).

The acute IL-6 response to resistance training varies between studies depending on muscle actions used, the intensity- and the total volume of the protocols. Protocols consisting of a

greater volume regardless of intensity seem to produce a more prominent acute response. No studies have directly compared the effects of either concentric- or eccentric-only muscle actions on the acute IL-6 response and no studies examined the effect of concentric-only muscle actions on the outcome.

2.6.2 Acute CRP responses to resistance training

The effects of resistance training on the acute response of CRP remain somewhat mixed. Most studies do not demonstrate significant increases or decreases in serum CRP levels following a bout of resistance training. The effects of resistance training on CRP remain somewhat unclear, as many studies report no acute increases in circulating amounts of CRP and long-term studies have failed to show reductions in basal CRP values (Ihalainen et al., 2018), while a good fitness level has a lowering effect on circulating CRP values (Aronson et al., 2004).

Peake et al. (2006) investigated the effects of 10 sets of 3 maximal eccentric repetitions with 3-minute rest intervals and 10 sets of 60 submaximal (10%RM) repetitions with 1-minute rest intervals on an isokinetic arm curl machine on the acute inflammation markers. Blood was sampled before, immediately after, 1 and 3 hours after, 1, 2, 3 and 4 days after the completion of the protocol. No differences in circulating CRP was noted on any of the measured time points (Peake et al., 2006).

Croisier et al. (1999) reported similar findings after performing 3 stages of 30 maximal eccentric repetitions on knee flexor and extensor muscles with 1-minute rest intervals. Blood was sampled before, immediately after, 30 minutes, 48, 72 and 96 hours after the protocol. The maximal experimental session was later reproduced after 3 weeks during which 5 eccentric training sessions with 5 sets of 10 repetitions and 75% of maximal force was used. No changes were detected in serum CRP values during any of the sampling points during the two different trials (Croisier et al., 1999).

Traditional resistance training has been shown to produce similar results. Volaklis et al. (2015) compared a resistance training session of 2 sets and 18 repetitions with 90 seconds of rest and

3 sets of 8 repetitions with 90 seconds of rest on chest press, shoulder press, leg press, lateral pull-downs, leg extension and leg flexion on patients with cardiovascular disease. Blood samples were drawn before, after and 60-minutes after the session. No changes were reported in circulating CRP among the two different Resistance training protocols (Volaklis et al., 2015). Similar results have been shown on elite weightlifters. Ammar et al. (2015) found no differences on CRP values after 5 sets of weightlifting exercises with varying intensities (Ammar et al., 2015).

Nakajima et al. (2010) investigated the effects of 4 sets of 70%RM to failure with 1-minute rest on leg press, leg extension and leg curl. Blood was sampled before, immediately after and 1-hour after the completion of the exercise bout. With an average of 52 repetitions completed for each exercise, a significant increase in CRP could be detected immediately after the RT session and returned to baseline one hour after the protocol (Nakajima et al., 2010).

Only one of the studies have reported an acute response of resistance training on the circulating amount of CRP. No studies have examined the difference between eccentric- and concentric-only effect on the response. The acute increases in CRP has mainly been demonstrated after strenuous endurance-type activities such as marathon running (Kasapis and Thompson, 2005) and it is possible that resistance training does not cause a similar acute response in CRP levels.

2.6.3 Acute CK responses to resistance training

When comparing submaximal or maximal eccentric muscle actions, it has been shown that maximal contractions produce a greater amount of muscle damage. Three sets of 10 eccentric repetitions for elbow flexors was performed with 50% of maximal isometric contraction force and compared to 3 sets of 10 eccentric maximal contractions with similar time under tension and rest intervals. The increase in blood serum CK activity was not significantly altered 24 hours after the protocol. However, significant increases were apparent in the serum CK levels after 2-5 days, with the maximal eccentric actions displaying over 80% higher values at those time periods (Nosaka and Newton, 2002). However, the total volume between the groups were not matched, which may have an impact on the big difference between the two groups.

Interestingly, Mavropalias et al. (2020) similarly investigated the difference between high and low intensity eccentric actions on muscle damage, with a volume-matched protocol. The high intensity group performed 12 sets of 10 maximal eccentric repetitions with 2-minute rest intervals. This was later performed again, but with 50% of the maximal eccentric force of the subject. The low intensity protocol performed repetitions until the same amount of work as in the high intensity session was completed. Difference in the CK response between the two sessions were observed only at the 24-hour post measurement point. Both sessions displayed significantly increased CK activity throughout 96 hours, with no significant differences between the bouts. However, the high intensity group showed significantly more decreases in muscle strength levels throughout the 96-hour follow up period, compared with the low intensity session (Mavropalias et al., 2020).

Uchida et al. (2009) investigated the effect of various intensities of 1RM and CK activity and muscle damage. Forty male soldiers were divided into 50%RM, 75%RM, 90%RM, 110%RM and a control group and performed volume matched bench-pressing ranging from 4-10 sets and 4-20 repetitions between the groups. The bench press consisted of both concentric and eccentric contractions, except for the 110%RM group, which performed only 3s long eccentric contractions. Creatine kinase activity was significantly increased on all performing groups between 24-48 hours after the bench press exercise. However, no significant differences were found in the peak amounts of CK, due to the large variability of the subjects (Uchida et al., 2009).

The CK response after resistance training is greater after eccentric muscle actions compared to concentric muscle actions. The total volume of work performed display a greater correlation with the increases in CK than the intensity used, with the highest amounts of CK measured generally between 24-96 hours after the protocols.

3 PURPOSE OF THE STUDY

A single bout of resistance training triggers a transient inflammatory response and stimulates both pro- and anti-inflammatory cytokine production (Freidenreich and Volek, 2012). Studies have mostly investigated the inflammatory responses of eccentric-only or isotonic-loading protocols of various muscle groups on muscle damage and inflammatory markers (Nakajima et al., 2010; Nosaka and Newton, 2002; Pedersen et al., 2003). The author has not found any studies directly comparing eccentric-only and concentric-only muscle actions during resistance training to the acute inflammatory response and muscle damage.

The purpose of this study was to evaluate the effects of an eccentric-only, concentric-only and a combined concentric-eccentric muscle action protocol on the acute inflammatory response and muscle damage after bench press exercising on an isokinetic bench press machine.

Question 1: Do eccentric-only muscle contractions cause a greater acute phase inflammatory response compared with concentric-only or both concentric and eccentric muscle contractions?

Hypothesis 1: The eccentric muscle contractions will cause a greater increase in inflammatory markers compared with the concentric muscle actions, as seen in previous studies utilizing eccentric loading protocols (Croisier et al., 1999; Peake et al., 2006; Smith et al., 2000). However, as concentric muscle actions have shown to increase the circulation of IL-6 (Brunsgaard et al., 1997) an increase after concentric muscle actions are possible.

Question 2: Do eccentric-only muscle contractions cause a greater amount of muscle damage compared with concentric-only or both concentric and eccentric muscle contractions?

