High- and low-load strength training to failure in trained men and women

A study investigating changes in muscle strength, muscle fiber size, satellite cells and myonuclear number in skeletal muscle after an eight-week period of strength training

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Ingrid Cecelia Elvatun

Abstract

The purpose of this study was to assess if either strength training modalities; high-load (HL) or low-load (LL), were equally functional in concern of provoking cellular adaptations and strength increases in well-trained men (n = 11) and women (n = 3), given that all sets were performed to volitional failure.

Fourteen previous resistance-experienced individuals participated in the study. The training intervention was set to two fully supervised training session per week for eight weeks, refraining from other strength training involving lower extremities. Each leg was randomly assigned to three sets of leg press and leg extension of either 3-5 repetitions for high-load training (HL; 90-95% of 1RM) or 20-25 repetitions of low-load training (LL; 40-60% of 1RM) performed to failure. A unilateral study design was adopted, meaning that each subject carried out both training protocols. Biopsy samples were collected from *vastus lateralis* prior to and after the eight-week training intervention before stained and analyzed using fluorescent light microscopy (Olympus BX61, Japan) followed by an image processing software (Fiji). After the intervention, maximal strength increased in leg press for both HL (170.9 ± 43.8 kg to 205.6 ± 49.8 kg; $p < 0.001$) and LL (169.8 ± 48.2 kg to 205.5 ± 50.3 kg), with no differences between protocols. The HL leg extension protocol showed an increase (68.4 ± 13.7 kg to 74.3 ± 15.9 kg; $p < 0.05$) but not the LL leg extension (70.4 ± 13.4 kg to 68.5 ± 14.3 kg; $p > 0.05$). The results after the training (post) displayed no significant difference between protocols. The cross-sectional area increased in neither HL nor LL. Both HL and LL had no changes in satellite cell or myonuclear number.

The data suggest that high- and low-load strength training protocols, may induce equally good increases in muscle strength but further studies are needed to establish whether an extended training intervention could yield other or similar cellular changes in both protocols.

Sammendrag

Målet med oppgaven var å vurdere, samt analysere, om en av treningsmetodene; styrketrening med høy (HL) eller lav (LL) belastning, var like funksjonelle med hensyn på cellulære tilpasninger og økning i styrke hos trente menn (n = 11) og kvinner (n = 3), gitt at alle settene blir gjennomført til utmattelse.

Totalt 14 tidligere styrketrente individer deltok i studiet. Treningsintervensjonen ble satt til to økter hver uke i totalt åtte uker, hvorav deltakerne måtte avstå fra annen styrketrening av beina. Beina ble tilfeldig tilordnet å gjøre tre sett av enten 3-5 repetisjoner med tung styrketrening (90-95% av 1RM) eller 20-25 repetisjoner med lett styrketrening (40-60% av 1RM) med benpress og kneekstensjon. Ettersom tidligere studier har vist fordeler ved denne testmetoden, ble det vedtatt et unilateralt (hvert bein separat) studiedesign, noe som innebar at hver forsøksperson gjennomførte begge treningsprotokollene. Biopsiprøver ble samlet fra m. *vastus lateralis* fra hvert bein hos hver enkelt forsøksperson før og etter den åtte uker lange treningsperioden. Snittene ble deretter merket og analysert ved hjelp av et lysmikroskop (Olympus BX61, Japan), etterfulgt av en bildebehandlingsprogramvare (Fiji). Etter treningsperioden hadde styrken økt i benpress for HL (170,9 ± 43,8 kg til 205,6 ± 49,8 kg, $p < 0,001$) and LL (169,8 ± 48,2 kg til 205,5 ± 50,3 kg), uten signifikante forskjeller mellom protokollene. Benet som gjennomførte HL kneekstensjon viste en økning (68,4 ± 13,7 kg til 74,3 ± 15,9 kg; $p < 0,05$), mens LL kneekstensjon viste ingen endring (70,4 ± 13,4 kg til 68,5 ± 14,3 kg; $p > 0,05$). Til tross for dette, var det ingen signifikant forskjell mellom protokollene etter endt treningsperiode. Det var ingen økning i muskeltverrsnitt. Både HL og LL førte til ingen endringer i antall satellittceller eller myokjerner.

