# Master Thesis

## Eight weeks of endurance training increases calcium release and uptake from skeletal muscle sarcoplasmic reticulum in sedentary adults

**Torkil Rogneflaaten**

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**Faculty of Social and Helath Sciences**

**Inland Norway University of Applied Sciences**

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# Acknowlegdements

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# Abstract

**Background**: Little is presently known about the effects of endurance training on release and uptake of calcium ions ($Ca^{2+}$) by the sarcoplasmic reticulum (SR) and the implications of such characteristics for muscular function and performance. Thus, the present study aimed to characterize this regulation in untrained men and woman following a period of endurance training and examine how the possible changes corresponds with changes in endurance performance.

**Methods**: Release and uptake by SR vesicles isolated from resting *m. vastus lateralis* muscle biopsies and endurance performance were determined before and after a period of eight weeks with moderate and high-intensity endurance training in sixteen females ($31.5 \pm 5.9$ mL $O_2 \cdot kg^{-1} \cdot min^{-1}$) and twenty-one males ($36.8 \pm 4.7$ mL $O_2 \cdot kg^{-1} \cdot min^{-1}$).

**Results**: Training led to improved 15-min endurance performance (10.0%, 95% confidence interval (CI): [7.9, 11.7]; $W_{15min}$) with concomitant increases in SR $Ca^{2+}$ release rate (7.3% [2.0, 12.8]) and SR $Ca^{2+}$ uptake (13.2% [3.4, 24.0]). SR $Ca^{2+}$ uptake was negatively associated with changes in $W_{15min}$ (-0.16 watt [-0.28, -0.04] per %-point increase in uptake), and SR $Ca^{2+}$ release was not associated with $W_{15min}$. SR $Ca^{2+}$ release did not differ between sexes, however, females displayed -16.4% [-29.8, -0.8] lower SR $Ca^{2+}$ uptake at baseline, yet, increased 11.9% [1.0, 23.7] more after training.

**Conclusion**: The rate of SR $Ca^{2+}$ release and uptake can be enhanced by eight weeks of endurance training in sedentary adults, which may have important implications for muscular performance and general health. Moreover, SR $Ca^{2+}$ release does in general not differ between sexes, with the exception of females displaying lower rates of SR $Ca^{2+}$ uptake prior to training, yet larger gains in response to training.

# Table of contents

# 1. Theory

## 1.1. Sarcoplasmic reticulum and the E-C coupling

The contractile function of skeletal muscle fibers depends on a multitude of specialized components and organelles within the skeletal muscle fibers, which collaborate to enable coordinated force production in response to signals from the central nervous system, i.e. the excitation-contraction (E-C) and relaxation coupling (Sandow, 1952). A central governor of the E-C and relaxation coupling is the specialized component of muscle cells endoplasmic reticulum, i.e. the sarcoplasmic reticulum (SR) (Volpe et al., 1992). The SR consists of a network of longitudinal tubules surrounding each myofibril (Porter, 1956; Porter & Palade, 1957). These tubules regularly merge into terminal cisternae, located at the junctions between A and I bands of sarcomeres, forming a "triad junction" with two terminal cisternae positioned opposite each other around a transverse tubule membrane (t-tubule) (Franzini-Armstrong, 1970). This distinct structure and localization of the SR within the skeletal muscle fibers, coupled with its calcium ion ($Ca^{2+}$) storing and release properties (Fleischer et al., 1985; Porter, 1956; Porter & Palade, 1957; Wuytack et al., 2002), allow the SR to facilitate the conversion of electrochemical signals of a action potential into the mechanical movement of the cross bridge cycle (Barone et al., 2015; Geeves et al., 2005). The SR $Ca^{2+}$ handling properties are primarily carried out by two distinct SR proteins, i.e. the ryanodine receptors type 1 (RyR1) $Ca^{2+}$ release channels, situated on the SR terminal cisternae, and the sarcoplasmic/endoplasmic reticulum $Ca^{2+}$ ATPase (SERCA), situated on the longitudinal SR and is responsible for transporting $Ca^{2+}$ from cytosol into the SR lumen (Dulhunty, 2006). These structural attributes of the SR are substantially different between fast type II fibers and slow type I fibers, wherein type II fibers roughly contain twice the volume and area of SR terminal cisternae, about four to six times higher RyR1 content and showcase roughly three times higher SR

$Ca^{2+}$ release than type I fibers Rüegg (1986). Moreover, SERCA exist in two isoforms: SERCA1 in fast type II fibers and SERCA2 in slow type I fibers (Lytton et al., 1992), with a significantly higher density observed in fast fibers compared to slow fibers (Everts et al., 1989a).

The remainder of this theory part will focus on the SR and its role in the E-C relaxation coupling, as well as addressing how the functional capacity of the SR is of importance for muscular fatigue and subsequently, important for general health and performance. Furthermore, the theoretical basis underlying how training adaptations in the SR $Ca^{2+}$ handling properties may induce a significant effect on muscular function will be addressed before presenting the current knowledge, together with knowledge gaps, of such adaptations.

### 1.1.1. The E-C and relaxation coupling

Voluntary muscle contractions is initiated in the brain by the genesis of action potentials which is transmitted via sequential electrical and chemical events through the spinal cord, motor nerve and neuromuscular junction to the muscle fiber. Depolarization of the muscle fiber triggers conformational changes to the dihydropyridine receptor (DHPR) (Schneider & Chandler, 1973), situated in t-tubules of the triad junction, and the subsequent opening of the RyR1 $Ca^{2+}$ release channels, by a mechanical interaction of DHPR and RyR1 (Dulhunty, 2006; Ríos, 2018; Tanabe et al., 1990). The subsequent SR $Ca^{2+}$ release through RyR1 leads to a significant increase in the concentration of free $Ca^{2+}$ in the cytosol of muscle fibers ($[Ca^{2+}]_i$), typically by 10-20 fold (Bruton et al., 2003; Ingalls et al., 1999). $Ca^{2+}$ within the cytosol bind to troponin C, triggering the movement of tropomyosin, thereby facilitating the cycling of cross-bridges, ultimately leading to the development of force (Ashley et al., 1991; Geeves et al., 2005). Concomitantly, $Ca^{2+}$ are continuously re-sequestered back into the SR by SERCA, causing muscle fiber relaxation as $[Ca^{2+}]_i$ declines. SERCA utilizes the energy generated by adenosine triphosphate (ATP) hydrolysis to shuttle two $Ca^{2+}$ ions from the cytosol into the SR lumen, while simultaneously transporting two protons from the SR lumen to the cytosol in exchange (Everts et al., 1989a; Yu et al., 1993).

## 1.2. SR $Ca^{2+}$ handling in fatigue

The contractile function of skeletal muscle fibers declines during intense or prolonged physical exercise, i.e., fatigue develops (Hill, 1925; Mosso et al., 1904). A fundamental principle in exercise physiology is that energy is essential for performing work (BASSETT, 2000). Accordingly, through repetitive muscle contraction, the energy demands within the working muscle fibers are heightened, and hence, necessitating efficient ATP resynthesis to match the substantially increased ATP consumption of the muscle fibers (Matheson et al., 1991). Within contracting muscle fibers, ATP is primarily utilized by molecular motors, such as actomyosin cross-bridges, ion pumps like SERCA, and to a lesser extent, the sarcolemmal $Na^+$-$K^+$-pumps. Ensuring adequate ATP supply to these ATP-consuming proteins is crucial for preserving normal cellular function and integrity, as ATP depletion will lead to detrimental outcomes, including the persistence of noncycling cross-bridges and the onset of rigor mortis, impaired SR $Ca^{2+}$ pumping resulting in uncontrolled elevation of $[Ca^{2+}]_i$, and compromised maintenance of $Na^+$ and $K^+$ gradients across the sarcolemma, leading to impaired action potential conductance and eventual loss of muscle fiber excitability (Cheng et al., 2018). Evidently, mechanisms aimed at averting these deleterious effects of ATP depletion during prolonged or intense physical exercise are present within the muscle fibers and includes; efficient metabolic pathways for ATP resynthesis and fatiguing mechanisms designed to reduce ATP consumption by the molecular motors. Accordingly, slow-twitch type 1 muscle fibers, characterized by a slower ATP consumption rate and a greater aerobic capacity, typically exhibit higher resistance to fatigue compared to fast-twitch type 2 muscle fibers (Bruton et al., 2006; Essén et al., 1975; Everts et al., 1989b; Lytton et al., 1992).

A substantial part of the exercise-induced fatigue development is related to impairments in the SR $Ca^{2+}$ handling associated steps of the E-C and relaxation coupling (Baker et al., 1993; Bigland-Ritchie et al., 1986; Byrd et al., 1989; Favero et al., 1993; Hostrup et al., 2014; Li et al., 2002; Ørtenblad et al., 2011). In which, the underlying fatiguing mechanisms involves a decrease in the rate of SR Ca2+ release, reduced maximal $Ca^{2+}$-activated force production, reduced $Ca^{2+}$ sensitivity, as well as reduced $Ca^{2+}$ re-sequestering rates, with the significance of each of these factors varying according to the pattern and duration of muscle activation (Allen et al., 2008; Westerblad & Allen, 1991). The most efficient method of decreasing the energy expended by contracting muscle fibers is by inhibiting

SR $Ca^{2+}$ release, thereby reducing the number of ATP-consuming cross-bridge cycles and diminishing the requirement for ATP-dependent SR Ca^2+ reuptake by SERCA (Cheng et al., 2018). However, this reduction in SR Ca^2+ release is accompanied by a decline in muscle force generating capability, leading to increased fatigue severity. During exercise, SR $Ca^{2+}$ release is reduced be numerous mechanisms, and the most prominent seem to be $P_i$ accumulation (Fryer et al., 1995; Westerblad & Allen, 1996), reduced ATP (Dutka & Lamb, 2004) and glycogen depletion (Nielsen et al., 2014; Ørtenblad et al., 2011).

## 1.3. Muscle fatigue - health and performance

### 1.3.1. General health

One facet of physical health encompasses the musculoskeletal system, comprising three key elements: muscular strength, endurance, and flexibility (Kell et al., 2001). Herein, the term muscular endurance is used to reflect the capacity of muscle or muscle groups to sustain repeated contractions against a load over an extended duration i.e. resilience the muscle fatigue. Higher levels of musculoskeletal fitness are in turn associated with numerous health benefits, e.g., reduced coronary risk factors (Durstine et al., 2001; Kelly et al., 1998; Kraus et al., 2002), heightened bone mineral density (Bérard et al., 1997), improved glucose tolerance (Pan et al., 1997; Ramachandran et al., 2006), and greater success in completion of activities of daily living (G. R. Hunter et al., 2004). Moreover, muscle fatigue is a very common side effect, and a nuisance for disease patients, in diseases like chronic obstructive pulmonary disease (Breslin et al., 1998), sarcopenia (Patino-Hernandez et al., 2017), cancer (Morrow et al., 2002; Ryan et al., 2007), obesity (Resnick et al., 2006; Vgontzas et al., 2000), and chronic fatigue syndrome (Kent-Braun et al., 1993). Hence, the skeletal muscle represents a crucial focal point for interventions aimed at promoting health throughout the entire lifespan, and in to improve a pleura of pathophysiological conditions (Wolfe, 2006). Yet, there is a notable gap in understanding the health benefits associated with training-induced improvements in muscle fatigue, and herein, the role of SR $Ca^{2+}$ handling properties.

### 1.3.2. Endurance performance

High rates of oxidative metabolism are required in order to sustain high absolute work rates during endurance sports, wherein the primary determining factors of endurance performance have been suggested to include the maximal rate of oxygen consumption ($VO_{2max}$) and fractional utilization of $VO_{2max}$ at lactate threshold, along with exercise economy (Joyner & Coyle, 2008). These parameters are typically assessed in well-rested individuals and have traditionally been regarded as static measurements. However, recent research indicates that these physiological factors may undergo shifts in response to prolonged exercise (Clark et al., 2018, 2019), highlighting the need to integrate this dynamic aspect into the endurance performance model. Consequently, there is increasing recognition of durability, which refers to the time of onset and magnitude of deterioration in physiological-profiling characteristics during prolonged exercise, as a significant limiting factor in endurance performance (Maunder et al., 2021). In contrast to the respective determinants of endurance performance, much less is know about the physiological determinants of durability, albeit that it is intrinsically connected to muscular fatigue (Matomäki et al., 2023), and thus, also SR $Ca^{2+}$ handling.

## 1.4. Exercise training and SR $Ca^{2+}$ handling

Over time, increasing the contractile demands placed on skeletal muscles during physical exercise will typically result in adaptations that improve functionality and performance towards the characteristics of the exercising stimulus (Bogdanis, 2012). Accordingly, a period of endurance training will improve the ability of skeletal muscles to sustain a given work-load for a longer period of time, through adaptations facilitating resilience to muscular fatigue. A salient response to endurance training aimed at enhancing resilience to muscular fatigue is an increase in the physiological capacity of ATP production, e.g. increased $O_2$ delivery to exercising muscles through expanded blood volume, an increased stroke volume, and greater quantity of hemoglobin (Lundby et al., 2017; Lundby & Montero, 2019), as well as peripheral adaptations, such as increased mitochondrial content and improved mitochondrial functions (Granata et al., 2018), increased capillarization surrounding the muscle fibers (Hoier & Hellsten, 2014), and improved muscle glycogen availability (Gejl et al., 2017). However, much less is known

about the possible adaptations at the level of the E-C and relaxation coupling and the factors underlying SR $Ca^{2+}$ handling properties, i.e. SR $Ca^{2+}$ uptake and release, SERCA ATPase activity, and SR protein contents (SERCA or RYR1).

The principal of symmorphosis posits that all components in a biological system is quantitatively coupled to functional demands and no singular parameter encompasses unnecessary surplus capacity (Taylor & Weibel, 1981; Weibel et al., 1991). Accordingly, given the known link between impaired SR $Ca^{2+}$ handling and muscular fatigue, it may be expected that the quantitative attributes and the functional capacity of the SR $Ca^{2}$ handling properties are inherently coupled to the functional demands of the biological system that is muscular fatigue. Thus, it might be anticipated that endurance training would reduce the SR impairment following a given training-load through adaptations in the SR $Ca^{2+}$ handling properties.

## 1.5. Methods to study SR $Ca^{2+}$ handling in skeletal muscle

Methods for analyzing SR $Ca^{2+}$ handling are confined to *in vitro* settings, involving techniques such as muscle biopsy from whole muscle (Bergström et al., 1967; Bergström, 1975) and dissection of single fibers from whole muscles (Allen et al., 2008; Lännergren & Westerblad, 1987). The intact single fiber dissection technique can be performed on animals and offer a robust approach for investigating muscle fiber force output during acute electrically stimulated contractions alongside fluorometric analysis of $[Ca^{2+}]_i$, i.e., indirect measure of SR $Ca^{2+}$ release and uptake. In addition, single fibers can be mechanically or chemically skinned, which allows for controlled analysis of the functionality of each of steps in the E-C and relaxation coupling (Lamb & Stephenson, 2018; Wood et al., 1975), in which, muscle fibers can be obtained from human muscle biopsies (Malisoux et al., 2006; Widrick et al., 1998). Single fiber techniques are, however, best suited for investigation force and $Ca^{2+}$ handling during acute exercise of the muscle fiber, and not for investigation of "chronic" training adaptations in response to exercise training.