Hypothesis 2: The eccentric muscle contractions will cause a greater amount of muscle damage, as eccentric muscle actions cause a greater amount of disturbances in the contracting muscle fibers after loading, compared with concentric muscle actions (Peake et al., 2017).

4 METHODS

4.1 Subjects

Twelve healthy resistance-trained men volunteered for this study. One of the subjects dropped out after the first loading session due to sensations of pain in the shoulder region, therefore 11 men completed all study requirements (mean + SD for age, weight, height and fat percentage; 26.6 ± 3.4 yrs, 89.4 ± 10.2 kg, 182.4 ± 6.36 cm and 13.8 ± 4.29%, respectively). All subjects had a background of regular resistance training (≥ 1 year). The subjects were recruited through flyers, the internal mailing system of The University of Jyväskylä and through local gyms. The requirements for participation were as follows: man, aged between 18-35 years old, 1RM bench press result ≥ 100 kg or 1.25 x bodyweight, generally healthy and no prescriptions to medication affecting cell metabolism.

Prior to participation, all subjects were informed about the potential risks of the study and the possible discomfort associated with high intensity resistance training and blood draws. All subjects gave their written informed consent to participate and filled out a PAR-Q+ form, to ensure readiness for strenuous physical activity. The procedures were approved by the University of Jyväskylä ethics committee and was carried out according to the declaration of Helsinki.

4.2 Study Design

The study consisted of a total of seven visits to the laboratory. Including one familiarization session, three loading sessions and three post-measurement sessions (24 hours after each loading session). The familiarization session and the first loading session was separated by at least 72 hours. All three loading sessions were separated by 14 days to minimize the repeated bout effect (McHugh, 2003; Nosaka and Clarkson, 1995; Peake et al., 2017). During the period of the study, subjects were informed to not participate in any resistance training activities targeting the pectoral- and triceps muscles.

Subjects reported to the laboratory in the morning between 8:00-10:00 am after a 12-hour fasted state and blood was drawn, followed by maximal isometric bench press trials. The subjects, thereafter, performed one of the three isokinetic bench press loading protocols and performed the isometric bench press trials again immediately after the last set. After 5 minutes of resting another blood sample was taken. The subjects returned the next day in a fasted state for another blood draw and maximal isometric bench press trials.

4.3 Familiarization

At the beginning of the familiarization session, subjects filled the PAR-Q+ form and signed informed consent. Subjects were instructed to withstand from eating and drinking for four hours prior the familiarization session. Anthropometric measurements and a body composition measurement were carried out with an InBody 770 (Inbody Co., Ltd. South Korea) bioimpedance scale. Subjects were adjusted in the isokinetic bench press machine in a manner, that they were not able to fully extend their arms in the top position, and a 2-3 cm clearance was set in the low-position to avoid the possibility of getting compressed between the bar and the bench. In a separate isometric bench press apparatus, the bar height was adjusted accordingly to allow the elbow joint to be in a 90° angle, measured with a hand-held goniometer. During the familiarization session, subjects performed maximal isometric repetitions on the isometric bench press and familiarized themselves with performing repetitions on the isokinetic bench press machine.

4.4 Maximal isometric force

To assess fatigue caused by the isokinetic bench press protocol, maximal isometric force was recorded before (pre), 45-seconds after (post-45s) and 24-hours (post-24h) after each isokinetic loading protocol. Maximal isometric force was measured with a custom-built isometric bench press rack (University of Jyväskylä, Finland), equipped with two strain gauge force transducers (Lahti Precision, Finland) in both ends of the bar. Maximal isometric exertions were recorded with a 90 ° flexion in the elbow joint, measured with a goniometer during the familiarization session. The subjects performed three maximal isometric contractions lasting between 3-5

seconds with a 15 second recovery period between the repetitions on all occasions. During the measurement, subjects kept their feet on the bench, mimicking the position on the isokinetic bench press machine. The highest force output of the three maximal contractions was registered and taken forward into further analyses. All maximal isometric force measurements were analyzed with Signal 4.11 software using a customized script (Cambridge Electronic Design Ltd, Cambridge, United Kingdom).

4.5 Isokinetic loading protocols

Subjects performed three different loading protocols on the isokinetic bench press machine (University of Jyväskylä, Finland). The loading modes consisted of concentric-only (CON) and eccentric-only (ECC) repetitions and both concentric and eccentric repetitions (COMB). The subjects performed all three loading protocols on separate occasions at the laboratory in a randomized order.

All three different protocols consisted of five sets of ten maximal repetitions. The sets were separated by a 2-minute inter-set recovery period during all three protocols. The subjects were verbally encouraged during every repetition and set. During all the protocols, the isokinetic machine operated with a constant 0.1 m/s speed throughout the predefined individual movement range. On the eccentric- and concentric-only protocols, the machine was programmed to stop at the top and bottom position for 500 ms, to aid with the pacing of the contractions. During the combined protocol, the isokinetic machine was programmed to stop only for 1 ms in the top and bottom position, to ensure maximal muscular contraction throughout the set. All loading protocols were completed with the subject's feet on the bench and back in flat position.

During the eccentric-only protocol, subjects exerted force maximally against the bar during each repetition before the bar started moving from the top position (i.e. straight arms). On the bottom position (i.e. bar close to the chest), subjects were instructed to relax and keep their hands gripping the bar, while the machine returned to the top position. During the concentric-only protocol, subjects kept their hands gripping the bar during the lowering, and in the bottom position started to maximally exert force to the bar before it started moving and continued to

push all the way to the top position. The subjects were instructed to remain relaxed as the bar returned to the bottom position and the cycle was repeated for all ten repetitions of the set. In the combined protocol, subjects were instructed to maximally exert force for the entire movement pattern including the concentric and eccentric actions.

Between the sets, subjects remained lying on the bench having their arms relaxed on their stomach. The subjects were instructed to avoid unnecessary arm movement during the recovery period between the sets. Subjects were given verbal instructions during the recovery period to pace their preparation for the following sets.

4.6 Warm up

The subjects performed a standardized warm up on the isokinetic bench press machine during the three loading sessions and the post 24-hour measurement points. The warm-up consisted of two sets of five repetitions including both concentric and eccentric muscle actions. The inter-set recovery was standardized to 1.5 minutes. During the first repetition, the subject was asked to follow the bar without exerting any force. On repetitions 2, 3, 4 and 5 the subjects were instructed to exert 50%, 60%, 70% and 80% respectively of their perceived maximal capacity.

4.7 Blood samplings

Blood was drawn from the antecubital vein (30 ml) with venipuncture and from the fingertip (20 µl) in a seated position before (Pre), 5 -minutes (Post-5min) and 24 -hours (Post-24h) after each loading session. The blood was collected in Vacutainer VACUETTE K3EDTA (Grenier Bio-One, Austria) tubes containing an anticoagulant to prevent blood clotting. The blood was centrifuged for 10-minutes at 2200g with a Heraeus megafuge 2.0R (Heraeus, Hanaus, Germany). Blood plasma was stored in -20°C for later analysis. Blood samples for lactate analysis were obtained from the fingertip and collected into capillary tubes (20µl) and inserted into the manufacturer specific tubes containing hemolyzing solution (1-mL) and mixed. All bloodwork was carried out by the laboratory staff at the laboratory of the Faculty of Sport and Health Sciences at the University of Jyväskylä.