Følgelig tyder dataene på at både høy- og lav treningsprotokoll kan gi tilsvarende gode muskulære adaptasjoner i skjelettmuskulaturen hos mennesket, men det er behov for ytterligere forskning for å fastslå om en forlenget treningsperiode kan fremkalle andre muskulære og cellulære forandringer i begge protokollene.

Abbreviations

NIH	The Norwegian School of Sport Sciences
GIH	The Swedish School of Sport and Health Sciences
CSA	Cross-sectional area
HL	High-load
LL	Low-load
1RM	1 repetition maximum
Pax7	Paired box 7
MRF	Myogenic regulatory factor
PCM1	Pericentriolar material 1
IGF-1	Insulin-like growth factor 1
MRF4	Muscle regulatory factor 4
MyoD	Myogenic differentiation factor
Myf5	Myogenic factor 5
DAPI	4’6-diamidino-2-phenylindole
BSA	Bovine serum albumin
ATP	Adenosine triphosphate
Cdc6	Cell division cycle 6
MAP kinases	Mitogen-activated protein kinase
NO	Nitric oxide
HGF	Hepatocyte growth factor
Akt	Protein kinase B
mTOR	Mechanistic target of rapamycin
FOXO	Forkhead box O
NCAM	Neural cell adhesion molecule
P70S6	Phosphoprotein 70 ribosomal protein S6 kinase
MHC	Myosin heavy chain

Contents

1 Introduction

Muscle strength and muscle mass have a large beneficial impact on an individual's health and performance. Studies have revealed evidence proposing that physical activity contribute to preventing certain chronic diseases (e.g. cardiovascular disease, cancer, obesity, hypertension) (Sothern et al., 1999, Janssen and Leblanc, 2010, Warburton et al., 2006). Among these, it has also been reported a greater ability to improve sleep and mental health, and delay the onset of sarcopenia (Seguin and Nelson, 2003, Agudelo et al., 2014). Several studies have shown improved physical function in elderly after strength training to counteract the age-related decrease in strength and muscle mass (i.e. sarcopenia) (Liu and Latham, 2009, Hunter et al., 2004, Hunter et al., 2000). These well-characterized, epidemiological challenges all seem to benefit from exercise, particularly strength training, as it contributes to increasing fiber size (i.e. muscle mass) and strength.

Improved musculoskeletal health following strength training is an important component of most sports conditioning programs, as well as being a major contributor in rehabilitation and to reduce injury (Escamilla and Wickham, 2003). Several studies have challenged the current recommendation that strength training with loads beyond 60-70% of the one repetition maximum (1RM) ensures an efficiently development of muscle mass and strength (Van Roie et al., 2013, Jenkins et al., 2016, Mitchell et al., 2012a, Ogasawara et al., 2013, Baar and Esser, 1999, Morton et al., 2016a). Nowadays, recent studies imply that low-load strength training to muscular failure may display equally good effects, though the effect on type 1 vs. type 2 fibers are unclear. The effects of strength training on fiber type composition is of interest considering previous research indicating both a fiber type-specific increase in satellite cells and hypertrophy in type 2 fibers (Verdijk et al., 2009b). Studies have also reported a high proportion of oxidative muscle fibers (type 1 and type 2B) in endurance-trained athletes (Andersen and Henriksson, 1977, Russell et al., 2003), whereas others have found correlations between the amount of fast-twitch fibers (type 2X) and the development of a higher power output (Tihanyi et al., 1982).Evidently, widespread results demonstrate a discrepancy concerning optimal loading range to augment muscle size and strength regarding performance.

Another aspect of strength training is its importance for performance- and strength athletes. Strength gains and cellular adaptations in the skeletal muscle are important traits, and

some researchers have, compared the differences in loading modalities between low, moderate and high load in untrained subjects (Ogasawara et al., 2013, Holm et al., 2008, Assuncao et al., 2016), and high load in untrained, recreationally active and trained individuals (Mangine et al., 2015, Schoenfeld et al., 2014c). Some researchers find it to be equally efficient doing high- and low-load strength training in untrained subject, with respect to hypertrophy and strength (Burd et al., 2010). Others conclude that high-load induce a greater hypertrophic and strength response, while a few have compared the differences between the loading modalities in resistance-trained individuals, suggesting a similar effect (Vinogradova et al., 2013, Morton et al., 2016b). Still, the general recommendations indicate that strength training athletes should follow a protocol of few repetitions with high-load, as many repetitions have been experienced as more painful.