Chronic training adaptations in SR $Ca^{2+}$ handling properties can either be studied for functional or quantitative changes in the specific SR $Ca^{2+}$ handling proteins, i.e. RyR1, SERCA1, SERCA2 and

DHPR, or by measuring the rate of $Ca^{2+}$ flux in and out of the SR in homogenized muscle tissue from human muscle biopsies. Each of these methods faces challenges due to muscles being composed of various fiber types with distinct properties. Consequently, there are issues either with the sample's representativeness or with how spatial and/or temporal averaging obscures interpretation. Analysis of function and content of SR proteins can be done on homogenized muscle tissue and includes analysis of SR protein content and concentration using immunoblotting (Ploug et al., 1993), determination of the total number of 3H-ouabain binding sites (Lunde & Sejersted, 1997; Nørgaard et al., 1984) and measurement of the $Ca^{2+}$-dependent steady state phosphorylation from ATP (Everts et al., 1989b), as well as analysis of SERCA activity using spectrophotometric techniques (Simonides & Van Hardeveld, 1990). However, the observed changes in quantity and function of SR proteins do not necessarily reflect a 1:1 change in the total functionality of SR $Ca^{2+}$ handling. Intriguingly, SR vesicles can be isolated from crude muscle homogenate, enabling the determination of rate of SR $Ca^{2+}$ release and uptake using fluorometric techniques (Ruell et al., 1995). In principle, this method can be used to study how alterations in metabolites affect SR $Ca^{2+}$ handling or, by using standard conditions, how SR $Ca^{2+}$ handling properties has been changed by the fatigue development or by a period of exercise training, assuming that the change is unaffected by isolation (Ruell et al., 1995). Accordingly, this technique can provide a 1:1 measure of SR function, and can be used to diteckt alterations in the rate of SR vesicle $Ca^{2+}$ release and uptake following a exercise training. However, investigations focusing on isolated cellular components can be highly productive in elucidating mechanisms but often lack the capacity to illustrate physiological significance.

## 1.6. Knowledge gap and research aims

To date, only a few studies have previously investigated the effect of a period with exercise training on SR $Ca^{2+}$ handling properties. Wherein, a period of resistance training has been demonstrated to enhance SERCA activity and SR vesicle $Ca^{2+}$ uptake in elderly women but not in young women (S. K. Hunter et al., 1999). Yet, resistance training has been associated with enhanced SERCA activity in young untrained males (Green et al., 1998). Moreover, resistance training has been shown to improve SR vesicle $Ca^{2+}$ release and uptake in young, trained men, albeit without alterations in SERCA,

RYR1, and DHPR contents (Jessen et al., 2021). Conversely, sprint training has been found to decrease SERCA activity while enhancing SR vesicle $Ca^{2+}$ uptake in sedentary adults (Harmer et al., 2014). Additionally, sprint training in moderately trained men has led to increased SR vesicle $Ca^{2+}$ release and SERCA and RYR1 contents, with no alterations observed in SR vesicle $Ca^{2+}$ uptake and SERCA activity (Ørtenblad et al., 2000). Collectively, both resistance and high-intensity sprint training appear to serve as robust stimuli for quantitative improvements in SR $Ca^{2+}$ handling properties.

However, previous research into the effects of endurance training on SR $Ca^{2+}$ handling have yielded somewhat inconsistent findings. For instance, in rats, aerobic exercise results in reduced SERCA content and activity (Green et al., 1984; Kim et al., 1981), due to a fast-to-slow fiber transition. Conversely, studies in trained men have shown no alterations in SERCA content and fiber type distribution following six weeks of high-intensity endurance training (Madsen et al., 1994). However, ten weeks of prolonged submaximal endurance training has been associated with reductions in SERCA1 content, while SERCA2 content remained unchanged, alongside reductions in SERCA activity and SR vesicle Ca^2+ release and uptake (Green et al., 2003). Additionally, four weeks of high-intensity endurance training have been shown to increase SR vesicle $Ca^{2+}$ release, but SR $Ca^{2+}$ vesicle uptake remained unchanged in highly trained triathletes and cyclist (Gejl et al., 2020).

This discrepancy in SR $Ca^{2+}$ handling responses following endurance training between training regimes (moderate vs high-intensity) and participant characteristics (old vs young and trained vs untrained) is poorly understood. And, whether SR $Ca^{2+}$ release and uptake rates are affected by high-intensity endurance training in untrained adults remains to be elucidated. Furthermore, the implications of training-induced alterations in SR $Ca^{2+}$ handling for improved muscular performance and general health has not been systematically delineated. In addition, to the best of my knowledge, only one study have previously investigated the interaction of sex on SR $Ca^{2+}$ handling properties, and found that total SERCA activity was lower in the females compared to the males, but that SR vesicle $Ca^{2+}$ uptake did not differ between sexes (Harmer et al., 2014). This sex difference in SERCA activity were hypothesized to be due to discrepancy in fiber type distribution (Harmer et al., 2014). However, it remains unclear if differences in SR $Ca^{2+}$ release exist between sexes, and further investigation is needed to ascertain whether potential variations in SR $Ca^{2+}$ release and uptake are attributable to differences in fiber type distribution among sexes.

Therefore, the present study was undertaken to further elucidate the effect of endurance training on SR $Ca^{2+}$ handling properties and subsequent implications for muscular function and performance, as well as sex interactions on SR $Ca^{2+}$ handling properties and fiber type distribution at baseline and in response to training. The primary aims of this study was to (1) investigate the effects of an eight-week endurance training intervention in untrained adult individuals on SR $Ca^{2+}$ release and uptake rates, and (2), examine how the possible changes in SR $Ca^{2+}$ release and uptake rates corresponds with changes in cycling performance and durability. Secondarily, the study aimed to (1) investigate possible sex differences in SR $Ca^{2+}$ release and uptake and MHC distribution at baseline and in response to the training intervention, and (2), investigate the importance of average percentage of $VO_{2max}$ during interval-sessions on training responses in SR $Ca^{2+}$ handling properties.

# 2. Introduction

The contractile function of skeletal muscle fibers relies on a complex interplay of intrinsic mechanical, electrochemical, and metabolic properties and processes, denoted as the excitation-contraction (E-C) and relaxation coupling (Sandow, 1952). Briefly, depolarization of the muscle fiber triggers the opening of calcium ion ($Ca^{2+}$) release channels (ryanodine receptor, (RyR1)) on the sarcoplasmic reticulum (SR), leading to a significant increase in the concentration of free $Ca^{2+}$ in the cytosol of muscle fibers ($[Ca^{2+}]_i$), typically by 10-20 fold (Bruton et al., 2003; Ingalls et al., 1999). This elevated $[Ca^{2+}]_i$ subsequently initiates the cross-bridge cycle, inducing muscle fiber contractions (Geeves et al., 2005). Simultaneously, $Ca^{2+}$ are continuously re-sequestered back into the SR by another SR membrane protein, the sarcoplasmic/endoplasmic reticulum $Ca^{2+}$ ATPase (SERCA), causing muscle fiber relaxation when $[Ca^{2+}]_i$ declines.

Through repetitive muscle contraction work, these SR $Ca^{2+}$ handling properties will be disturbed (Baker et al., 1993; Bigland-Ritchie et al., 1986; Byrd et al., 1989; Favero et al., 1993; Hostrup et al., 2014; Li et al., 2002; Ørtenblad et al., 2011). As a consequence, this will result in a progressive loss of force generating capacity, referred to as muscular fatigue (Hill, 1925; Mosso et al., 1904). The underlying fatiguing mechanisms in SR $Ca^{2+}$ handling includes primarily a decline in the rate of SR $Ca^{2+}$ release, along with, in certain cases, diminished $Ca^{2+}$ re-sequestering rates (Allen et al., 2008). Higher levels of musculoskeletal fitness, which includes the ability of skeletal muscles to sustain repeated contractions against a external load for an extended period of time (Kell et al., 2001), are associated with both numerous health benefits, e.g., reduced coronary risk factors (Durstine et al., 2001; Kelly et al., 1998; Kraus et al., 2002), improved glucose tolerance (Pan et al., 1997; Ramachandran et al., 2006), and greater success in completion of activities of daily living (G. R. Hunter et al., 2004), as well as

superior endurance performance (Maunder et al., 2021). In addition, muscle fatigue is a very common side effect, and a nuisance for disease patients, in chronic obstructive pulmonary disease (Breslin et al., 1998), sarcopenia (Patino-Hernandez et al., 2017), cancer (Morrow et al., 2002; Ryan et al., 2007), obesity (Resnick et al., 2006; Vgontzas et al., 2000), and chronic fatigue syndrome (Kent-Braun et al., 1993) Yet, there is a notable gap in understanding the health and performance benefits associated with training-induced improvements in SR $Ca^{2+}$ handling properties.

Over time, increasing the contractile demands placed on skeletal muscles during physical exercise will typically result in adaptations that improve functionality and performance towards the characteristics of the exercising stimulus (Bogdanis, 2012). Accordingly, a period of endurance training will improve the ability of skeletal muscles to sustain a given work-load for a longer period of time, through adaptations facilitating resilience to muscular fatigue. A salient response to endurance training aimed at enhancing resilience to muscular fatigue is an increase in the physiological capacity of ATP production, e.g. increased $O_2$ delivery to exercising muscles through expanded blood volume, an increased stroke volume, and greater quantity of hemoglobin, (Lundby et al., 2017; Lundby & Montero, 2019), as well as peripheral adaptations, such as increased mitochondrial content and improved mitochondrial functions (Granata et al., 2018), increased capillarization surrounding the muscle fibers (Hoier & Hellsten, 2014), and improved muscle glycogen availability (Gejl et al., 2017). However, much less is known about the possible adaptations at the level of the E-C and relaxation coupling, and herein the SR $Ca^{2+}$ handling properties *per se*. The principal of symmorphosis posits that all components in a biological system, such as for muscular fatigue, is quantitatively coupled to functional demands (Taylor & Weibel, 1981; Weibel et al., 1991). Thus, given the known link between impaired SR $Ca^{2+}$ handling and muscular fatigue, it might be anticipated that endurance training would reduce the SR impairment following a given training-load through adaptations in the SR $Ca^{2+}$ handling properties.

To date, previous studies investigating the effect of endurance exercise training on SR $Ca^{2+}$ handling properties have shown rather inconsistent results. For instance, studies investigating content and function of SR proteins, have shown that aerobic exercise results in reduced SERCA content and activity in rats (Green et al., 1984; Kim et al., 1981). In humans, SERCA content have been shown to remaine unaltered following high-intensity endurance training (Madsen et al., 1994), however, moderate-intensity endurance training has been associated with reductions in SERCA1 content and total SERCA activity,

while SERCA2 content remained unchanged (Green et al., 2003).

Intriguingly, SR vesicles can be isolated from crude muscle homogenate, and the functional capacity of SR $Ca^{2+}$ uptake and release can be measured directly (Ruell et al., 1995). Using this technique, sprint training in sedentary and moderately trained adults, have indicated no significant alteration in SR $Ca^{2+}$ uptake (Harmer et al., 2014; Ørtenblad et al., 2000), whereas SR $Ca^{2+}$ release has been observed to increase by 9% (Ørtenblad et al., 2000). Two studies has previously investigated the effect of endurance training on SR $Ca^{2+}$ release and uptake. Whereby, moderate-intensity endurance training have been shown to reduce SR Ca^2+ release and uptake in untrained men by 26% and 18%, respectively (Green et al., 2003). Conversely, high-intensity endurance training have been shown to increase SR $Ca^{2+}$ release by 10%, while SR $Ca^{2+}$ uptake remained unchanged in highly trained endurance athletes (Gejl et al., 2020). This discrepancy in SR $Ca^{2+}$ handling responses following endurance training between training regimes (moderate vs high-intensity) and participant characteristics (trained vs untrained) is poorly understood. And, whether SR $Ca^{2+}$ release and uptake rates are affected by high-intensity endurance training in untrained adults remains to be elucidated. In addition, to the best of my knowledge, only one study have previously investigated the interaction of sex on SR $Ca^{2+}$ handling properties, and found that total SERCA activity was lower in females compared to males, but that SR vesicle $Ca^{2+}$ uptake did not differ between sexes (Harmer et al., 2014). This sex difference in SERCA activity were hypothesized to be due to discrepancy in fiber type distribution (Harmer et al., 2014). However, it remains unclear if differences in SR $Ca^{2+}$ release exist between sexes, and further investigation is needed to ascertain whether potential variations in SR $Ca^{2+}$ release and uptake are attributable to differences in fiber type distribution among sexes.

Therefore, the primary aims of this study was to (1) investigate the effects of an eight-week endurance training intervention in untrained adult individuals on SR $Ca^{2+}$ release and uptake rates, and (2), examine how the possible changes in SR $Ca^{2+}$ release and uptake rates corresponds with changes in cycling performance and durability. Secondarily, the study aimed to (1) investigate possible sex differences in SR $Ca^{2+}$ release and uptake and myosin heavy chain (MHC) distribution at baseline and in response to the training intervention, and (2), investigate the importance of average percentage of maximal oxygen consumption ($VO_{2max}$) during interval-sessions on training responses in SR $Ca^{2+}$ handling properties.

# 3. Methods

## 3.1. Study design

The current thesis was part of a larger research project (The Repeat Study, Mølmen et al., 2024, pre-registered (OSF)). The study was approved by the Regional Committee for Medical and Health Research Ethics - Southeast Norway (aod. 2023-6-9, reference number, 591218) and the Norwegian Agency for Shared Services in Education and Research (data processing and storage, reference number, 499593). In the study, sedentary adults conducted two identical eight-week endurance training periods ("period 1" and "period 2"), separated by eight weeks of no training. An extensive test-battery was conducted before and after each training period to evaluate changes in physiological, hematological, and performance-related measures. The current study will solely focus on training period 1 and methods relevant to investigate the defined research questions.

The participants performed 24 interval sessions of indoor cycling training over a period of eight weeks. The endurance training consisted of three different interval sessions (Figure 1B), with the number of interval sessions per week varying between 2-4 sessions in a cyclic manner (Figure 1A). During the first week of training, the power output of the interval sessions corresponded to a percentage of the individual participant's mean power output during a 15 minute cycling trial ($W_{15min}$), but was replaced and repeatedly updated by the individual mean power output during the 4x5 minute all-out effort session ($W_{4x5min}$) throughout the eight weeks of training (Figure 1A,D). Oxygen consumption ($VO_2$) was measured during one of the interval sessions in the third and the sixth week of the training intervention (Figure 1A). Resting muscle micro-biopsies were sampled ∼one week before the training intervention and >48 hours after the training intervention (Figure 1A). Endurance testing, consisting of

a incremental cycling test to determine maximal oxygen consumption ($VO_{2max}$) and maximal 1-minute aerobic power output ($W_{max}$) and a 15-minute cycling trial, was conducted once ~two weeks prior to the training intervention to get familiarized with the laboratory equipment and the test procedure (Edgett et al., 2018), as well as twice over two consecutive days approaching the training intervention and once after the training intervention (Figure 1A).