4.8 Blood analyses

Blood lactate was measured to determine the metabolic demands of work performed on the different protocols. The blood lactate concentrations were analyzed immediately after completion of the protocol according to manufacturer instructions with an EKF Biosen C-Line lactate analyzer (EKF Diagnostics, United Kingdom).

Blood drawn with venipuncture from the antecubital vein was analyzed for CRP, IL-6 and CK activity. Serum IL-6 and CRP was assessed by utilizing chemiluminescence immunoassays with an Immulite 2000 XPi (Siemens, Llanberis, United Kingdom) according to the manufacturer instructions by The University of Jyväskylä laboratory staff. The coefficient of variation for the CRP assays was 3.84% and for IL-6 assays 3.68%. Creatine kinase activity was assessed by colorimetric analysis with Konelab 20 (Thermo electron Oy, Vantaa, Finland) with coefficient of variation of 1.5%.

4.9 Nutrition

All loading sessions were performed in a 12-hour fasted state. Prior to the first loading session, subjects filled out a food diary consisting of all foods consumed 24-hours prior to the first loading session. Subjects were instructed to repeat the exact same meal plan according to the food diary during the following loading sessions. Failure to follow the instructions resulted in a reschedule of the loading session. No subjects reported to have disobeyed the nutritional instructions prior to the loading sessions. Prior to every loading session, subjects were given a glass of water to drink (75 ml).

4.10 Statistical analyses

All data is presented as means ± SD. All data was analyzed using IBM SPSS version 26 (IBM, Chicago, IL, USA). Normality of the data was assessed using the Shapiro-Wilk test for normality. The criterion level for significance was set at $\alpha = < 0.05$. Data was assessed for sphericity with Mauchly's Test of Sphericity and if sphericity was not met, the highest Epsilon[b]

value was used in further analyses. A repeated-measures analysis of covariance (ANCOVA) was used to analyze the differences between the protocols, using the baseline values as the covariate. When significant main effects were observed, a repeated-measures analysis of variance (ANOVA) was used to analyze the individual protocols and time-points, using pairwise comparisons and the Bonferroni correction for post hoc testing.

5 RESULTS

5.1 Maximal isometric force

Pre-loading values for the maximal isometric force are displayed in Table 1 and absolute values between the groups in Figure 1. No significant main effects for time were found ($p > 0.05$). A significant interaction effect for time*baseline was found ($F = 20.3$, $p < 0.001$). A significant effect on time*protocol was found ($F = 19.8$, $p < 0.001$) and a significant effect for protocol was found ($F = 13.5$, $p < 0.001$). Post hoc pairwise comparisons revealed a significant difference in maximal isometric force between the CON and COMB protocol at the post-45s time-point ($p < 0.001$) and between ECC and COMB ($p < 0.001$) at the post-45s time-point. No significant differences were found between the CON and ECC protocol ($p > 0.05$).

In the CON protocol, maximal isometric force decreased -27.9 ± 6.8% at the post-45s time-point from the pre-loading values ($p < 0.001$) and remained decreased -4.3 ± 3.6% at the post-24h time-point compared with the pre-measurement values ($p = 0.013$). In the ECC protocol, maximal isometric force decreased -21.5 ± 4.7% at the post-45s time-point ($p < 0.001$) and remained -10.0 ± 7.2% decreased at the post-24h time-point ($p = 0.002$). In the COMB protocol, maximal isometric force decreased -40.6 ± 5.9% at the post-45s time-point ($p < 0.001$) and remained -10.5 ± 5.1% decreased at the post-24h time-point ($p < 0.001$).

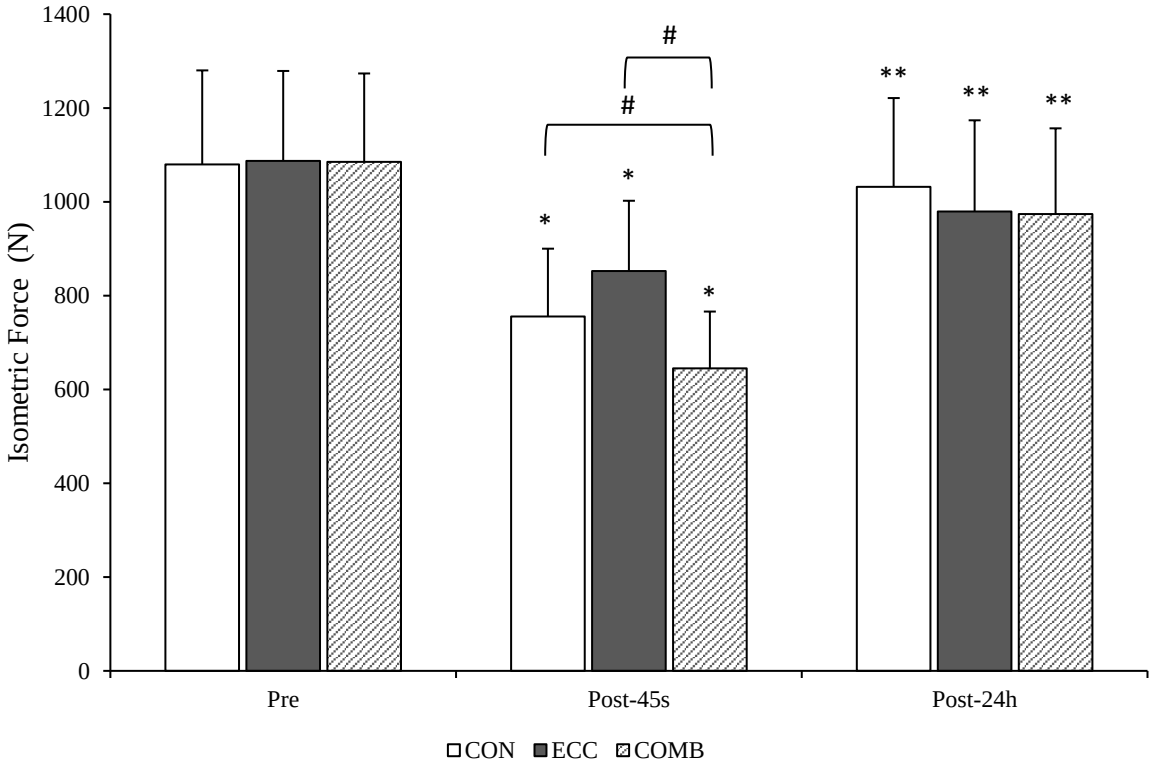

FIGURE 1: Mean ± SD in maximal isometric force between the three time-points. CON = Concentric-only, ECC = Eccentric-only & COMB = Combination protocol. * = Significant difference $p < 0.05$ from pre- values, ** = significant difference $p = < 0.01$ from pre- values. # = significant difference between groups $p < 0.05$.