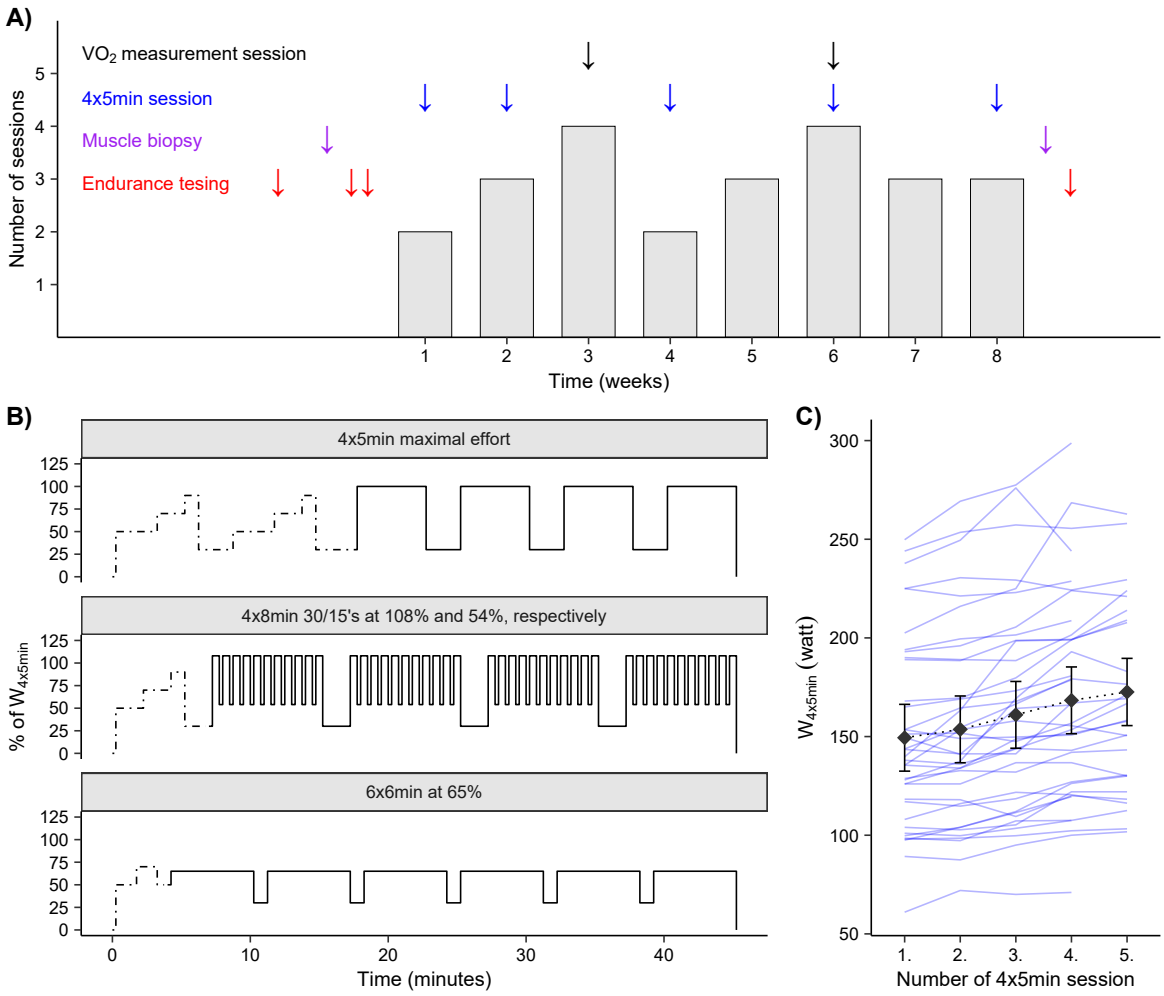



**Figure 1**. A) Study overview showing number of sessions per week together with time points for muscle micro-biopsy sampling, endurance testing, 4x5min all-out sessions and training sessions with $VO_2$ measurement. B) Overview and design of training sessions, showing warmup (dot-dashed lines) and training (solid black lines). C) Individual (blue lines) and average group (black point and 95% CI error lines) $W_{4x5min}$ during the 8-week training period. Abbreviations: $W_{4x5min}$, average power output

from the 4x5min session; $VO_2$, oxygen consumption.

## 3.2. Participants

Initially, 58 volunteers were recruited to the study. Eligible participants were healthy adults between 30 and 65 years of age with a training history of less than one endurance training session per week during the last 12 months leading up to the study. Exclusion criteria were symptoms or history of disease, injuries affecting their ability to cycle with high intensity, and known adverse reactions to local anesthetics. Of the recruited participants, 51 completed the training intervention along with physiological testing. However, 14 were excluded from the results due to missing data in $\Delta$ $Ca^{2+}$ release and uptake rates, leaving a total of $n$ = 37 participants (females: $n$ = 16, males: $n$ = 21; see Table 1 for participant characteristics). Prior to inclusion, participants were informed of any potential risks and discomfort associated with participation in the study, and they all gave their written informed consent prior to data collection (Appendix A). The study was conducted according to the Declaration of Helsinki of 1975.

**Table 1**. Participant characteristics

| | Female ($n$ = 16) | Male ($n$ = 21) |
|---|---|---|
| Age (years) | 54.0 ± 8.9 | 49.2 ± 8.5 |
| Body height (cm) | 167.6 ± 6.5 | 180.9 ± 7.9 |
| Body mass (kg) | 72.1 ± 15.8 | 96.8 ± 19.4 |
| Body mass index ($kg \cdot m^2$) | 25.7 ± 5.6 | 29.5 ± 5.1 |
| $VO_{2max}$ ($mL \cdot min^{-1}$) | 2217 ± 453 | 3513 ± 618 |
| $VO_{2max}$ ($mL \cdot kg^{-1} / min^{-1}$) | 31.5 ± 5.9 | 36.8 ± 4.7 |
| $W_{max}$ (watt) | 179 ± 37 | 273 ± 51 |
| $W_{15min}$ (watt) | 111 ± 26 | 169 ± 41 |

Abbreviations: $VO_{2max}$, maximal oxygen uptake from the highest 30 second time interval during the incremental cycling test; $W_{max}$, maximal aerobic power measured as the average power output from the last minute of the incremental cycling test; $W_{15min}$, average power output during the 15 minute cycling trial

## 3.3. Endurance training

The interval training sessions were performed in training facilities at Inland Norway University of Applied Sciences, campus Lillehammer, on a stationary cycling trainer device (Tacx NEO T8000 Bike Smart, Wassenaar, the Netherlands). The seat height, handlebar position, and horizontal distance between of the seat and bottom bracket on the stationary cycling trainer were adjusted according to each participant's preference at the first session and replicated during subsequent sessions. All interval sessions were completed as supervised group sessions (2-7 participants). The power output during the interval sessions corresponded to a percentage of the individual $W_{4x5min}$, which was controlled by pre-programmed workouts using an app (Tacx Training-app, version 4.52.1, Garmin Ltd., the Netherlands) connected to the trainer device. The interval sessions consisted of three different 45 minutes sessions, i.e. a 4x5min session with maximal effort, a 4x8min session with interchanging 30's and 15's work

efforts at 108% and 54% of $W_{4x5min}$ respectively, and a 6x6min session with sustained effort at 65% of $W_{4x5min}$ (see Figure 1B).

The 4x5min session began with a warm up protocol consisting of two identical incremental intervals of three minutes at 50% of $W_{4x5min}$, two minutes at 70% and one minute at 90% with inter-interval active recovery periods of three minutes at 30%. For the 4x5min sessions, the power output during the five minute intervals were set to 100% of the previous $W_{4x5min}$. However, the participants received instructions to adjust the power output "freely" to achieve the highest possible mean power output from all four interval bouts, and they received strong verbal encouragement throughout the session. Inter-interval active recovery periods were set to 2.5 minutes at 30%. Power output during the interval bouts were recorded using a cycling computer (Garmin Edge 530 or Garmin Edge 1040, Garmin Ltd., Olathe, Kansas, USA) for calculation of $W_{4x5min}$.

The 4x8min session began with a warm up protocol consisting of two minutes at 50% of $W_{4x5min}$, two minutes at 70%, one minute at 90% and two minutes at 30%. The eight minute intervals were set to average 90% with interchanging 30's and 15's work efforts at 108% and 54% respectively, with inter-interval active rest periods of two minutes at 30%. Oxygen consumption ($VO_2$) was measured in two of the 24 interval sessions (both being a 4x8min session) with sampling time every $10^{th}$ s using a metabolic system with mixing chamber (Vyntus CPX, Erich Jaeger, Hoechberg, Germany) during the working intervals ($VO_2$ measurement session; Figure 1A). The metabolic system was calibrated before each of the two $VO_2$ measurement sessions. Briefly, using certified calibration gasses of known concentrations to calibrate the internal gas analyzers and an internal pump to calibrate airflow through a digital volume transducer (DVT, Erich Jaeger). During the $VO_2$ measurement sessions, blood samples from the fingertip were obtained immediately after each work-interval for determination of blood lactate concentration ([La-]; Biosen C-line Lactate analyzer, EKF Diagnostic GmbH, Barleben, Germany).

The 6x6min session began with a warmup-protocol consisting of 1.5 minutes at 50% of $W_{4x5min}$, 1.5 minutes at 70% and one minute at 50%. The six minutes intervals were set to sustain 65% with inter-interval active recovery periods of one minute at 30%.

Rate of perceived exhaustion (RPE) was recorded immediately after each work-interval during all

interval-sessions using Borg's 6-20 scale (Borg, 1982). If the power output was perceived as "too easy" or "too hard" (4x8min: RPE < 15 after 1st interval, or RPE < 16 after 2nd interval, or RPE > 19, 6x6min: RPE < 11 after 1st interval, or RPE < 12 after 2nd interval, or RPE > 15), the power output was adjusted up or down by 5 %-points in regard to % of $W_{4x5min}$, respectively. Heart rate (HR) and power output was recorded continuously throughout all interval-sessions by the Tacx NEO T8000 Bike Smart and Taxc Training-app. Data from the interval sessions are presented in Table 2.

**Table 2**. Interval session data

| | 4x5min | 4x8min | 6x6min |
|---|---|---|---|
| Average HR (beats · $min^{-1}$) | 136 ± 13 | 141 ± 14 | 130 ± 13 |
| Maximal HR (beats · $min^{-1}$) | 168 ± 17 | 164 ± 14 | 150 ± 16 |
| Average power output (watt) | 116 ± 34 | 128 ± 39 | 104 ± 31 |
| Average RPE (6-20) | 17.1 ± 1.2 | 16.2 ± 1.1 | 13.1 ± 1.1 |
| Average [La-] (mmol · $L^{-1}$) | NA | 6.8 ± 1.9 | NA |
| Average $VO_2$ (% of $VO_{2max}$) | NA | 81.2 ± 7.6 | NA |

Abbreviations: HR, heart rate; RPE, rate of perceived exhaustion (BORG scale); [La-], blood lactate concentrations; $VO_2$, oxygen consumption

## 3.4. Endurance testing

### 3.4.1. Testing procedures

Endurance testing was conducted at the physiological test laboratory at Inland Norway University of Applied Sciences, campus Lillehammer. The participants were instructed to refrain from any exercise training during the two days prior to the endurance testing, and replicate their nutrient intake in the day leading up to the test day (self-reported at familiarization testing; Figure 1A). The individual participant had the same test leader at all time points, and strong verbal encouragement during the maximal tests were given to ensure maximal effort. All endurance testing was performed on an electromagnetically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), and metabolic responses

were measured with a metabolic system with mixing chamber, Vyntus CPX (previously described; Endurance training protocol 3.3). The seat height, handlebar position, and horizontal distance between the seat and bottom bracket on the electromagnetically-braked cycle ergometer was adjusted according to each participant's preference at the familiarization test and replicated during subsequent tests. All cycling tests were performed in a seated cycling position. Endurance testing was initiated by a standardized warm up protocol, consisting of two 5.5-minutes submaximal cycling bouts at 25% and 40% of $W_{max}$ from the previous endurance test timepoint. At familiarization testing, power output of these warm up bouts were set to pre-defined values of 60 and 100 W for males and 40 and 60 W for females, respectively.

### 3.4.2. Maximal oxygen consumption and maximal aerobic power output

$VO_{2max}$ and $W_{max}$ were determined by an incremental cycling test performed to exhaustion. The test started at the 20 W increment closest to the individual participant's 40% of $W_{max}$ and increased by 20 W until exhaustion, defined as the cadence dropping < 60 revolutions per minute. $VO_2$ was measured every $5^{th}$ s, and $VO_{2max}$ was defined as the highest 30 s average during the test (i.e., highest rolling average of six consecutive 5 s measures). $W_{max}$ was defined as the average power output during the last minute of the incremental test. The participant's adjusted the cadence freely during the entirety of the test.

### 3.4.3. 15-minute cycling trial

The participants were given 10 minutes of active recovery cycling after the incremental test before starting the 15-minute cycling trial. The test started at 60% of $W_{max}$, however, the participants adjusted the power output freely during the test with an external control unit placed by the handlebar, with the aim of achieving the highest average power output throughout the test. $W_{15min}$ was defined as the average power output during the entire 15-minute cycling trial. Based on the ratio in performance from the incremental test to the 15-minute cycling trial, was a durability index calculated as the percentage of $W_{15min}$ in relation to the $W_{max}$.

## 3.5. Muscel biopsy sampling

Muscle specimens (10-25 mg per sample) were obtained approximately 15 cm above the knee joint of the *m. vastus lateralis* muscle under local anesthesia of the skin, subcutaneous tissue, and muscle fascia (Xylocain 10 mg $mL^{-1}$, AstraZeneca, Aspen Pharma Trading Limited, Dublin, Ireland). This muscle was preferred because it is highly active during cycle exercise (Henriksson & Bonde-Petersen, 1974). The micro-biopsy technique was used, in which, a disposable 12 gauge needle (Universal Plus, Mermaid medical A/S, Stenløse, Denmark) operated with a spring-loaded biopsy gun (Bard Magnum, Bard Norway A/S, Oslo, Norway) was passed into the muscle two to four times to collect enough material. The material was blotted on filter paper, dissected free from visible fat and connective tissue and homogenized in ice-cold homogenizing buffer (300 mM sucrose, 1 mM ethylene diamine-tetra-acetic acid (EDTA), 10 mM $NaN_3$, 40 mM Tris-base, 40 mM l-histidine, pH 7.8) (O'Brien, 1990). Homogenization was done in $\sim$ 30 s, without under/over pressure, using a Potter-Elvehjem homogenizer (Fischerbrand, Thermo Fisher Scientific, Waltham, Massachusetts). The muscle tissue was kept ice cold during the whole procedure. The obtained homogenate was immediately frozen and stored at −80°C until further analysis.

### 3.5.1. SR vesicle $Ca^{2+}$ release and uptake rates in muscle homogenate

The obtained muscle homogenate was transferred to- and analysed for SR vesical $Ca^{2+}$ release and uptake rates at the scientific laboratory of the University of Southern Denmark, Odense. SR $Ca^{2+}$ vesical uptake and release rates were analyzed *in vitro* using the $Ca^{2+}$-binding fluorescent dye "indo-1" on a fluorometer (20 Hz, Ratiomaster RCM, Photon Technology International, Brunswick, NJ, USA). The excitation wavelength was 355 nm, and the emission wavelength was continuously measured at 400nm (emission peak of $Ca^{2+}$-saturated indo-1) and 470 nm (emission peak of $Ca^{2+}$-free indo-1). The ratiometric data were collected every $0.5^{th}$ s in a thermostated cuvette holder at 37°C with continuous stirring by a magnetic bar. The reaction was initiated by adding 30 $\mu$ of muscle homogenate to a 2 ml assay buffer consisting of 165 mM KCl, 22 mM HEPES, 5.5 $\mu$M*N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), 7.5 mM oxalate, 11 mM $NaN_3$, 20 $\mu$M $CaCl_2$, 2 mM $MgCl_2$,

and indo-1 to a final concentration of 1 $\mu$M (pH 7.0). TPEN was added to prevent perturbation of heavy metal ions in the indo-1 measurements without disturbing free $Ca^{2+}$ concentrations ($[Ca^{2+}]_{free}$). Oxalate facilitated the SR $Ca^{2+}$-accumulation, and the addition of $NaN_3$ blocked mitochondrial $Ca^{2+}$ sequestering activity. $[Ca^{2+}]_{free}$ was derived from the subsequent ratiometric data according to the equation: $[Ca^{2+}]_{free} = K_d \times \frac{R-R_{min}}{R_{max}-R} \times (S_{f2}/S_{b2})$ (Grynkiewicz et al., 1985), where $K_d$ is the dissociation constant of indo-1 and $Ca^{2+}$ (measured with a $Ca^{2+}$ calibration buffer kit (Molecular Probes, Eugene, OR)), R is the ratio value between emission wavelengths, $R_{max}$ is the limiting ratio value between emission wavelengths when all the indo-1 is saturated with $Ca^{2+}$, $R_{min}$ is the limiting ratio value between emission wavelengths when all the indo-1 is in the $Ca^{2+}$ free form, and the factor $S_{f2}/S_{b2}$ is the fluorescence intensity measured at 470 nm when all the indo-1 is free from or saturated with $Ca^{2+}$, respectively. $R_{max}$ and $R_{min}$ was determined in each test to calibrate the fluorescence signal.

$[Ca^{2+}]_{free}$ in the buffer was above 1000 nM, but decreased to $\sim$ 800 nM immediately after the muscle homogenate was injected, because of EDTA and protein binding of $Ca^{2+}$ (Ruell et al., 1995). Thereafter, SR $Ca^{2+}$ uptake was initiated by adding 2 mM ATP to a final concentration of 5 mM and the ATP-driven oxalate-supported SR $Ca^{2+}$ uptake was followed until the $[Ca^{2+}]$ plateaued, $\sim$ 300 s. Then, SR $Ca^{2+}$ uptake was blocked by addition of cyclopiazonic acid (CPA), and subsequent inactivation of SERCA, to the assay buffer. The CPA was incubated in the assay buffer for 30 s before SR $Ca^{2+}$ release was initiated by the addition of 5 mM 4-chloro-M-Cresol (4-CmC) to the assay buffer, which followed for at least 30 s (Figure 2). Subsequent raw-data of $[Ca^{2+}]_{free}$ over time was imported into Matlab version 7.0.1 (The MathWorks, Natick, MA, USA) and the resulting curve was smoothed over 15 points using mono-exponential equations based on Savitsky-Golay algorithm (Curve Fitting Toolbox version 1.1.1; The MathWorks).

The SR $Ca^{2+}$ uptake rate ($uptake_{tau}$) was defined as the time (s) for $[Ca^{2+}]_{free}$ to decrease by 63% (using data-points between 700 nM $[Ca^{2+}]_{free}$ and $[Ca^{2+}]_{free}$ upon CPA addition), and calculated as 1/b using the following equation: $[Ca^{2+}]_{free} = ae - b\tau + c$. In addition, SR $Ca^{2+}$ uptake rates were determined at 600 and 200 nM $[Ca^{2+}]_{free}$ from the derivative of the curve ($uptake_{600nM}$ and $uptake_{200nM}$, respectively). This roughly translates to the physiological levels of $[Ca^{2+}]_i$ during tetanic contractions and in resting muscle fibers, respectively (Bruton et al., 2003; Ingalls et al., 1999). The SR $Ca^{2+}$ leak rate was estimated during the period between addition of CPA into the buffer assay and initiation of

$Ca^{2+}$ release, and defined as the derivative of the curve. SR $Ca^{2+}$ release rate was calculated during the first 30 s after 4-CmC stimulated $Ca^{2+}$ release and was defined as the derivative of the initial release. Values obtained for SR $Ca^{2+}$ uptake and release rates are relative and expressed as arbitrary units; $\mu$mol · g protein$^{-1}$ · min$^{-1}$. Uptake$_{tau}$ is expressed as s$^{-1}$ · mg protein$^{-1}$, i.e. higher value for a faster reuptake. Changes from baseline to after training in SR vesicle $Ca^{2+}$ release and uptake is expressed as percentage changes using the following equation; $change = exp(log(change) - 1) \times 100$, because of the large inter-individual variation in SR vesicle $Ca^{2+}$ release and uptake rates. The whole procedure was performed in duplicate. Protein content in the muscle homogenate was also measured in duplicate using a standard kit (Pierce BCA protein reagent no. 23225).

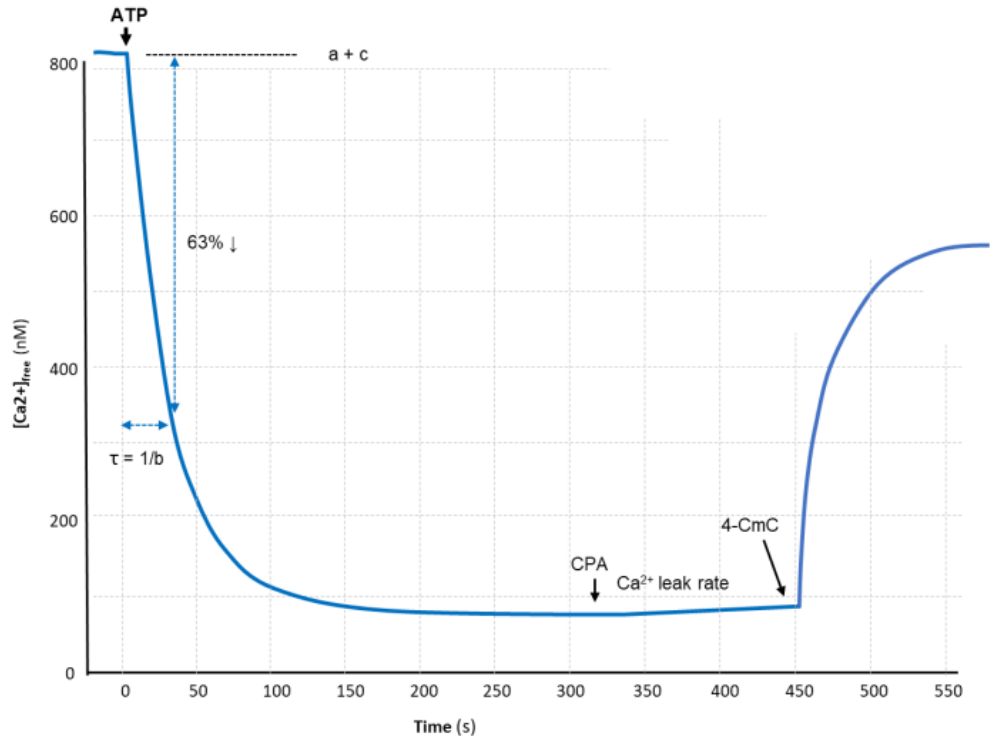



**Figure 2**. Representative example of measurements of parameters associated with SR vesicle $Ca^{2+}$ release and uptake rates. The $[Ca^{2+}]_{free}$ was determined fluorometrically and the SR vesicle $Ca^{2+}$ uptake was initiated by adding ATP (5mM). After approximately 300 s, CPA was added in order to block SR vesicle uptake and to estimate SR $Ca^{2+}$ leak rate. SR $Ca^{2+}$ release was initiated by the addition of 5 mM 4-CmC to the assay buffer, which followed for at least 30 s. The time (τ) for $[Ca^{2+}]_{free}$ to decrease by 63% during ATP-driven SR vesicle $Ca^{2+}$ uptake was calculated as 1/b from the equation; $[Ca^{2+}]_{free} = ae\text{-}bt+c$. SR $Ca^{2+}$ release rate was obtained by mathematically fitting the data points during the first 30 s of release and then determine the rate of $Ca^{2+}$ release as the derivative of the initial release. The graph is from the scientific laboratory of the University of Southern Denmark, Odense and used with permition from Niels Ørtenblad (Professor, Head of Research Unit). Abbreviations: SR, sarcoplasmic reticulum; $Ca^{2+}$, calcium; $[Ca^{2+}]_{free}$, concentration of free calcium; ATP, adenosine triphosphate; CPA, cyclopiazonic acid; 4-CmC, 4-chloro-M-Cresol.

### 3.5.2. MCH distribution

MHC analysis was performed on the same muscle homogenate as for the analysis of SR vesicle $Ca^{2+}$ release and uptake rates. 200 $\mu$l of lysine buffer containing 10% glycerol, 5% 2-mercaptoethanol and 2.3% sodium dodecyl, 62.5 mM Tris-base and 0.2% bromophenolblue (pH 6.8) was mixed with 80 $\mu$l muscle homogenate and boiled in water at 100°C for 3 minutes. 10–40 $\mu$l of the sample-buffer was loaded on to a sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gel with 6% polyacrylmide (100:1 acrylmid : bis-acrylmid), 30% glycerol, 0.4% sodium dodecyl, 67.5 mM Tris-base, and 0.1 M glycine. SDS-PAGE gels were run at 80 V for at least 42 hours at 4°C, followed by 2–4 hours of 200 V at 4°C. Subsequently, the gels were stained with Coomassi and MHC bands made visible. The gels were scanned (Lino-scan 1400 scanner, Heidelberg, Germany) and the relative proportions of MHC type I (MHC-I), MCH type IIA (MHC-IIA), and MCH type IIX (MHC-IIX) isoforms were determined densitometrically (Phoretix 1D, non-linear, Newcastle, United Kingdom). The whole procedure was performed in duplicate and the average of the two values was used to define relative proportions in MHC-I, MHC-II and MHC-IIx.

## 3.6. Statistics and data analysis

Descriptive data are presented as mean and standard deviation (mean $\pm$ SD), unless otherwise stated. All data analysis was performed in R (version 4.4.0). To assess the effect of endurance training on SR $Ca^{2+}$ release and uptake rates, linear mixed-effects models (LMMs) were specified with time as the main fixed effect and sex as a co-variate. Sex differences in SR $Ca^{2+}$ release and uptake rates at baseline as well as the effect of endurance training was also assessed using LMMs specified with time and time to sex interaction as the fixed effects. Relative interactions between sexes were estimated as females compared to males, and models with a robust sex or sex to time interaction, i.e. 95% confidence intervals (CI) not including 0, were refitted with the relative proportion of MHC-II as co-variate to asses potential effects of MHC distribution on the observed interactions. Log-transformed SR $Ca^{2+}$ release and uptake rates were specified as the dependent variable.

The general efficacy of the training intervention, i.e. $\Delta$ $VO_{2max}$, $\Delta$ $W_{max}$, $\Delta$ $W_{15min}$, $\Delta$ durability index

and $\Delta$ $W_{4x5min}$, were assessed using LMMs specified with time as the fixed effect. The potential effect of changes in SR $Ca^{2+}$ release and uptake rates on $\Delta$ $W_{15min}$, $\Delta$ $W_{max}$, $\Delta$ $VO_{2max}$ and $\Delta$ durability index was assessed using linear regression models (LMs) with absolute changes in $W_{15min}$, $W_{max}$, $VO_{2max}$ and durability index as the response variable and percentage changes in $Ca^{2+}$ release and uptake rates as the predictor variable. Baseline values and sex was defined as co-variates. LMs specified with $W_{15min}$, $W_{max}$, $VO_{2max}$, and durability index as the response variable and normalized SR $Ca^{2+}$ release and uptake rates ($value = x_i/max(x) \times 100$) as the predictor variable, and sex as co-variate was used to explore the relationships between baseline values in SR $Ca^{2+}$ release- and uptake rates and cycling performance, $VO_{2max}$ and durability index.

The general change in MHC distribution was assessed using LMMs specified with time and MHC isoform (i.e., MHC-I, MHC-IIa and MHC-IIx) as the fixed effect. Subsequently, the MHC data were pooled into relative distribution of MHC-II (MHC-IIa and MHC-IIx) vs MHC-I and a LMM specified with time as the fixed effect and sex as a co-variate was used to assess the effect of training on relative proportion of MHC-II. Potential sex differences in relative proportion of MHC-II at baseline and in change after training was assessed with a LMM specified with time and time to sex interaction as fixed effects. LMs specified with absolute baseline values in SR $Ca^{2+}$ release and uptake response and the relative proportion of MHC-II at baseline as the predictor variable, was used to asses potential effects of relative MHC-II distributions on SR $Ca^{2+}$ release and uptake.

The effect of potential changes in both the relative proportion of MHC-II and percent of $VO_{2max}$ during interval sessions on changes in SR $Ca^{2+}$ release and uptake rates was assessed using LMs specified with percentage change in $Ca^{2+}$ release and uptake rates from baseline as the response variable and absolute change in relative proportion of MHC-II and percent of $VO_{2max}$ during interval sessions as the predictor variables. Baseline values and sex was used as co-variates. In addition, potential relationships between baseline values in relative proportion of MHC-II and percent of $VO_{2max}$ during interval session was assessed using a LM specified with percent of $VO_{2max}$ during interval session as the response variable and relative proportion of MHC-II as the predictor variable.

All LMMs were fitted using the lme4-package written for R (Bates et al., 2015) and specified with random intercept for participants, and random intercepts for time at the level of participants to ac-

count for duplicate measures in SR $Ca^{2+}$ release and uptake and MHC distribution. Inference about effects of interest was drawn based on point estimates and their 95% CI (Calin-Jageman & Cumming, 2019). CI not containing null effects was interpreted as robust effects. The adjusted $R^2$ value ($R^2_a$) was calculated to analyze the proportion of the variance in the response variable that can be explained by the predictor variables in all respective LMs. All LMMs were assessed for uniformity of variance over the fitted range by visual inspection of residuals plotted over the fitted values and normal distribution of residuals were evaluated by inspecting a standardized normal probability plot. All LMs were visually inspected for normal distribution and homoscedasticity of residuals using plots of residuals and fitted values. Complete datasets and scripts can be downloaded here: (https://github.com/Rogneflaaten/repeatData)

# 4. Results

## 4.1. General efficacy of the training intervention

All participants sufficiently completed their prescribed endurance training, whereby 99.1 ± 2.6% of the training sessions were completed. $W_{4x5min}$ increased by 23.2 watt, 95% confidence interval (CI): [18.6, 27.8], from 149 ± 48 watt at the first 4x5 min session to 167 ± 47 watt at the last 4x 5min session (Figure 1C). Concomitantly, the 8-week training intervention led to robust increases in cycling performance, an improvement of 10.5% [9.3, 11.5] for $W_{max}$ (232 ± 65 to 257 ± 68 watt) and 10.0% [7.9, 11.7] for $W_{15min}$ (144 ± 45 to 159 ± 47 watt), as well as of 8.4% [7.3, 9.3] in $VO_{2max}$ (2953 ± 849 mL · $min^{-1}$ to 3201 ± 915 mL · $min^{-1}$), see Figure 3A-C and E). However, the durability index did not change following the training intervention (-0.0%-points [-1.2, 1.2]; baseline, 61.9 ± 4.5%-points; after training, 61.7 ± 3.8%-points; Figure 3D,E).

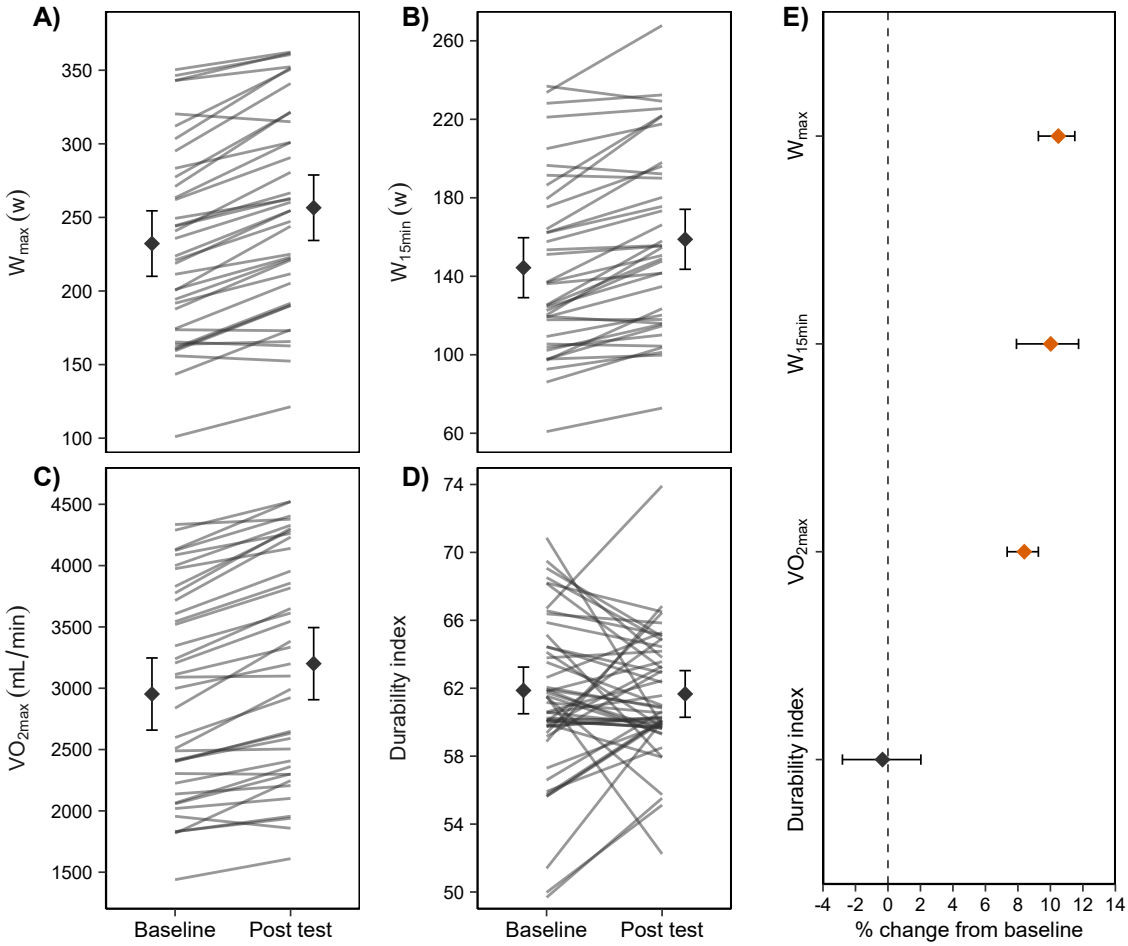



**Figure 3**. A,B,C,D displays baseline and post-test values for $W_{max}$, $W_{15min}$, $VO_{2max}$ and the durability index, respectively. Individual training responses in A, B, C, and D are shown as grey lines and group averages are shown as black points. E) group average percent changes in $VO_{2max}$, $W_{max}$, $W_{15min}$ and the durability index from pre- to post testing. Striped line in E indicates 0. Statistically robust results are presented as orange points (i.e., 95% CI not containing 0). Error lines indicates 95% CI. Abbreviations; $VO_{2max}$, maximal oxygen uptake from the highest 30 second time interval during the incremental cycling test; $W_{max}$,maximal aerobic power measured as the average power output from the last minute of the incremental cycling test; $W_{15min}$, average power output during the 15-minute cycling trial; durability index, the percentage of $W_{15min}$ in regard to $W_{max}$.