TABLE 1. Maximal isometric force measured before each loading session. Values are presented as mean ± SD.

Concentric-only	Eccentric-only	Combination
1079 ± 201 N	1087 ± 192 N	1085 ± 188 N

5.2 Interleukin-6

The absolute measured values of IL-6 between the different time-points and protocols are presented in figure 2. A significant main effect for time in the circulating IL-6 was found (*F* =

4.2, $p = 0.27$). A significant interaction effect for time*baseline was found ($F = 3.7$, $p = 0.034$). No significant interaction effects for time*protocol or protocol were found ($p > 0.05$).

In the CON protocol, no significant differences were found between any of the measured time-points ($p > 0.05$). In the ECC protocol, no significant differences were found between any of the measured time-points ($p > 0.05$). In the COMB protocol, no significant differences were found between any of the measured time-points ($p > 0.05$).

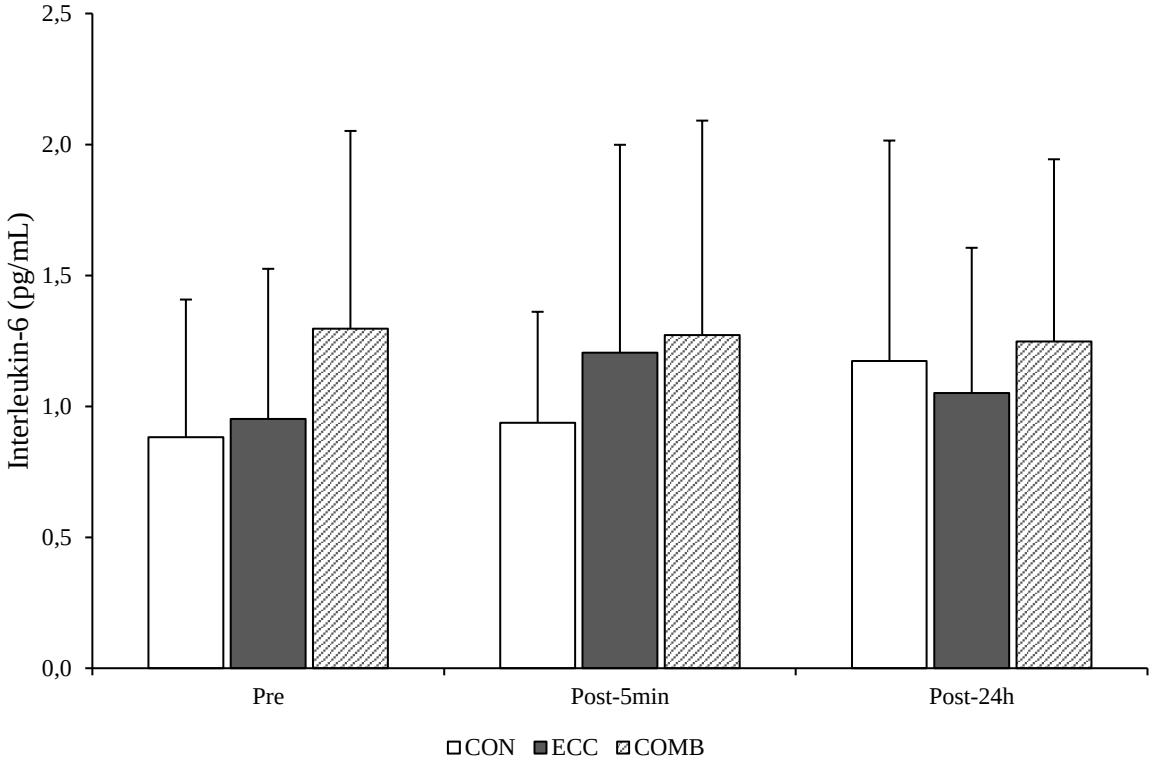

FIGURE 2: Mean ± SD in circulating IL-6 between the three time-points. CON = Concentric-only, ECC = Eccentric-only & COMB = Combination protocol.

5.3 C-reactive protein

The absolute measured values of CRP between the different time-points and protocols are presented in figure 3. A significant main effect for time was found ($F = 8.2$, $p = 0.006$). A

significant interaction effect for time*baseline was found ($F = 19.0$, $p < 0.001$). No Significant effect for time*protocol or protocol were found ($p > 0.05$).

In the CON protocol, no significant differences between any of the measured time-points ($p > 0.05$). In the ECC protocol, CRP values increased by 5.0 ± 5.3% at the post-5min time-point ($p = 0.019$) but not at the post-24h time-point (11.2 ± 35.4%, $p > 0.05$). No significant differences were found between the post-5min, post-24h or pre-loading and post-24h time-points ($p > 0.05$). In the COMB protocol, no significant differences were found between any of the measured time-points ($p > 0.05$).

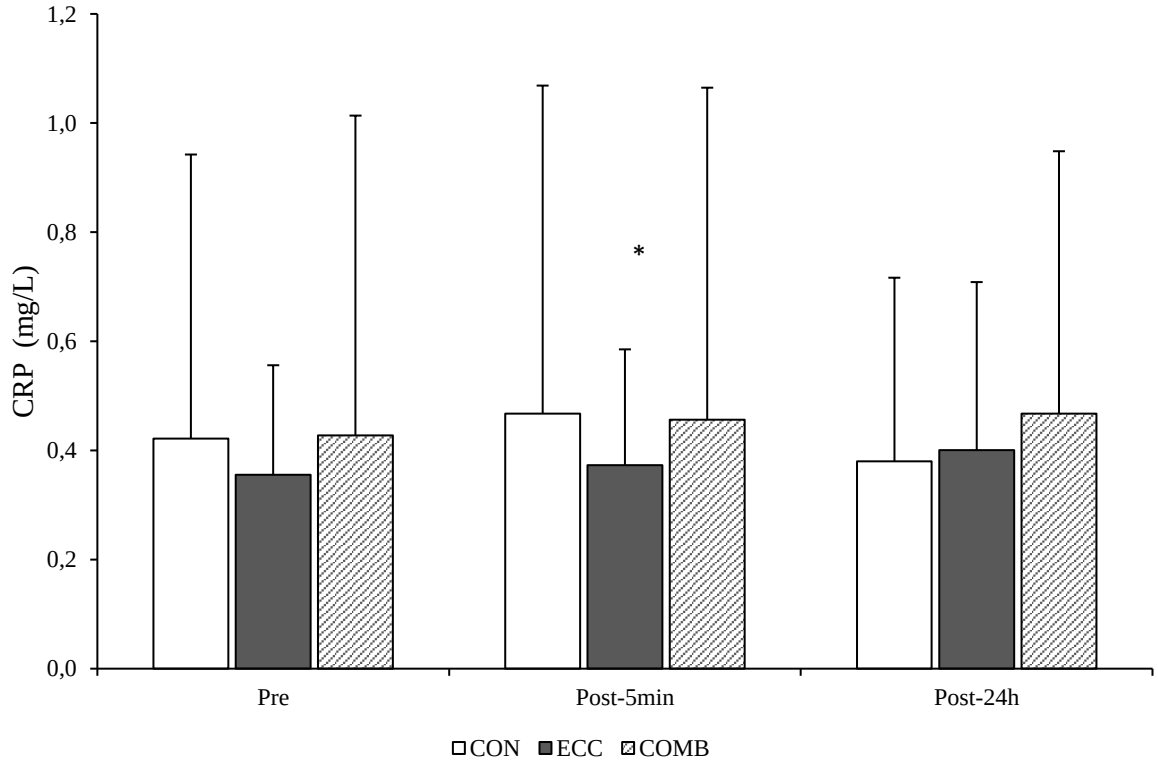

FIGURE 3: Mean ± SD in circulating CRP between the three time-points. CON = Concentric-only, ECC = Eccentric-only & COMB = Combination protocol. * = significant difference from pre- values $p < 0.05$.

5.4 Creatine kinase

The absolute measured values of CK between the different time-points and protocols are presented in figure 4. A significant main effect for time was found (F = 7.8, p = 0.006). A significant interaction effect for time*baseline was found (F = 4.1, p = 0.045). No significant interaction effect for time*protocol or for protocol was found ($p > 0.05$).

In the CON protocol, CK levels increased by 14.2 ± 10.3% at the post-5min time-point (p = 0.013) but not at the post-24h time-point (34.7 ± 67.1%, $p > 0.05$). No significant differences were found between the post-5min and post-24h time-point ($p > 0.05$). In the ECC protocol, CK levels increased by 15.1 ± 10.0% at post-5min time-point (p = 0.002) but not at the post-24h time-point (31.6 ± 41.1%, $p > 0.05$). No significant differences were found between the post-5min and post-24h time-point ($p > 0.05$). In the COMB protocol, CK levels increased by 17.6 ± 10.4% at the post-5min time-point (p = 0.002) and but not at the post-24h time-point (64.9 ± 111.0%, $p > 0.05$). No significant differences were found between the post-5min and post-24h time-point ($p > 0.05$).

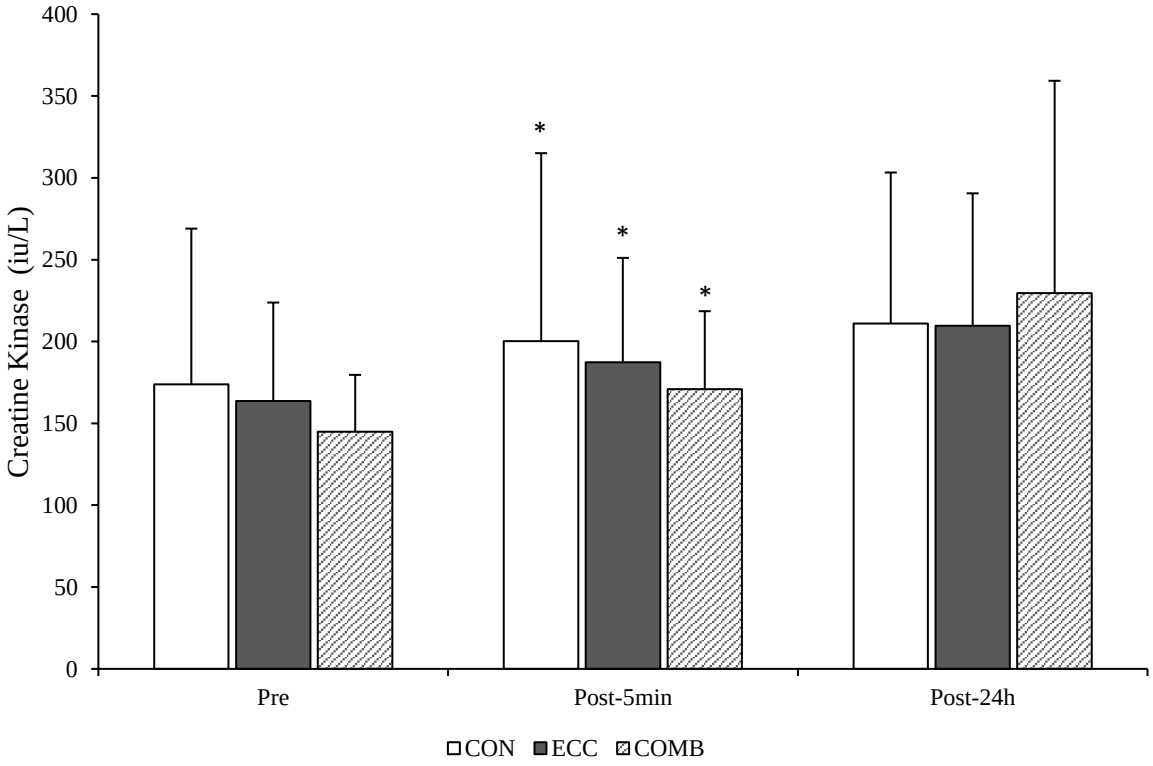

FIGURE 4: Mean ± SD in CK activity between the three time-points. CON = Concentric-only, ECC = Eccentric-only & COMB = Combination protocol. * = significant difference from pre-values $p < 0.05$.

5.5 Blood lactate

The absolute measured values of blood lactate concentration between the different time-points and protocols are presented in figure 5. A significant main effect for time was found ($F = 47.0$, $p < 0.001$). No significant interaction effect for time*baseline was found ($p > 0.05$). A significant effect for time*protocol ($F = 8.1$, $p < 0.001$) and a significant effect for group ($F = 8.9$, $p < 0.001$) was found. A post hoc pairwise comparisons test showed a significant difference in blood lactate values between the ECC and COMB protocol at the post-5min time-point ($p = 0.001$). No significant differences were found between the ECC and CON or CON and COMB protocols ($p > 0.05$).

In the CON group, blood lactate levels increased by 410.5 ± 189.4% at the post-5min time-point ($p < 0.001$) and returned to baseline values at the post-24h time-point ($p > 0.05$). In the ECC group, blood lactate levels increased by 301.9 ± 169.7% at the post-5min time-point ($p < 0.001$) and returned to baseline at the post-24h time-point ($p > 0.05$). In the COMB group, blood lactate levels increased by 515.4 ± 244.3% at the post-5min time-point ($p < 0.001$) and returned to baseline at the post-24h time-point ($p > 0.05$).