The 8-week endurance training intervention led to robust alterations in the MHC distribution, i.e., the relative distribution of MHC-I was reduced by -2.3% [-4.3, -0.4]; baseline, 51.8 ± 10.0; after training, 49.5 ± 8.7 (Figure 4A,D), and the relative distribution of MHC-IIa was increased by 3.2% [1.3, 5.0]; baseline, 35.3 ± 7.4; after training, 38.5 ± 6.9 (Figure 4B,D). However, the relative distribution of MHC-IIx did not change (-0.8% [-2.4, 0.7]; baseline, 12.9 ± 6.8; after training, 12.0 ± 4.8; Figure 4C,D).

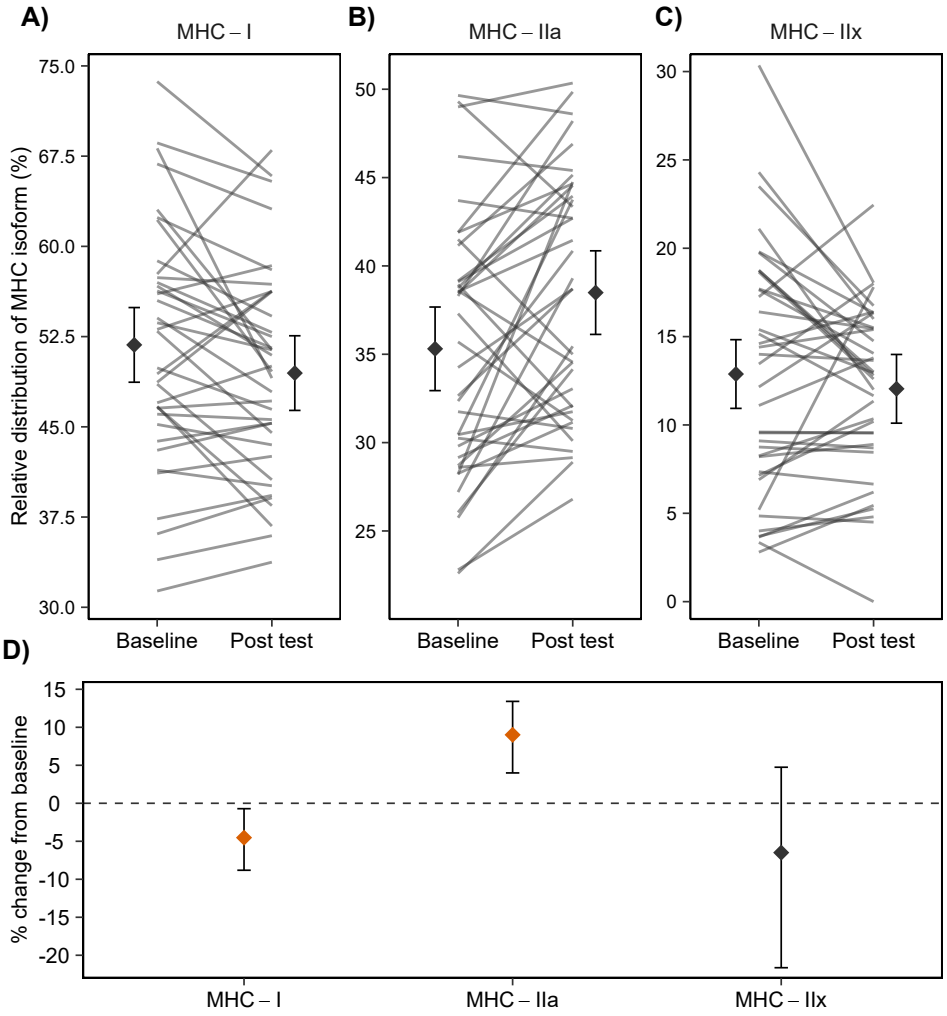
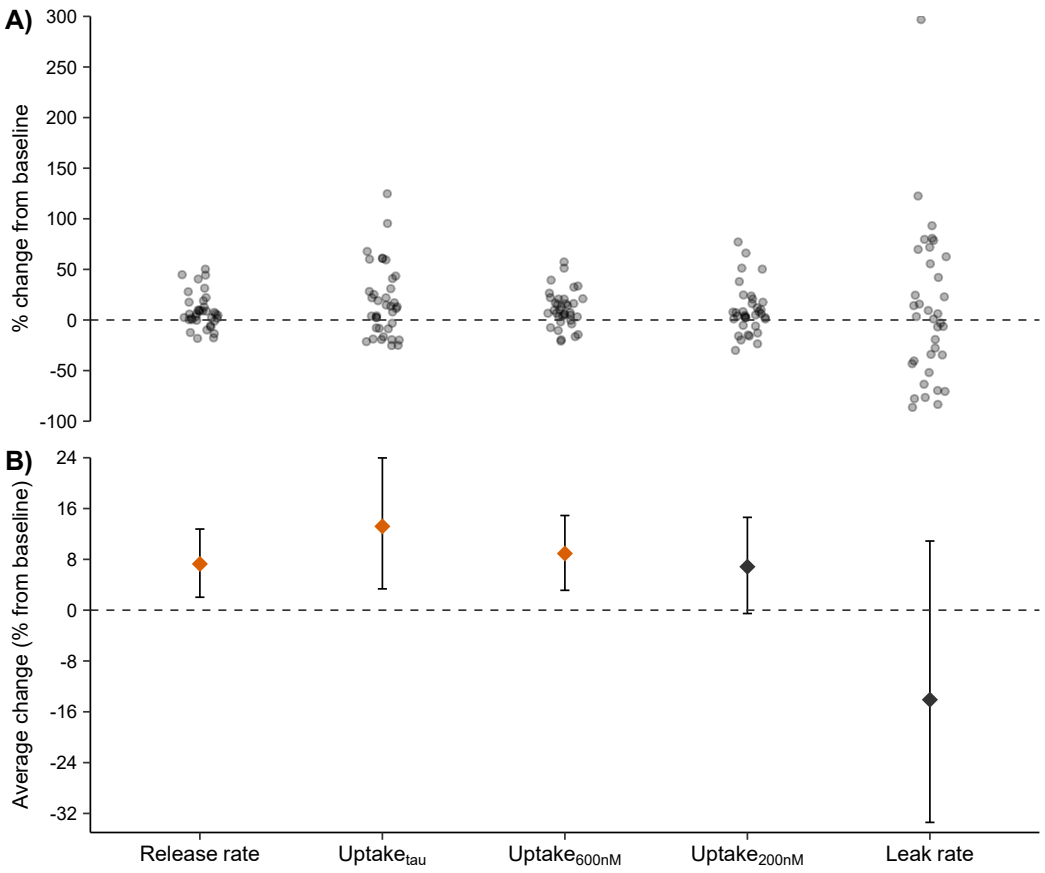



**Figure 4**. A,B,C displays the relative distribution of MHC-I, MHC-IIa and MHC-IIx at baseline and post-test, respectively. Individual MHC distribution in A, B, C, and D are shown as grey lines and group averages are shown as black points. D) group average percent changes in MHC-I, MHC-IIa and

MHC-IIx from baseline to post testing. Striped line in D indicates 0. Statistically robust results are presented as orange points (i.e., 95% CI not containing 0). Error lines indicates 95% CI. Abbreviations; MHC, myosin heavy chain.

## 4.2. Effects of endurance training on SR $Ca^{2+}$ release and uptake

The 8-week endurance training intervention led to a robust increase in SR $Ca^{2+}$ release of 7.3% [2.0, 12.8]; baseline, 0.91 ± 0.18; after training, 0.97 ± 0.18 $\mu$mol · g $protein^{-1}$ · $min^{-1}$ (Figure 5A,B). In addition, endurance training led to a robust increased in $Uptake_{tau}$ of 13.2% [3.4, 24.0]; baseline, 1.70 ± 0.40; after training, 2.00 ± 0.76 $s^{-1}$ · mg $protein^{-1}$ (Figure 5A,B), as well as a robust increase in $Uptake_{600nM}$ of 8.9% [3.1, 14.9]; baseline, 1.71 ± 0.44; after training, 1.84 ± 0.47 $\mu$mol · g $protein^{-1}$ · $min^{-1}$ (Figure 5A,B). However, the endurance training intervention did not alter $uptake_{200nM}$ (6.8% [-0.5, 14.6]; baseline, 0.50 ± 0.16; after training, 0.51 ± 0.15 $\mu$mol · g $protein^{-1}$ · $min^{-1}$; Figure 5A,B), or SR $Ca^{2+}$ leak rate (-14.1% [-33.4, 10.9]; baseline, 28.6 ± 24.6; after training, 21.1 ± 12.5 nmol · g $protein^{-1}$ · $min^{-1}$; Figure 5A,B).




**Fgure 5**. A) individual changes in SR $Ca^{2+}$-handling variables. B) group average changes from baseline to post-training in SR $Ca^{2+}$-handling variables. Striped lines indicates 0. Statistically robust results are presented as orange points (i.e., 95% CI not containing 0). Error lines are 95% CI. Abbreviations: Release rate; SR $Ca^{2+}$ release rate (µmol · g protein$^{-1}$ · min$^{-1}$); Uptake$_{tau}$, half-life (time to decrease by 63%) of $[Ca^{2+}]_{free}$ during SR $Ca^{2+}$ uptake (s$^{-1}$ · mg protein$^{-1}$); Uptake$_{600nM}$, rate of SR $Ca^{2+}$ uptake at 600 nM $[Ca^{2+}]_{free}$ (µmol · g protein$^{-1}$ · min$^{-1}$); Uptake$_{200nM}$, rate of SR $Ca^{2+}$ uptake at 200 nM $[Ca^{2+}]_{free}$ (µmol · g protein$^{-1}$ · min$^{-1}$); Leak rate, rate of SR $Ca^{2+}$ leak (nmol · g protein$^{-1}$ · min$^{-1}$).

## 4.3. Sex differences in SR $Ca^{2+}$ release and uptake

$Uptake_{600nM}$ was -16.4% [-29.8, -0.8] lower in females compared to males at baseline (males, 1.81 ± 0.44; females, 1.59 ± 0.42 $\mu$mol · g $protein^{-1}$ · $min^{-1}$; Figure 6A), however, females increased 11.9% [1.0, 23.7] more than males from baseline to after training (Figure 5B). The robustness of the sex interaction observed in $uptake_{600nM}$ at baseline and in response to training was not altered by accounting for the relative proportion of MHC-II at baseline (-17.3% [-31.1, -1.1]) or change from baseline to after training (11.8% [0.9, 23.6]), in females compared to males respectively. However, neither $uptake_{tau}$ or $uptake_{200nM}$ were different between sexes at baseline (-11.4% [-23.4, 2.4] and -6.5% [-25.8, 17.1], respectively; Figure 6A) or in change from baseline to after training (-9.2% [-24.2, 8.8] and 2.7% [-11.2, 18.5], respectively; Figure 6B). Moreover, SR $Ca^{2+}$ release did not differ between sexes at baseline (-9.9% [-20.0, 1.4]; males, 0.95 ± 0.16; females, 0.86 ± 0.19 $\mu$mol · gr $protein^{-1}$ · $min^{-1}$; Figure 6A) or in change from baseline to after training (-3.5% [-12.7, 6.7]; Figure 6B). In addition, $uptake_{200nM}$ did not differ between sexes at baseline (-6.5% [-25.8, 17.1]; males, 0.50 ± 0.18; females, 0.49 ± 0.14 $\mu$mol · g $protein^{-1}$ · $min^{-1}$; Figure 6A) or in change from baseline to after training 2.7% [-11.2, 18.5] (Figure 6B).

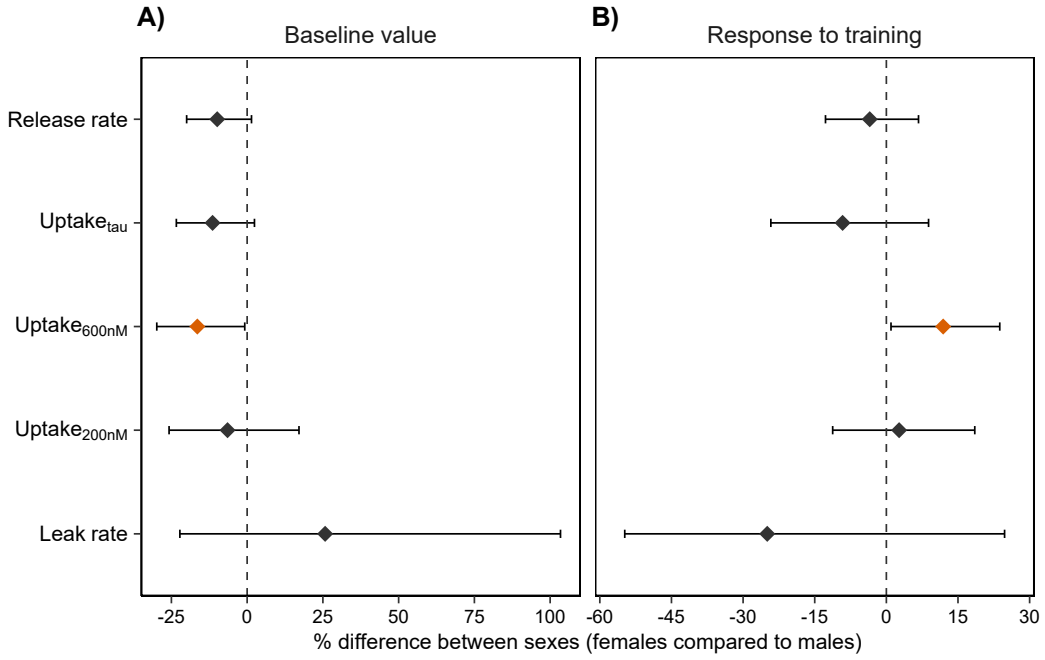


**Figure 6**. A) difference in SR $Ca^{2+}$-handling between sexes at baseline. B) difference between sexes in SR $Ca^{2+}$-handling training effect. Striped lines indicates 0. Statistically robust results are presented as orange points (i.e., 95% CI not containing 0). Error lines are 95% CI. Abbreviations: Release rate; SR $Ca^{2+}$ release (μmol · g protein · $min^{-1}$); $Uptake_{tau}$, half-life (time to decrease by 63%) of $[Ca^{2+}]_{free}$ during SR $Ca^{2+}$ uptake ($s^{-1}$ · mg $protein^{-1}$); $Uptake_{600nM}$, rate of SR $Ca^{2+}$ uptake at 600 nM $[Ca^{2+}]_{free}$ (μmol · g $protein^{-1}$ · $min^{-1}$); $Uptake_{200nM}$, rate of SR $Ca^{2+}$ uptake at 200 nM $[Ca^{2+}]_{free}$ (μmol · g $protein^{-1}$ · $min^{-1}$); Leak rate, rate of SR $Ca^{2+}$ leak (nmol · g $protein^{-1}$ · $min^{-1}$).