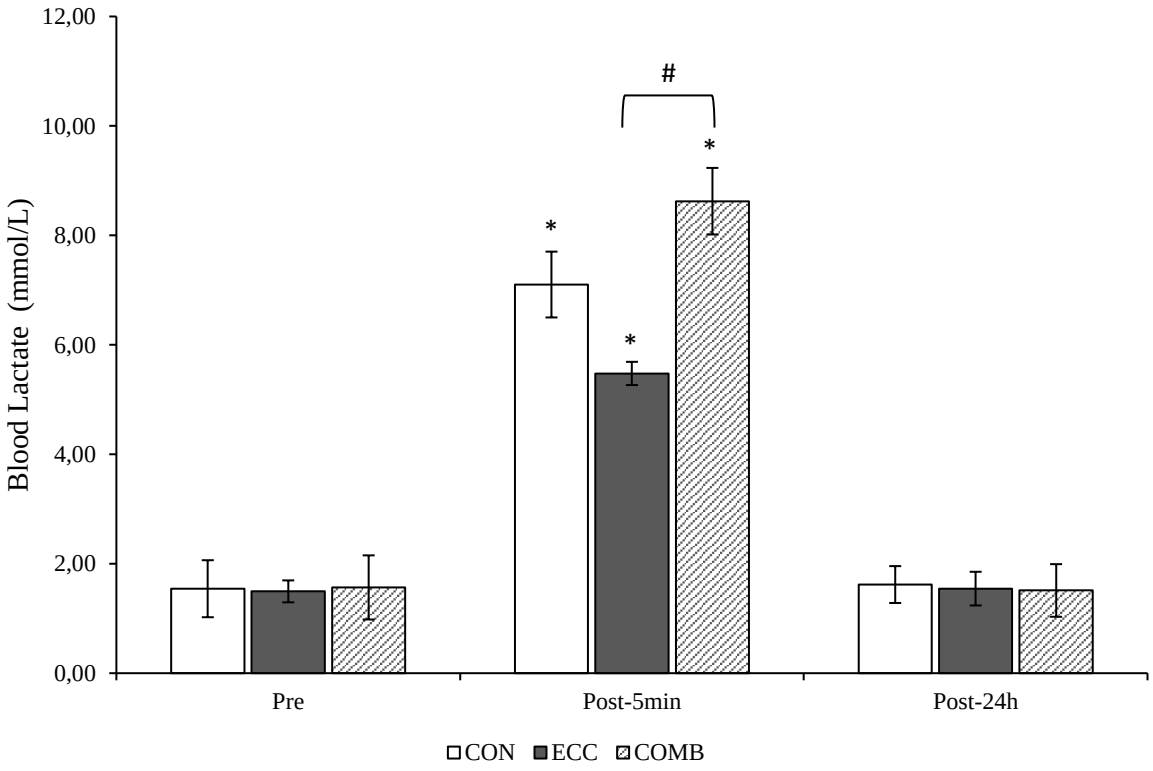

FIGURE 5: Mean ± SD in blood lactate concentration between the three time-points. CON = Concentric-only, ECC = Eccentric-only & COMB = Combination protocol. * = significant difference from pre- values $p < 0.05$. # = significant difference between groups $p < 0.05$.

6 DISCUSSION

The purpose of this study was to evaluate the effects of an eccentric-only, concentric-only and a combined concentric-eccentric loading protocol consisting of five sets of ten repetitions on the acute inflammatory markers IL-6 and, CRP, and muscle damage marker CK responses after maximal bench press exercise using an isokinetic bench press machine.

The main findings of this study demonstrate a significant decrease in maximal isometric force immediately after, with the COMB protocol displaying a significantly greater decrease in force loss compared to the ECC and CON protocol. All three protocols displayed a significant decrease in maximal force production at the post-24h time-point compared with the pre-measured values. Although significant decrease in maximal isometric force at the post-24h time-point, no significant increases in CK activity was found at the post-24h time-point following any protocol. No significant increases in the acute phase inflammatory marker IL-6 was present at any of the measured time-points during the study. A significant increase in CRP was observed at the post-5min measurement point after the ECC loading only, however, this might not be considered a meaning physiological change given the low concentrations observed.

6.1 Maximal isometric force and muscle damage

A significant decrease in maximal isometric force was seen immediately after all three loading protocols. The combination protocol displayed the greatest drop in maximal isometric force followed by CON and then ECC. Interestingly, the ECC protocol displayed the smallest drop in maximal isometric force immediately after loading (post-45s). Although possible, the drop in maximal force of the CON group is mostly explained by central and peripheral fatigue at a cellular level, and not due to muscle damage. Generally, concentric muscle actions do not generate clear muscle damage, if at all (Lavender and Nosaka, 2006; Peake et al., 2017). However, a significant increase in circulating CK levels was evident at the post-5min measurement point in all the groups. This is probably induced by metabolic stress during the concentric contractions in the CON protocol which lead to free radical formation and calcium

overload and damage to cell membranes and an increased circulating CK levels (Koch et al., 2014). The mechanical stress instead occurring during the ECC and COMB protocols disrupts the muscle fibers causing leakage of CK to the circulation. However, the magnitude of the CK response was relatively small, averaging around 200 U/L, which would not indicate any physiologically significant muscle damage.

Eccentric muscle actions typically cause prominent muscle damage to the contracting muscle fibers (Clarkson and Hubal, 2002). The indirect measurement of amount of force loss after eccentric exercise has been used as a tool to classify the amount of muscle damage from eccentric exercise. Decreases of ≤ 20% can be classified as mild exercise induced muscle damage, 20-50% decrease in maximal force can be classified as moderate and the loss of ≥ 50% can be classified as severe exercise induced muscle damage. In association with force loss (~21% in the present study), the CK activity can be accompanied to this with amounts of ≤ 1500 IU/L as mild 1500-10 000 IU/L as moderate and ≥ 10 000 IU/L as severe (Paulsen et al., 2012). In this study, it was expected for the eccentric-only protocol to produce a significant amount of muscle damage. Interestingly, after the ECC protocol, subjects in this study displayed only a force drop of 21%, which is on the border of "mild" and "moderate" amount of muscle damage, but the CK amounts after the protocol remained below 250 IU/L, which would suggest only a low amount of damage. It seems that five sets of ten maximal eccentric repetitions on isokinetic bench press is only capable of inducing a low to moderate response of muscle damage. However, it could be argued as the population in this study had a background in strenuous resistance training, the subjects might have been accustomed to eccentric actions and therefore the CK response did not reach the moderate level. Also, the CK response has been shown to peak at around 48-72 hours after exercise (Koch et al., 2014), and these time-points were not measured in this study.

At the post-24h time-point, the ECC and COMB group displayed a 10% force drop and the CON group displayed a 5% reduction in maximal isometric force compared with the baseline values. Previous studies using submaximal eccentric loadings of the upper body musculature with eccentric repetitions ranging between 30-72 repetitions, have shown maximal isometric force decrements below 20% after 24 hours of recovery (Hirose et al., 2004; Jamurtas et al., 2005). This strengthens the assertion that the maximal protocol used in this study did not induce

a big response in muscle damage. While the decrease in maximal isometric strength can be classified as mild to moderate, the relative change in CK activity can be seen as small. Only one of the subjects displayed a value above 500 IU/L and all other measured values at any measured time-points remained below 500 IU/L, while studies indicate a moderate response to be between 1500-10 000 IU/L (Paulsen et al., 2012).

Eccentric exercise performed with upper body musculature has shown significantly bigger decreases in maximal isometric strength compared to the leg musculature (Jamurtas et al., 2005). This is probably due to the leg musculatures being utilized more commonly in all day activities (downhill walking & descending stairs etc.), which provides a repeated bout effect and attenuates the incidence of muscle damage. Although a bench press protocol utilizing the upper body musculature was used in this study, the subjects in this group had a background in regular heavy resistance training, and thus might have a prior adaptation effect (repeated bout) against eccentric exercise induced muscle damage, and thus showing a response of lower magnitude. The CK response has been shown to peak between 48 to 72 hours after cessation of exercise (Koch et al., 2014), thus the 24-hour timeframe used in this study may have been non-optimal to detect the highest CK response between the groups.

6.2 Acute inflammatory response

6.2.1 Interleukin-6

No significant differences were found in the circulating levels of IL-6 after the three loading protocols at any measured time-point in this study. Previous studies demonstrating an increase in circulating IL-6 after physical activity have mostly utilized activities classified as cardiopulmonary training lasting long periods of time, such as marathon running (Ostrowski et al., 1998). An acute increase in IL-6 measured in the bloodstream has been a constant finding in cardiopulmonary-type training, and IL-6 has been shown to be acting as a myokine and being released from the contracting muscle fibers (Pedersen et al., 2003). As presented in the study by Steensberg et al. (2000), increases of circulating IL-6 starts to increase after 60 minutes of one legged concentric isokinetic muscle contractions and at the 120-minute time-point the

amount reaches statistical significance (Steensberg et al., 2000). This type of exercise, however, is more related to cardiopulmonary training, although the movement is done in a similar manner as during resistance training.

No study has investigated the effect of resistance type training on the effect of circulating IL-6 consisting of only concentric muscle actions. Most studies investigating the effects of resistance training on IL-6 expression also include the eccentric component in their treatment groups. Protocols of this kind, consisting of five sets of then repetitions with 10RM load on a leg press machine have shown to increase the circulating IL-6 over 100% from pre-measurement values after 45 minutes (Izquierdo et al., 2009). Others have demonstrated increases in IL-6 measured from muscle biopsies after three sets of 12 maximal repetitions performed with knee extensor muscles three hours after the loading (Trennery et al., 2011). However, on a bench press protocol with varying intensities from 50%RM to 110%RM, 4-10 sets and 4-20 repetitions Uchida et al. (2009) demonstrated no significant increases in IL-6 at 24-48 hours after the interventions (Uchida et al., 2009). The release of IL-6 as a myokine from the contracting muscle fibers seems to be linked to the intensity of the exercise, and therefore also indirectly linked to the amount of muscle mass activated. This might help to explain the differences in the incidence of circulating IL-6 between lower and upper body exercises, with generally higher increases seen after lower-body exercises activating a bigger amount of muscle mass (Pedersen and Febbraio, 2008).

Previous studies have shown conflicting results on the IL-6 response and eccentric muscle actions. Studies have shown significantly increased amounts of circulating IL-6 after 4 sets of 12 repetitions with 100% of the concentric 1RM with significant increases after 12, 24 and 72 hours after the protocol, with increases of over 100% from the pre-measurement values (Smith et al., 2000). A bout of 3 sets of 30 eccentric repetitions on both leg extensor and flexor muscles has shown to elicit a significant increase in the amount of circulating IL-6 immediately and 30 minutes post exercise (Croisier et al., 1999). However, a protocol of 10 sets and 3 eccentric maximal repetitions on an arm dynamometer failed to elicit any increases on circulating IL-6 at any time-point varying from immediately post to 1, 3 hours and 4 days after the protocol, while an increase three hours post was shown after 10 sets and 60 repetitions with 10% of the maximal voluntary force (Peake et al., 2006). Hence, an important aspect of resistance training

protocols in eliciting a robust IL-6 response seems to be related to the utilization of upper or lower body musculature and the total amount of performed repetitions, with the lower body musculature and higher amount of total repetitions eliciting a response more commonly.

In this study, IL-6 was sampled at five minutes post- and 24-hours post-loading, which might not have been suitable to see the effects on circulating IL-6, as it has mostly been shown to peak 45 minutes to 3 hours after intense resistance training regimens. However, previous studies have shown increased amounts of circulating IL-6 at the post 24h time-point, which this study did not show. Previous studies have mostly utilized designs targeting the lower body musculature, which generally includes larger amounts of muscle mass compared to the upper body musculature. It may be that the generally smaller pectoralis and triceps musculature fail to stimulate IL-6 during resistance training in such manner, especially in men accustomed to such exercise and high-intensity resistance training, that it would be significantly increased when measured form the bloodstream.

6.2.2 C-Reactive Protein

A significant increase in circulating CRP was found after the ECC protocol at the post-5min time-point, but no significant differences occurred at any other measurement point. Examining the actual values in this study, it can be stated that this is not likely a meaningful physiological change. Previous studies have classified a high amount of basal circulating CRP to be > 3.0 mg/L and among the only studies demonstrating an effect of resistance training on CRP showed an increase of > 0.2 mg/L after resistance training (Nakajima et al., 2010). While the mean increase in this study between the pre and post-5min time-points on the ECC group is 0.046 mg/L, it can be seen as a statistical significant difference between the time-points, but not as a meaningful physiological change likely influencing adaptation or immune function. In studies where physiologically meaningful increases have been seen (such as marathon running), increases in CRP of over 400% from basal levels have been measured immediately and 24 hours after the race, with absolute values reaching over 22 mg/L (Weight et al., 1991).

Studies using similar loading protocols have generally not shown an effect on the circulating CRP after the loadings. Previous studies performing isokinetic eccentric-only muscle actions with the total amount of maximal repetitions ranging between 30 to 90, have not shown increased amounts of circulating CRP in close relation to the loadings (Croisier et al., 1999; Peake et al., 2006). The ECC protocol in this study is in agreement with these previous findings. A previous study did not find any significant increases in CRP levels after 2 sets of 18 repetitions with 50%RM or 3 sets of 8 repetitions with 75%RM performed with chest press, shoulder press, leg press, lateral pull-downs, leg extension and leg flexion on patients with cardiovascular disease (Volaklis et al., 2015). While the intensity remains lower than in the present study, the total amount of work (volume) performed, due to the multiple exercises increases to a higher degree and would hypothetically lead to a greater immunological response, which seems not to be the case. These results mimic the combined loading protocol of this study and are in an agreement.