## 4.4. SR $Ca^{2+}$ release and uptake in relation to cycling performance, $VO_{2max}$ and durability

There was a robust inverse relationship between training response in $uptake_{tau}$ and training responses in $W_{15min}$ and $W_{max}$, with one percent increase in $uptake_{tau}$ coinciding with -0.16 watt [-0.28, -0.04] $R^2_a$ = 0.16 lower training response in $W_{15min}$ and -0.15 watt [-0.28, -0.01] $R^2_a$ = 0.13 lower training response in $W_{max}$ (Table 3). No other relationships where found between training responses in SR $Ca^{2+}$ handling variables and training responses in $W_{15min}$, $W_{max}$, $VO_{2max}$ or durability index (Table 3).

**Table 3**. Effect of training responses in SR $Ca^{2+}$ handling on training responses in cycling performance, $VO_{2max}$ and durability.

| | Estimate[a] | Standard..Error | Lower 95% CI | Upper 95% CI | $R^2$ adjusted |
|---|---|---|---|---|---|
| Change in power output during 15min cycling trial | | | | | |
| Release rate | −0.17 | 0.13 | −0.43 | 0.10 | 0.00 |
| $Uptake_{tau}$ | −0.16* | 0.06 | −0.28 | −0.04 | 0.16 |
| $Uptake_{600nM}$ | −0.18 | 0.14 | −0.46 | 0.10 | 0.00 |
| $Uptake_{200nM}$ | −0.04 | 0.11 | −0.27 | 0.18 | −0.05 |
| Leak rate | −0.04 | 0.03 | −0.10 | 0.02 | 0.01 |
| Change in power output of the last minute during incremental cycling test | | | | | |
| Release rate | −0.23 | 0.15 | −0.53 | 0.06 | 0.07 |

| | | | | | |
|---|---|---|---|---|---|
| $Uptake_{tau}$ | −0.15* | 0.07 | −0.28 | −0.01 | 0.13 |
| $Uptake_{600nM}$ | −0.23 | 0.15 | −0.53 | 0.08 | 0.06 |
| $Uptake_{200nM}$ | −0.19 | 0.11 | −0.42 | 0.04 | 0.08 |
| Leak rate | −0.03 | 0.03 | −0.10 | 0.04 | 0.02 |
| Change in maximal oxygen consumption | | | | | |
| Release rate | −0.96 | 1.56 | −4.14 | 2.23 | 0.07 |
| $Uptake_{tau}$ | −1.01 | 0.74 | −2.51 | 0.49 | 0.11 |
| $Uptake_{600nM}$ | −0.18 | 1.64 | −3.52 | 3.16 | 0.06 |
| $Uptake_{200nM}$ | −0.52 | 1.23 | −3.03 | 1.99 | 0.05 |
| Leak rate | −0.01 | 0.36 | −0.74 | 0.73 | 0.06 |
| Change in durability index | | | | | |
| Release rate | −0.04 | 0.04 | −0.11 | 0.04 | 0.40 |
| $Uptake_{tau}$ | −0.02 | 0.02 | −0.06 | 0.01 | 0.43 |
| $Uptake_{600nM}$ | −0.07 | 0.04 | −0.15 | 0.01 | 0.44 |
| $Uptake_{200nM}$ | −0.01 | 0.03 | −0.07 | 0.05 | 0.39 |
| Leak rate | 0.00 | 0.01 | −0.02 | 0.01 | 0.39 |

[a]Percent change in SR calcium release and uptake as depandant variable

*statistically robust results (i.e., 95% CI not containing 0)

Abbreviations: Release rate; SR $Ca^{2+}$ release (μmol · g protein$^{-1}$ · min$^{-1}$); $Uptake_{tau}$, half-life (time to decrease by 63%) of $[Ca^{2+}]_{free}$ during SR $Ca^{2+}$ uptake (s$^{-1}$ · mg protein$^{-1}$); $Uptake_{600nM}$, rate of SR $Ca^{2+}$ uptake at 600 nM $[Ca^{2+}]_{free}$ (μmol · g protein$^{-1}$ · min$^{-1}$); $Uptake_{200nM}$, rate of SR $Ca^{2+}$ uptake at 200 nM $[Ca^{2+}]_{free}$ (μmol · g protein$^{-1}$ · min$^{-1}$); Leak rate, rate of SR $Ca^{2+}$ leak (nmol · g protein$^{-1}$ · min$^{-1}$); SR, sarcoplasmic reticulum.

Exploratory analysis of potential relationships between baseline values in SR $Ca^{2+}$ handling and baseline values in $W_{15min}$, $W_{max}$, $VO_{2max}$ and durability index showed that one normalized unit higher $uptake_{600nM}$ at baseline was related to 0.011 watt · kg$^{-1}$ [0.001, 0.021] $R^2_a$ = 0.14 higher $W_{15min}$, 0.015 watt · kg$^{-1}$ [0.002, 0.029] $R^2_a$ = 0.19 higher $W_{max}$ and 0.16 mL · kg$^{-1}$ · min$^{-1}$ [0.03, 0.29] $R^2_a$ = 0.29

higher $VO_{2max}$ values at baseline. Similarly, one normalized unit higher $uptake_{200nM}$ at baseline was related to 0.010 watt · $kg^{-1}$ [0.001, 0.019] $R^2_a$ = 0.14 higher $W_{15min}$, 0.014 watt · $kg^{-1}$ [0.003, 0.025] $R^2_a$ = 0.19 higher $W_{max}$ and 0.13 mL · $kg^{-1}$ · $min^{-1}$ [0.02, 0.24] $R^2_a$ = 0.26 higher $VO_{2max}$ values at baseline. In contrast, one normalized unit increase in SR $Ca^{2+}$ leak rate at baseline was related to -0.007 watt · $kg^{-1}$ [-0.013, -0.001] $R^2_a$ = 0.16 lower $W_{15min}$, -0.010 watt · $kg^{-1}$ [-0.017, -0.003] $R^2_a$ = 0.23 lower $W_{max}$ and -0.08 mL · $kg^{-1}$ · $min^{-1}$ [-0.16, -0.01] $R^2_a$ = 0.27 lower $VO_{2max}$ values at baseline.

Taken together, SR $Ca^{2+}$ release rate do not show any clear relationship with endurance performance at baseline or changes in endurance performance following the training intervention. SR $Ca^{2+}$ uptake rates ($uptake_{600nM}$ and $uptake_{200nM}$) are positively related to endurance performance at baseline, however, a negative relationship with changes in cycling performance following the training intervention ($uptake_{tau}$). SR leak rate at baseline show a negative relationship with baseline endurance performance.

## 4.5. SR $Ca^{2+}$ release and uptake in relation to MHC distribution and exercise intensity

The 8-week endurance training intervention led to a robust increase in the relative proportion of MHC-II isoforms (pooled MHC-IIa and MHC-IIx) of 2.3%-points [0.4, 4.3]; baseline, 48.2 ± 10.0%; after training, 50.5 ± 8.7% (Figure 3). Females had -7.3%-points [-12.8, -1.7] lower proportion of MHC-II at baseline than males (44.1 ± 9.6% vs 51.3 ± 9.3%, respectively). However, the change in relative proportion of MHC-II from baseline to after training did not differ between sexes (-1.0%-points [-4.9, 3.0]). There was a robust positive relationship between the change in relative proportion of MHC-II and change in $uptake_{200nM}$ of 7.9% [0.8, 15.5], $R^2_a$ = 0.43 increase in $uptake_{200nM}$ coinciding with one %-point increase in the relative proportion of MHC-II (Table 4). Changes in SR $Ca^{2+}$ release rate, $Uptake_{tau}$, $uptake_{600nM}$ and SR $Ca^{2+}$ leak rate did not relate to change in MHC distribution (Table 4). In addition, exploratory analysis of potential relationships between relative proportion of MHC-II at baseline and baseline values in SR $Ca^{2+}$ handling variables did not yield any robust interactions.

Exercise intensity, defined as the percent of $VO_{2max}$ during interval bouts measured at two separate

4x8min session (Figure 1A), ranged from 65.0% to 95.1%. There was a robust negative relationship between exercise intensity and increase in SR $Ca^{2+}$ release rate from baseline to after training of -8.7% [-15.3, -1.6], $R^2_a$ = 0.14 increase in SR $Ca^{2+}$ release coinciding with one %-point increase in exercise intensity (Table 4). However, exercise intensity did not relate to changes in SR $Ca^{2+}$ uptake or SR $Ca^{2+}$ leak rate (Table 4). In addition, there was no relationship between of the relative distribution of MHC-II at baseline and exercise intensity (0.23% [-0.02, 0.48], $R^2_a$ = 0.06).

**Table 4**. Effects of percent of $VO_{2max}$ during interval sessions, and change in relative proportion of MHC-II on SR $Ca^{2+}$ release and uptake.

| | Estimate[a] | Standard Error | Lower 95% CI | Upper 95% CI | $R^2$ adjusted |
|---|---|---|---|---|---|
| Change in relative proportion of MHC-II | | | | | |
| Release rate | 6.54 | 4.23 | −2.20 | 16.07 | 0.01 |
| $Uptake_{tau}$ | −2.07 | 4.34 | −10.35 | 6.97 | −0.04 |
| $Uptake_{600nM}$ | 3.32 | 2.61 | −2.04 | 8.97 | −0.02 |
| $Uptake_{200nM}$ | 7.91* | 3.34 | 0.79 | 15.53 | 0.43 |
| Leak rate | −7.26 | 4.89 | −16.24 | 2.69 | −0.02 |
| Percent of maximal oxygen consumption during interval training | | | | | |
| Release rate | −8.73* | 3.69 | −15.33 | −1.62 | 0.14 |
| $Uptake_{tau}$ | −2.12 | 3.68 | −9.20 | 5.51 | −0.03 |
| $Uptake_{600nM}$ | −1.92 | 2.35 | −6.53 | 2.92 | −0.06 |
| $Uptake_{200nM}$ | −1.34 | 2.80 | −6.84 | 4.47 | 0.29 |
| Leak rate | −3.95 | 7.21 | −17.20 | 11.41 | −0.17 |

[a]percent change in SR calcium release and uptake rates as the dependent variable

[*]statistically robust results (i.e., 95% CI not containing 0)

Abbreviations: Release rate; SR $Ca^{2+}$ release (μmol · g protein$^{-1}$ · min$^{-1}$); $Uptake_{tau}$, half-life (time to decrease by 63%) of $[Ca^{2+}]_{free}$ during SR $Ca^{2+}$ uptake (s$^{-1}$ · mg protein$^{-1}$); $Uptake_{600nM}$, rate of SR $Ca^{2+}$ uptake at 600 nM $[Ca^{2+}]_{free}$ (μmol · g protein$^{-1}$ · min$^{-1}$); $Uptake_{200nM}$, rate of SR $Ca^{2+}$ uptake at 200 nM $[Ca^{2+}]_{free}$ (μmol · g protein$^{-1}$ · min$^{-1}$); Leak rate, rate of SR $Ca^{2+}$ leak (nmol · g protein$^{-1}$ · min$^{-1}$); MHC, myosin heavy chain.

# 5. Discussion

The present findings yield novel insights into the effects of endurance training on SR $Ca^{2+}$ handling properties and support the idea of training adaptations in SR $Ca^{2+}$ handling properties to counteract muscle fatigue and thus enhance muscular function and performance. The present study demonstrate that eight weeks of endurance training enhances SR $Ca^{2+}$ release and uptake in the leg muscles of previously untrained adults. Moreover, as the first study to investigate sex differences in SR $Ca^{2+}$ release, the present findings showcase that females and males does not differ in SR $Ca^{2+}$ release at baseline or in response to training. However, females displayed lower $uptake_{600nM}$ at baseline, and larger enhancement in response to training.

Contrary to prior beliefs that enhanced SR $Ca^{2+}$ release and uptake would subsequently improve cycling performance, the present findings did not demonstrate such relationships with changes in SR $Ca^{2+}$ release, and a negative relationship between change in $uptake_{tau}$ and change in cycling performance was observed. In contrast, $uptake_{600nM}$ and $uptake_{200nM}$ at baseline, were positively associated with higher baseline levels of cycling performance.

## 5.1. Effect of endurance training on SR $Ca^{2+}$ release

The observed increase of 7.3% in SR $Ca^{2+}$ release rate is in accordance with previously reported training responses to resistance, sprint and high-intensity endurance training, performed on trained, moderately-trained, and highly-trained individuals (Gejl et al., 2020; Jessen et al., 2021; Ørtenblad et al., 2000). The present finding expands upon this knowledge by showcasing that eight weeks of endurance training enhances SR $Ca^{2+}$ release in thirty-seven previously untrained adults. However, this

is in contrast to results from a study comprising prolonged submaximal endurance training, in which, SR $Ca^{2+}$ release was reduced (Green et al., 2003). Considering that Gejl et al. (2020) and Ørtenblad et al. (2000) reported slightly larger improvements in SR $Ca^{2+}$ release (9% and 10%, respectively) compared to the 7.3% observed in the present study, following just four and five weeks of training that included intermittent all-out sprint training, high-intensity training may be essential for inducing enhancements in SR $Ca^{2+}$ release. Accordingly, the discrepancy between the present result and the reduction in SR $Ca^{2+}$ release following prolonged submaximal endurance training (Green et al., 2003), could be a consequence of the current endurance training protocol, which included some high-intensity and maximal effort exercises. Intriguingly, in the present study, an inverse relationship between exercise intensity, i.e. percent of $VO_{2max}$ during interval sessions, and improvements in SR $Ca^{2+}$ release was observed. Thus, the importance of exercising at high intensities to elicit enhancements in SR $Ca^{2+}$ release may be questioned. However, the relevance of this relationship remains questionable considering that $VO_2$ only was measured during one of the three interval types (i.e., 4x8min session) and only at two of the twenty-four total interval sessions.

Since the analysis of the rate by which the SR releases $Ca^{2+}$ are done *in vitro* under apparently constant conditions, and under no influence from the intracellular milieu nor under physiological voltage sensor control, the observed increase in SR $Ca^{2+}$ release rate has to be due to either permanent changes in the content of RyR1 in the muscle tissue and/or modulation of the RyR1 opening probability. Consecutively, elevated content of RyR1 in the muscle tissue could be due to one or a combination of the following three hypotheses: modifications in fiber type distribution (specifically, from MHC-I to MHC-II), increased density of RYR1 and/or elevated SR content *per se* within the existing fiber types. In order to differentiate between some of these possibilities, the relative proportion of MHC isoforms was measured in the present study, which during the training period shifted towards a higher relative proportion of MHC-II. Considering the substantial quantitative differences in structural attributes of the SR between the two fiber types, wherein type II fibers roughly contain twice the volume and area of SR terminal cisternae, about four to six times higher RyR1 content and showcase roughly three times higher SR $Ca^{2+}$ release than type I fibers (Baylor & Hollingworth, 2003; Delbono & Meissner, 1996; Eisenberg & Kuda, 1976; Margreth et al., 1993; Rüegg, 1986), SR $Ca^{2+}$ release rate might have been enhanced due to the increase in the relative proportion of MHC-II. However, the observed change

in relative proportion of MHC-II was not associated with changes in SR $Ca^{2+}$ release, implying that the enhancement in SR $Ca^{2+}$ release was caused by alternative mechanisms. This assertion was further supported by the additional absence of an association between the initial MHC-II distributions and the initial rates of SR $Ca^{2+}$ release, and consequently, raising doubt on the significance of MHC distribution alone on SR $Ca^{2+}$ release rates.

The relative importance of the other mechanisms that might elevate RyR1 content, i.e. enhanced RyR1 density and enhanced SR content, or the importance of alterations in RYR1 opening time and/or conductance, can only be speculated. Ørtenblad et al. (2000) concluded that the observed increase in SR $Ca^{2+}$ release after five weeks of intermittent 20 x 10-s all-out sprints were associated with increased RyR1 content as a consequence of an overall expansion of the SR volume, however, whether this is translatable the present situation is unclear. In theory, the observed increase in SR $Ca^{2+}$ release might also be due alterations in RyR1 opening probability, through alterations in the structure or gating dynamics of RyR1. The significance of this hypothesis remains speculative as previous studies have not explored the training effects on this aspect. Nevertheless, acute high-intensity training has been shown to induce structural alterations of RyR1 (i.e., fragmentation) in recreationally active individuals, with subsequent reductions in SR $Ca^{2+}$ release and increases in SR $Ca^{2+}$ leak, which persisted 24 hours post-training (Place et al., 2015). However, considering that muscle biopsies were sampled before endurance testing at pretest and more than 48 hours following the last training session, as well as no observed alterations in SR $Ca^{2+}$ leak in the present study, it is unlikely that this effect influenced the observed increase in SR $Ca^{2+}$ release rate; however, if it did, it would likely result in an underestimation of the effect.