Interestingly, only one study has showed an increase in circulating CRP after completing a total of 157 repetitions of lower body exercises until exhaustion. Circulating CRP values were upregulated immediately after cessation of the training bout (Nakajima et al., 2010). The subjects performed the repetitions to exhaustion, similarly as in the combined protocol of this study, however the total amount of repetitions performed was three times higher. Also, the protocol consisting of multiple exercises targeting the lower body musculature, utilizing a greater amount of muscle mass compared to this study and the subjects classification as untrained, compared to resistance-trained subjects in this study, may explain the different findings. Resistance trained (more fit) persons have generally shown lower basal levels of CRP (Donges et al., 2010; Kelley and Kelley, 2006; Lakka et al., 2005), which may also have an effect on the CRP response after resistance training. This might be due to regular exercising affects the release of cytokines from adipose tissue, muscles and white blood cells which affect the synthesis of CRP from the liver and indirectly by improving insulin sensitivity and endothelial function in subjects which has an effect on the CRP levels (de Salles et al., 2010). The lack of a clear acute phase response (upregulation of IL-6) in this study might also partially explain the lack of any meaningful increases in CRP levels after the protocols in this study, as IL-6 stimulates the production of CRP in the liver whereafter it is released to the bloodstream during the acute inflammation response (Del Giudice and Gangestad, 2018).

6.3 Blood lactate

In this study, blood lactate concentrations increased significantly after all three different loading protocols at the post-5min measurement point and returned to baseline values at the 24-hour time-point. The most prominent increases were noted in the COMB protocol, followed by CON protocol and the lowest increase was obtained immediately after the ECC protocol.

Lactate is a metabolic bi-product, which can be used to determine the dominance of the metabolic energy systems. Anaerobic energy metabolism plays an important role during resistance exercising which imposes a high energy demand and restricted blood flow during the performed repetitions. Blood lactate concentrations have been shown to progressively increase with increasing amounts of sets performed during the recovery phases between the sets, when blood is permitted to flow more freely to the circulation from the muscles. Interestingly, during the sets, lactate concentrations have a tendency to decrease, due to the elimination of lactate, transportation to other tissues and lactate being utilized as an energy substrate for anaerobic metabolism (Wirtz et al., 2014). The highest amounts of lactate are generally measured during the minutes following cessation of exercise. The lactate response seems to be of similar magnitude when the loading is done according to the relative intensity of 1RM of the subject instead of an absolute intensity, with greater intensities demonstrating a greater peak lactate production (Reynolds et al., 1997).

Eccentric muscle actions generate a lower energy demand and require fewer motor units for a given mechanical power output during activation compared with concentric muscle actions (Douglas et al., 2017). The proposed different contraction model of eccentric muscle actions in relation to unique neural activation of eccentric muscle actions results in the lesser magnitude of metabolic demand and accumulated metabolites (Douglas et al., 2017). This leads to a lower degree of metabolic stress and accumulated metabolites such as lactate and to a lower oxygen consumption during eccentric loading of the muscles for a given load. As showed previously, significantly less lactate was accumulated after four sets of 12 eccentric muscle actions compared to concentric muscle actions (Durand et al., 2003), in a similar fashion as in this study after the ECC protocol.

The highest amount of lactate accumulated was demonstrated in this study after the COMB protocol, where the total amount of work and therefore the total amount of energy utilized through anaerobic pathways is the highest. The findings in this study are in a high agreement with previous studies demonstrating the lowers amount of blood lactate accumulated after the ECC protocol, followed by the CON protocol and greatest amounts of excess lactate after the COMB protocol, where the total amount of work is highest (Douglas et al., 2017).

Metabolic stress, exercise-induced muscle damage and mechanical tension are all important stimuli in inducing exercise induced adaptations, especially mechanical tension to initiate hypertrophic adaptations through activation of the mTOR pathway (Wackerhage et al., 2019). The production of lactate and its possible utilization in skeletal muscle hypertrophy has recently been recognized in rodents as a potential molecule to stimulate hypertrophy through the activation of satellite cells (Ohno et al., 2019). Exercise-induced muscle damage and increases in the circulating amounts of cytokines, either produced by the white blood cells during the inflammatory response or as a myokine from the contracting muscles, e.g. the IL-6, may potentially play a role in the adaptation processes of skeletal muscle after training. This is due to the potential of IL-6 to induce satellite cell-induced muscle hypertrophy (Serrano et al., 2008). However, exercise-induced muscle damage and metabolic stress probably work as indirect stimuli toward adaptations of the muscles, as the exercise induced muscle damage response decreases with prolonged training. The exercise-induced damage response has been reported to be blunted after an initial bout of eccentric exercise, resulting in a less severe incidence of markers of muscle damage and inflammation after training, known as the repeated bout-effect (McHugh, 2003; Nosaka and Clarkson, 1995; Peake et al., 2017). Similar to this study, with subjects adapted to high intensity resistance training, no clear effects of muscle damage (indicated by CK concentrations) were present after the loadings up to post-24h time-point, even when all the repetitions were performed with maximal voluntary contraction. The absence of increased amounts of IL-6 after the loadings, as a marker of muscle tissue damage, reinforce the findings that no clear muscle damage was formed during the loadings, although muscle cells have been shown to be capable of producing cytokines (myokines) during activation (Pedersen et al., 2003).

Yet, training utilizing long term eccentric muscle actions have been reported to cause significant amount of muscle growth (Douglas et al., 2017). Therefore, it may be, that mechanical tension plays a greater role in the skeletal muscle adaptations compared to metabolic stress and exercise induced muscle damage. Eccentric exercise, which requires a lower metabolic demand resulting in lower amounts of blood lactate and other metabolites (metabolic stress) and a decreased amount of muscle damage acquired through the repeated bout-effect, could be a viable training option for people suffering from pulmonary and coronary disease or the elderly. This recommendation would likely achieve the positive muscle strengthening and hypertrophic adaptations, as suggested in previous studies (Peñailillo et al., 2013), without exacerbating the disease-related symptoms or age-related limitations.

6.4 Study limitations

The population in this study was relatively small and may, therefore, easily affect the results. Since the population had a background in regular heavy resistance training, this may have affected the magnitude of the responses of the measured inflammatory markers. This study only measured the outcomes at two time-points after the loadings, which limits the investigation of the measured markers that have been shown to increase at later time-points. The total volume of the loading protocols mimics loading that are generally utilized in practical resistance training, but this may have been too small to cause a large reaction, when measured from the bloodstream.

Future studies should examine the differences of concentric and eccentric muscle actions with either an untrained population, or a bigger total volume of the protocols utilizing more measurement points with longer periods in between the measurements.

6.5 Conclusions

This study is among the few which have directly measured concentric and eccentric muscle actions with an identical set and repetition protocol on acute inflammatory markers and muscle damage. It was demonstrated that five sets of ten maximal repetitions on an isokinetic bench

press protocol provokes a significant decrease in maximal isometric force immediately after the protocols, persisting also up to 24 hours, but no protocol provoked a significant increase on the measured acute phase inflammatory marker IL-6 and circulating CRP after either concentric, eccentric or combined muscle actions. A significant increase in blood lactate values were observed immediately after all protocols, with eccentric muscle actions displaying a significantly lower metabolic cost compared to the other protocols.

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