## 5.2. Effect of endurance training on SR $Ca^{2+}$ uptake

To my knowledge, the present study is the first to demonstrate that SR $Ca^{2+}$ uptake rates are enhanced following a period of endurance training, evident by the observed 13.2% and 8.9% increase in $uptake_{tau}$ and $uptake_{600nM}$, respectively. In previous studies, SR $Ca^{2+}$ uptake have been shown to remain unaltered following high-intensity endurance training (Gejl et al., 2020) and sprint training (Ørtenblad

et al., 2000), as well as been reduced following moderate-intensity endurance training (Green et al., 2003). However, in the latter study Green et al. (2003) observed reductions in SR $Ca^{2+}$ uptake rates at $[Ca^{2+}]_{free}$ levels between 1000 nM and 2000 nM, which correspond to supra-physiological levels, and SR $Ca^{2+}$ uptake rates closer to physiological levels (i.e., <1000 nM) remained unaltered, and thus, questioning the relevance of these results. Since the measurements on the rate by which SR re-sequesters $Ca^{2+}$ are done *in vitro* under constant conditions, potential changes in SR $Ca^{2+}$ uptake could arise from alterations in the number of active SERCA, SERCA hydrolysis rates and/or properties of the SERCA ATP binding site. None of the respective SR $Ca^{2+}$ handling properties were measured in the present study, however, type II muscle fibers generally encompass four to six times higher SERCA activity and contents than MHC-I fibers (Eisenberg & Kuda, 1976; Rüegg, 1986). Accordingly, the observed changes in SR $Ca^{2+}$ uptake could be attributed to the observed increase in relative proportion of MHC-II. Yet, change in $uptake_{tau}$ and $uptake_{600nM}$ was not related to changes in the relative proportion of MHC-II, and neither was initial SR $Ca^{2+}$ uptake rates and initial relative proportion of MHC-II related. Thus, SR $Ca^{2+}$ uptake properties seem to a large degree to be uninfluenced by MHC distributions and the observed elevation in SR $Ca^{2+}$ uptake may rather be due to alterations of the respective attributes within the muscle fibers irrespective of general fiber types. Additionally, and in contrast to what was observed on SR $Ca^{2+}$ release, exercise intensity was not associated with changes in SR $Ca^{2+}$ uptake.

In the present study, a positive relationship between changes in $uptake_{200nM}$ and changes in the relative proportion of MHC-II was observed, however, the relevance of this relationship in regard to muscular performance might be questioned by the absence of an increase in $uptake_{200nM}$ following the training intervention. Nevertheless, this relationship implicates that a increase in relative proportion of MHC-II improves the capacity of SR $Ca^{2+}$ uptake at resting $[Ca^{2+}]_{free}$ levels.

## 5.3. Sex differences in SR $Ca^{2+}$ release and uptake

The present study is, to my knowledge, the first study to highlight that SR $Ca^{2+}$ release is similar in previously between sexes, and that the subsequent responses to exercise training does not differ between sexes. However, females showcased lower $uptake_{600nM}$ at baseline, and larger enhancements

in response to training in comparison to males, which is in contrast to results from a previous study showing no effect of sex on SR $Ca^{2+}$ uptake (Harmer et al., 2014). However, the same study did observe lower SERCA activity in females compared to males, which they proposed was due to known sex differences in fiber type composition (Roepstorff et al., 2006; Simoneau & Bouchard, 1989) with subsequent fiber type differences in SERCA activity (Li et al., 2002). However, despite a -7.3% difference in baseline values of relative proportion of MHC-II in females compared to males in the present study, the observed sex interaction in $uptake_{600nM}$ was practically unchanged after accounting for MHC distribution in the same model. Suggesting that the observed difference is not explained by differences in MHC distribution between sexes at baseline. Moreover, the observed sex interaction on training response in $uptake_{600nM}$ was not altered after accounting for MHC distribution in the same model, nor did the changes of relative proportion of MHC-II in response to training differ between sexes. Therefor, the observed sex differences in $uptake_{600nM}$ may rather be due to differences in attributes within the muscle fibers irrespective of fiber types, i.e. content of active SERCA or SERCA hydrolysis rates and/or properties of the SERCA ATP binding site.

## 5.4. Implications of SR $Ca^{2+}$ handling properties on performance and health

The present study demonstrated a robust increase in SR $Ca^{2+}$ release and uptake rates following eight weeks of endurance training including twenty-four training sessions. This effect, in line with the principal of symmorphosis (Taylor & Weibel, 1981; Weibel et al., 1991), might be anticipated to enhance the muscle‘s maximal exercise capacity. This theory is supported by the concomitant improvements in cycling performance (i.e., $W_{15min}$, $W_{max}$) along with improvements in $VO_{2max}$ following the endurance training period. However, an inverse relationship between enhancements in $uptake_{tau}$ and improvements in both $W_{15min}$ and $W_{max}$ was observed in the present study. The overall relevance of these relationships may be questioned by the rather poor model fit, evident by $R^2_{adjusted}$ values of 0.16 and 0.13, respectively. Previous studies have consistently highlighted acute deteriorations in SR $Ca^{2+}$ handling as an important mechanism underlying the development of muscle fatigue, however, the impairment is primarily related to declining SR $Ca^{2+}$ release (Duhamel et al., 2006; Gejl et al., 2014; Li et al., 2002; Place et al., 2015). Hence, SR $Ca^{2+}$ uptake might not be among the main limiting com-

ponents in muscular fatigue, and thus, increasing SR $Ca^{2+}$ uptake rate may therefore not be expected to improve resilience to muscular fatigue. This is supported by previous findings suggesting that SR $Ca^{2+}$ uptake is less trainable in response to high-intensity endurance and high-intensity intermittent sprint training (Gejl et al., 2020; Ørtenblad et al., 2000). Interestingly, $uptake_{600nM}$ and $uptake_{200nM}$ at baseline were positively related to baseline values in $W_{15min}$, $W_{max}$ and $VO_{2max}$, indicating that the mechanisms of SR $Ca^{2+}$ uptake in regulating endurance performance might differ between initial performance and changes in performance.

The observed increase in SR $Ca^{2+}$ release rate showed no associations with the observed improvements in $W_{15min}$, $W_{max}$ and $VO_{2max}$, which is in accordance with previous findings (Gejl et al., 2020; Ørtenblad et al., 2000). However, it is also crucial to acknowledge that establishing significant correlations or regressions between two delta parameters (change scores) tend to require very large sample sizes or vary strong associations, i.e. doubles the variation for each parameter by introducing twice the sources of measurement error. Thus, considering that endurance performance is highly multifactorial with the main determinants being $VO_{2max}$, fractional utilization of $VO_{2max}$ at lactate threshold and exercise efficiency (Joyner & Coyle, 2008), it is probable that the influence of the SR $Ca^{2+}$ release at the level of the E-C coupling, although positive, is mitigated when considering the overall body's response to endurance training, owing to numerous confounding factors that impact endurance performance. Additionally, initial levels of SR $Ca^{2+}$ release showed no relationship with $W_{15min}$, $W_{max}$ or $VO_{2max}$.

The ability to maintain performance over time, i.e., durability, is also considered to be among the main limiting factors of endurance performance (Maunder et al., 2021). Thus, and in consideration with the prior idea of training adaptations in SR $Ca^{2+}$ handling properties to counteract muscle fatigue, a durability index was calculated as the percentage of $W_{15min}$ in regard to $W_{max}$. Improving the durability index may therefore reflect an improved ability to sustain high relative work-load in an already fatigued state. However, at the group level, the durability index did not change following the training period, nor was the change in SR $Ca^{2+}$ release and uptake associated with changes in durability index. This might be explained by large variation in individual change scores of durability index, evident by the bigger range of 95% CI (Figure 3E), possibly due to inexperience of the participants in conducting cycling trials to exhaustion and by the introduction of measurement error in two separate tests.

Taken together, the observed improvement in SR $Ca^{2+}$ release may have contributed to enhancements in muscular function and performance, due to improved resilience to muscular fatigue, which may have important implications for general health an performance. In contrast, enhancements in SR $Ca^{2+}$ uptake may be of less importance. Interestingly, enhanced contractile force in non-fatigued muscle in trained men have previously been associated with pharmacological-induced increase of SR $Ca^{2+}$ release rate via β2-adrenergic stimulation (Hostrup et al., 2014). Thus, suggesting that in addition to the potential effects on resilience to muscle fatigue, enhanced SR $Ca^{2+}$ release *per se* might also be associated with an increase in contractile function.

## 5.5. Methodological considerations

A significant limitation of ecological validity in the present study stems from the absence of a control group. As described previously (see section "Methods"), the present investigation of training responses in SR $Ca^{2+}$ handling properties constituted a segment of a larger scientific project that tracked the same participants through two consecutive and identical training interventions aimed at examining intra- and interindividual variations in training responses. Nonetheless, a prior study on 5-week sprint training has shown that the control subjects exhibited no alterations in SR $Ca^{2+}$ handling properties (Ørtenblad et al., 2000). If this could translate to the present setting can only be speculated. Additionally, analysis of SR $Ca^{2+}$ release and uptake are limited to *in vitro* settings, which fail to capture interactions of the among various intracellular sites and conditions. Consequently, while changes in SR $Ca^{2+}$ release and uptake after exercise training are evident under isolated *in vitro* conditions, it's imperative to evaluate the physiological significance of these changes for overall muscle function. This methodology hinges on the crucial assumption that the SR vesicle utilized for *in vitro* SR $Ca^{2+}$ release and uptake measurements is functionally representative of *in vivo* muscle conditions.

## 5.6. Conclusion

In conclusion, the present study demonstrates that the rate of SR $Ca^{2+}$ release and uptake can be enhanced by eight weeks of endurance training in sedentary adults, which may have important implications for muscular performance and general health. Moreover, SR $Ca^{2+}$ release does in general not differ between sexes, with the exception of females displaying lower rates of SR $Ca^{2+}$ uptake prior to training, yet larger gains in response to training, possible due to mechanisms other than fiber type composition.

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# A. Appendix: Written informed consent

# VIL DU DELTA I FORSKNINGSPROSJEKTET:

## *«REPEAT» – HVORFOR HAR UTRENTE PERSONER ULIK EFFEKT AV FYSISK TRENING?*

### FORMÅLET MED PROSJEKTET OG HVORFOR DU BLIR SPURT

Dette er et spørsmål til deg om å delta i et forskningsprosjekt der formålet er å undersøke hvorfor utrente personer har ulik effekt av trening, og om treningseffekten kan repeteres hvis det samme treningsopplegget gjentas flere ganger. Når vi trener utholdenhets- og styrketrening forbedrer vi kroppens evne til energiomsetning og kraftutvikling, hvorav begge faktorene er avgjørende for opprettholdelse av god helse og normal kroppslig funksjon. Imidlertid ser man at hvor stor effekt man oppnår varierer mellom personer, til tross for at man utsetter kroppen for det samme arbeidet Man vet i dag lite om hva som forårsaker disse forskjellene, og det er derfor et behov for å gjennomføre studier som undersøker hva som forårsaker disse forskjellene i treningseffekt. Kunnskapen slike studier gir oss vil man i fremtiden kunne bruke for å tilpasse treningsprogrammer til den enkelte slik at de gir best mulig effekt, altså forbedringer i kroppslig funksjon og helse, samt god forebyggende effekt mot livsstilsykdommer. I dette forskningsprosjektet ønsker vi at du som deltager skal trene utholdenhetstrening på sykkel over to identiske 8-ukersperioder som er separert av en 8-ukers periode uten trening. Før og etter treningsperiodene vil det gjennomføres omfattende testing for å undersøke hva slags effekter treningsperioden gav. Dersom du blir med som deltager i denne studien, vil du være med i en studie som ønsker å besvare spørsmål om 1) i hvilken grad kan forskjeller i treningseffekt mellom personer repeteres, 2) hva er det som bestemmer størrelsen på treningseffektene hos en person, samt treningseffektforskjellene mellom personer, og 3) hvilke faktorer er avgjørende for å forbedre helsen og utholdenhetsprestasjon?

Til denne studien søker vi deg som tidligere har gjennomført styrketreningsstudien «Alpha & Omega i regi av Seksjon for helse og treningsfysiologi ved Høgskolen i Innlandet, campus Lillehammer. Vi søker også deg som ikke har gjennomført en slik type treningsstudie tidligere. For å være med i studien kan du ikke ha trent mer enn én utholdenhetstreningsøkt per uke det siste året, du må være frisk og i alderen 30-65 år. Vi ønsker å rekruttere totalt 45 personer til dette prosjektet.

Om du etter å ha lest denne informasjonen ønsker å delta i studien ber vi deg skrive under og returnere den siste siden til oss. Du kan når som helst i etterkant trekke deg fra studien uten å oppgi grunn.

Ansvarlig for studien er Høgskolen i Innlandet og prosjektleder for studien er førsteamanuensis Knut Sindre Mølmen. Ph.d.-student Ingvill Odden vil ha det praktiske ansvaret for den daglige driften underveis i studien. Studien vil inngå i hennes doktorgradsavhandling, samt i flere master- og bacheloroppgaver.

### HVA INNEBÆRER PROSJEKTET FOR DEG?

Dersom du blir med i denne studien vil du fra juni 2023 til mars 2024 gjennomføre to identiske 8-ukers utholdenhetstreningsperioder, separert av en 8-ukers periode uten trening (Figur 1). Før og etter begge treningsperiodene skal du gjennomføre et standardisert testprogram bestående av målinger på to ulike testdager; se «Testdag 1 og 2» i Tabell 1 for innhold og rekkefølge på tester under disse testdagene, samt blå og grønne piler i Figur 1 for når disse testdagene skal gjennomføre Overordnet sett vil de fysiske testene gjennomføres på Testdag 1 (dvs. styrke- og

utholdenhetstesting, mens de testene som utføres hvilende og i fastende tilstand (dvs. ikke spist eller drukket annet enn vann på siden kvelden før) vil gjennomføres på Testdag 2. Du skal gjennomføre Testdag 2 totalt fire ganger; én gang før og én gang etter hver treningsperiode. Testdag 1 skal du gjennomføre totalt 11 ganger; to ganger før og to ganger etter hver treningsperiode, samt tre ganger i 8-ukersperioden som er forut for periode 1. De tre testene forut for første treningsperiode blir gjennomført for at du skal tilvenne deg denne testen, slik at dataene vi samler inn i prosjektet blir så nøyaktige som mulig. Treningen under de to 8-uker lange treningsperiodene vil gjennomføres på sykkel med tre til fire treningsøkter per uke. Alle treningsøkter vil bli veiledet av instruktør.


***Figur 1.*** *Grovoversikt over studiens tidslinje, inkludert trening og testprosedyrer. Totalt vil det bli gjennomført to 8-ukers treningsperioder, fire Testdag 1 og elleve Testdag 2.*

I prosjektet vil vi innhente og registrere ulike personopplysninger om deg som er direkte relevante for formålet med studien. Dette vil være informasjon som alder, kroppshøyde og -vekt, opplysninger om treningen du gjør under og utenom forsøket, samt data fra de ulike testene og målingene som skal gjøres.

## MER DETALJERT INFO OM TRENINGEN OG TESTINGEN

*Treningen:* All treningen som du skal være med på vil gjennomføres med en instruktør i spinningsalen på Høgskolen i Innlandet, studiested Lillehammer. Fellestreninger med inntil 10 personer vil bli satt opp både på morgenen, midt på dagen og ettermiddagen hver dag, slik at du har mulighet til å tilpasse treningen til din hverdag. Under de 8-uker lange treningsperiodene skal du gjennomføre tre til fire 45 minutters spinningøkter per uke. Øktene vil inneholde en standardisert oppvarming, 3-5 serier av 4-7 minutters intervaller hvor du sykler på en moderat til høy intensitet, samt en rolig nedkjøringsperiode mot slutten. Under den 8-uker lange perioden uten trening vil du bli bedt om å etterstrebe å ha det samme aktivitetsnivået som du hadde før den første treningsperioden.

*Tester før og etter treningsperiodene:* All testingen som du skal gjøre i prosjektet vil bli gjennomført ved Høgskolen i Innlandet, studiested Lillehammer sitt idrettsfysiologiske testlaboratorium. Tilvenningstestene er for at du skal kunne venne deg til utstyret og protokollene, slik at de fysiske testene gjennomføres på en best mulig måte. Testene blir fordelt over to ulike testdager, en testdag som involverer fysisk aktivitet (Testdag 1) og en testdag uten fysisk aktivitet, men i fastende tilstand, altså at du ikke har spist eller drukket annet enn vann siden kvelden før (Testdag 2). For oversikt over når testdagene skal gjennomføres og innholdet til testdagene; se Figur 1 og Tabell 1.

**Tabell 1:** Oversikt over innhold og rekkefølge på forskjellige testdagene.

| | |
|---|---|
| **Testdag 1 – ca. 2 timer** | |
| 1. | Styrketest |
| 2. | Laktatprofil (4-5 5-min arbeidsperioder med stigende arbeidsintensitet med måling av bl.a. hjertefrekvens og melkesyre) |
| 4. | Maksimal aerob kapasitetstest ($VO_{2maks}$-test) |
| 5. | 20 minutters sykkelprestasjonstest (*kun før og etter periode 1)* |
| 6. | Spørreundersøkelse om helserelatert livskvalitet (SF-36) |
| **Testdag 2 – ca. 3 timer** | |
| 1. | Fastende test av kroppssammensetning (målt med DXA) |
| 2. | Fastende test av muskeltykkelse (målt med ultralyd) og arteriell stivhet |
| 3. | Fastende mikrobiopsi av lårene på begge bein (m. Vastus lateralis) |
| 4. | Fastende blodprøve |
| 5. | Fastende glukosetoleranse-test |
| 6. | Fastende bestemmelse av blodvariabler (målt med karbonmonoksid-gjenpustingstest) |

**På Testdag 1** gjennomføres en test av din maksimale muskelstyrke og to-tre utholdenhetstester. Utholdenhetstestingen vil bli gjennomført på sykkel og starter med at du skal gjennomføre en laktatprofiltest som innebærer at arbeidsbelastningen øker gradvis, etterfulgt av en maksimal test for å måle ditt maksimale oksygenopptak ($\dot{V}O_{2maks}$). Før og etter den første treningsperioden vil utholdenhetstestingen også inkludere en 20 minutters sykkelprestasjonstest der du skal prøve å sykle på så høy intensitet som mulig. Testdag 1 avsluttes med at du skal fylle ut et spørreskjema som omhandler helserelatert livskvalitet. Totalt skal du i prosjektet gjennomføre Testdag 1 elleve ganger.

På den siste testdagen etter den første treningsperioden skal sertifisert helsepersonell tappe ut den eksakte mengden blod du tilegnet deg gjennom treningsperiode 1 før du gjennomfører utholdenhetstestene (Figur 1). Blodvolumet endres ulikt mellom individer som følge av trening, men det er antatt at det i dette prosjektet vil øke med mellom 1-4 dl gjennom den 8-uker lange treningsperioden. Det er denne eksakte mengden blod som skal fjernes (til sammenligning tappes ca. 5 dl blod ved bloddonasjon i blodbanken). Dette gjøres for å undersøke om det ekstra blodet du har fått i treningsperioden påvirker hvor høy intensitet og arbeidsbelastning du klarer å sykle på under sykkeltesten. Dette er et spørsmål som forskningen i dag ikke har et konkret svar på.

Før utholdenhetstestene på den siste testdagen etter den andre treningsperioden skal det ikke tappes blod, men det skal gjennomføres en gjenpustingsprosedyre med en liten dose karbonmonoksid. Karbonmonoksidgassen vil binde seg til en andel av dine røde blodceller. Karbonmonoksiddosen vil bli kalkulert til å tilsvare den oksygenbærende effekten som den mengden røde blodceller du tilegnet deg gjennom treningsperioden har. Dette gjøres for å undersøke om det maksimale oksygenopptaket endres dersom blodets oksygenbærende kapasitet reduseres, selv om blodvolumet og hjertets pumpeegenskaper ivaretas. Dette er også er også et spørsmål som det per i dag ikke finnes svar på i vitenskapslitteraturen.

**På Testdag 2** skal du møte opp fastende, altså at du ikke har spist eller drukket annet enn vann siden kvelden før. Testdagen starter med en DXA-skanning (dual-energy X-ray absorptiometry) for måling

av kroppssammensetningen din (deriblant måling av total mengde muskler, fett og ben i kroppen din), etterfulgt av ultralydundersøkelse for måling av tykkelsen på lårmuskulaturen din, samt måling av arteriell stivhet. Deretter vil det bli tatt to mikrobiopsier fra hvert av lårene dine, før du til slutt tar en blodprøve og en glukosetoleransetest. Testdag 2 avsluttes med en karbonmonoksid-gjenpustingstest for måling av blodvolumet ditt og andre variabler i blodet ditt.

## INFORMASJON OM DELTAKELSE I STUDIEN

*Fordeler ved deltagelse i studien:* Som deltager i denne studien vil du få god innsikt i din egen helse, samt kunnskap om hva slags effekter utholdenhetstrening vil gi deg. Du vil få veiledet trening og oppfølging gjennom to 8-ukers treningsperioder og vil gjennomgå flere avanserte tester som man vanligvis ikke får mulighet til, bl.a. ulike tester med måling av oksygenopptak på sykkel, kroppssammensetning, måling av blodvolum, samt inngående analyser av hva som karakteriserer muskulaturen din. Du vil med andre ord få kunnskap om hvordan du skal trene på en effektiv måte, samt få nøyaktige svar på hvordan din fysiske form er. Du vil også få et innblikk i hvordan forskning foregår og tilegne deg kunnskap om hvordan blod og muskulatur endrer seg med trening.

*Potensielle ulemper ved deltagelse i studien:* Deltagelse i prosjektet vil kreve en del tid og oppmerksomhet da du må møte opp til treningsøkter tre til fire ganger i uken gjennom to 8-ukers perioder, i tillegg til oppmøtene for tilvenning og testing. Vi ønsker at alle deltar på fellesøktene som blir satt opp, men dersom du er forhindret fra å møte på disse tidspunktene så vil vi være så fleksible som vi klarer.

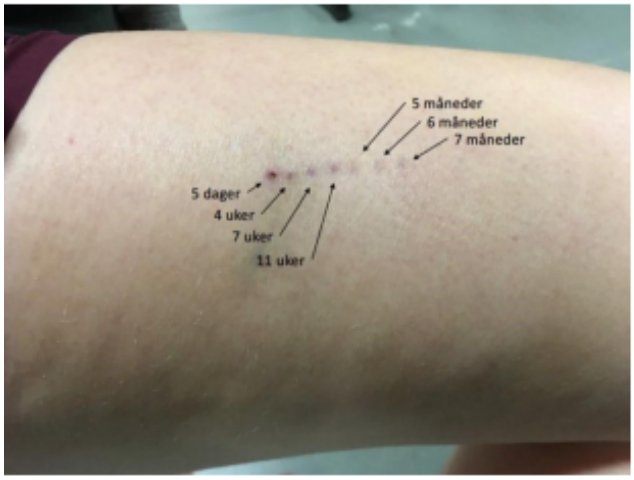
Enkelte av sykkeløktene og de fysiske testene skal gjennomføres på en relativt høy intensitet. Dette kan oppleves som anstrengende, og du bør regne med at du kan bli litt støl/sår i muskulaturen etter de første testene og treningene.

Tapping av blod assosieres med minimale negative konsekvenser når det omfatter en så liten mengde som i denne studien (bestemmes individuelt ut ifra hvor mye blodvolumet øker gjennom treningsperioden, men estimert til mellom 1-4 dl). Til sammenligning tapper man ca. 5 dl blod per gang ved bloddonasjoner hos blodbanken. Enkelte kan i ettertid av slik blodtapping oppleve noe slapphet, trøtthet og/eller svimmelhet, men dette er forbigående. I tillegg kan det tenkes at noe av treningseffekten som du fikk gjennom den første treningsperioden forsvinner etter blodtappingen, men at denne treningseffekten vil komme tilbake når du går i gang med den andre treningsperioden. Det kan forekomme litt misfarging på innstikkstedet ved blodprøve og blodtapping som tegn på blødning under huden, men dette reduseres ved at det holdes et trykk på innstikkstedet i en periode etter at nålen er fjernet.

Karbonmonoksid-gjenpusting som benyttes for å måle blodvariabler og forbigående tilbakestille den økte oksygenbærende kapasiteten i blodet ved trening, kan oppleves som litt ubehagelig for noen. Under disse målingene skal du puste inn en liten mengde karbonmonoksid (tilsvarende det å røyke en håndfull sigaretter), men denne mengden er så liten at den ikke anses som helseskadelig. Karbonmonoksid forsvinner relativ fort ut av kroppen, så innen 12-14 timer vil hemoglobin-karbonmonoksidnivået i blodet ditt være tilbake til normale nivåer.

Muskel- og blodprøvene i prosjektet vil tas av sertifisert personell ved bruk av prosedyrer som er veletablerte ved testlaboratoriet vårt ved Høgskolen i Innlandet, studiested Lillehammer. Muskelprøven tas med den svært skånsomme mikrobiopsimetoden. Noen synes likevel biopsier er ubehagelig. Du vil typisk bli litt støl i muskelen en til to dager i etterkant, først og fremst på grunn av små blødninger i muskulaturen. Inngrepet vil etterlate små arr, men disse vil forsvinne hos de fleste med tiden (se Figur 2). I svært få tilfeller vil biopsitaking føre til at følelsen i huden rundt biopsien

forsvinner over en lengre periode. Biopsitaking er også forbundet med en viss infeksjonsfare. Risikoen for disse komplikasjonene er imidlertid svært liten ved bruk av prosedyrene som benyttes i dette prosjektet. Biopsiene tas fra lårmuskelen på utsiden av låret ca. midt mellom kneet og hoften. Vi setter først en dose lokalbedøvelse (samme variant som hos tannlegen) før vi steriliserer området. Selve biopsien tas med en nål med en diameter på 2,1 millimeter som føres inn i lårmuskelen. For å få nok vev må vi inn to til tre ganger i samme hull. Du vil få klare instrukser om hvordan du skal behandle såret i etterkant av prøvetagningen. Blodprøvene som skal tas er ikke forbundet med noen risiko. Hvis det skulle oppstå noen uforutsette hendelser kan du kontakte medisinsk ansvarlig i prosjektet Inger Johanne Løkkevik på telefon: 45151929.




**Figur 2.** Typisk arrdannelse etter mikrobiopsitaking. De angitte tidspunktene indikerer tid siden biopsitaking.

Opplysningene vi samler inn om deg i prosjektet vil bli behandlet uten navn og avidentifisert (se avsnittet «Hva skjer med opplysningene om deg?»). Daglig leder i prosjektet (Ingvill Odden) vil være den eneste som er involvert i innsamlingen og behandlingen av spørreskjemaene.
Vi leter i utgangspunktet ikke etter helseutfordringer. Skulle vi likevel oppdage noe som avviker fra det vi forventer og/eller gir oss mistanke om helseutfordringer vil det bli tatt initiativ til videre medisinsk oppfølging. Du vil da bli kontaktet av medisinsk ansvarlig i prosjektet eller autorisert helsepersonell ved Seksjon for Helse og Treningsfysiologi (fysioterapeut Anne Mette Rustaden, tlf: 61288023, e-post: anne.rustaden@inn.no). Denne personen vil veilede deg videre om hvordan du bør håndtere situasjonen. Samme person kan også kontaktes av deg dersom det skulle dukke opp uheldige opplevelser i prosjektet som du ikke ønsker å dele med prosjektleder eller andre i prosjektgruppen.

## FRIVILLIG DELTAKELSE OG MULIGHET FOR Å TREKKE DITT SAMTYKKE

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Det vil ikke ha noen negative konsekvenser for deg hvis du ikke vil delta eller senere velger å trekke deg. Du kan også kreve dataene dine slettet så lenge de er identifiserbare i datamaterialet. Dersom du ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte prosjektleder (se kontaktinformasjon på siste side).

## HVA SKJER MED OPPLYSNINGENE OM DEG?

Opplysningene som registreres om deg skal kun brukes slik som beskrevet under formålet med prosjektet, og planlegges brukt til og med prosjektslutt 31.12.2030. Etter prosjektslutt skal opplysningene oppbevares i fem år for dokumentasjonshensyn. Eventuelle utvidelser i bruk og oppbevaringstid kan kun skje etter godkjenning fra Regional Komité for Medisinsk og Helsefaglig Forskningsetikk og andre relevante myndigheter.
Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert. Du har også rett til å få innsyn i sikkerhetstiltakene ved behandling av opplysningene. Alle data skal oppbevares på sikker server, Tjenester for sensitive data (TSD), ved Universitetet i Oslo som Høgskolen i Innlandet har databehandleravtale med. Du kan klage på behandlingen av dine opplysninger til Datatilsynet og institusjonen sitt personvernombud.
Vi behandler opplysningene konfidensielt og i samsvar med personvernregelverket. Det er bare medlemmer i prosjektgruppa som får tilgang på disse dataene. Navnet og kontaktopplysningene dine vil erstattes med en kode som lagres på egen navneliste adskilt fra øvrige data. Det er kun anonyme testresultater som publiseres, slik at du ikke vil kunne gjenkjennes i publikasjoner.

## HVA SKJER MED PRØVER SOM BLIR TATT AV DEG?

Muskelvevet og blodprøven som tas av deg skal oppbevares i en forskningsbiobank tilknyttet prosjektet ved Høgskolen i Innlandet campus Lillehammer. Ansvarlig for denne biobanken er Knut Sindre Mølmen.

## FORSIKRING

Som deltager i studien er du forsikret gjennom Høgskolen i Innlandets forsikring hos Gjensidige.

## GODKJENNINGER

Etter ny personopplysningslov har behandlingsansvarlig Høgskolen i Innlandet og prosjektleder Knut Sindre Mølmen et selvstendig ansvar for å sikre at behandlingen av dine opplysninger har et lovlig grunnlag. Dette prosjektet har rettslige grunnlag i EUs personvernforordning artikkel 6 nr. 1a og artikkel 9 nr. 2a og ditt samtykke. Du har rett til å klage på behandlingen av dine opplysninger til Datatilsynet.
Vi behandler opplysningene basert på ditt samtykke.

## KONTAKTOPPLYSNINGER

Dersom du har spørsmål til prosjektet eller ønsker å trekke deg fra deltagelse, kan du kontakte:
Prosjektleder: Knut Sindre Mølmen, telefon: 94860805, e-post: knut.sindre.molmen@inn.no
Daglig leder i prosjektet: Ingvill Odden, telefon: 94895112, e-post: ingvill.odden@inn.no

Dersom du har spørsmål om personvernet i prosjektet, kan du kontakte personvernombudet ved institusjonen:
https://www.inn.no/om-hogskolen/personvern/

JEG SAMTYKKER TIL Å DELTA I PROSJEKTET OG TIL AT MINE PERSONOPPLYSNINGER OG MINE DATA BRUKES SLIK DET ER BESKREVET

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Sted og dato

Deltagers signatur

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Deltagers navn med trykte bokstaver

For deg som tidligere har deltatt i styrketreningsprosjekt i regi av Høgskolen i Innlandet:
Jeg samtykker til at data som omhandler treningsrespons i det prosjektet kan overføres til dette prosjektet (det innebærer informasjon om endringer fra før til etter styrketreningsperioden på variabler som glukosetoleranse, samt muskelstyrke, -funksjon, -kvalitet og -masse)

☐ Ja ☐ Nei