Yet, low-load strength training is a good alternative to high load, especially for individuals in which certain conditions prevent them from loading high forces upon the musculoskeletal system (Wernbom et al., 2008). It may be that a strength training-combination of both high- and low-loading is the best approach to achieve maximized muscular strength adaptations. Thus, promoting stress in both type 1 and type 2 fibers (Schoenfeld et al., 2016b). Additionally, elderly and people suffering from other musculoskeletal conditions may benefit from more easily tolerated strength training with lighter loads. Regardless, the number of studies covering this field is limited, as most research is conducted on individuals with little or no strength training experience.

As such, the main purpose of this study was to determine if either training modalities (high- or low-load strength training) were equally functional or if they induced different adaptations and strength increases in well-trained individuals. A secondary aim was to investigate whether previous findings with regards to similar studies, could be applied to induce cellular changes along with strength and hypertrophy.

Based on evidence and suggestions from previous research of high- vs. low-load strength training performed on well-trained subjects, we propose the following research questions and hypothesis:

Will low-load strength training yield similar cellular adaptations (i.e. fiber size, satellite cell number, myonuclear number) as high-load strength training in well-trained individuals, given that all sets are performed to volitional failure.

H_0: eight weeks of high- or low-load strength training will result in an equal increase in muscle strength, fiber cross-sectional area, and the number of satellite cells and myonuclei. The training modalities will cause no significant difference in fiber type 1 or type 2 regarding fiber size, satellite cell number or myonuclear number.

H_1: eight weeks of high- or low-load strength training will result in a different increase in muscle strength, fiber cross-sectional area, and the number of satellite cells and myonuclei. These differences exist in that the changes are incomparable when looking at fiber specific adaptations. With respect to fiber size, satellite cell number and myonuclear number, type 1 fibers will respond greater to low-load, whereas type 2 fibers will respond greater to high-load.

2 Background

2.1 Skeletal muscle

2.1.1 Muscle plasticity

The skeletal muscle is comprised of muscle fibers. Muscle fibers are made up of myofibrils which are composed of thousands of tandem repeated units called sarcomeres. The sarcomeres are stacked with the muscle's contractile proteins actin and myosin. Together with actin and myosin, sarcomeres are the functional, contractile unit of the muscle fiber. Due to its characteristics, actin and myosin are part of a continuous process of production and degradation, determining if there will be an increase or decrease in muscle mass/volume (Wilborn and Willoughby, 2004).

The mammalian skeletal muscle makes up a high proportion (~50%) of the total body mass and is an exceptional heterogenous tissue (Roy et al., 1991). Also, the remarkable feature of the skeletal muscle as a plastic tissue is the foundation in all the training protocols which exploit the muscle's ability to adapt. Hence, muscle mass is the major determinant of muscle strength. These properties make the skeletal muscle more competent to accommodate specific demands, altering its functional, morphological, and metabolic characteristics (Allen et al., 1999). Thus, the structural design of the muscle is impacted by the specific condition under which the skeletal muscle function (Hoppeler and Fluck, 2002).

Strength training exercises are external stimuli, differing from endurance exercises, with specific duration and intensity of contraction. The difference in intensity and duration reflects specific patterns which may cause a phenotypic switch in fiber type composition (Liu et al., 2003). Due to its abundance and contractile significance, a multitude of signaling mechanisms (e.g. quantitative and qualitative) can cause changes in the myosin isoforms which significantly impact muscle strength (Qaisar et al., 2016). This may carry its advantageous when exposing the skeletal muscle to a certain type of exercise.

The differences in fiber types are due to their protein isoform profile based on their myosin heavy chain (MHC) composition. The type of myosin heavy chain corresponds to the various fiber types as it serves the site functioning as the ATPase necessary during muscle contraction (Fry et al., 1994b). Additionally, Barany (1967) revealed a correlation between the contraction speed and the ATPase activity. Further, studies have suggested that expression of MHC in the major fiber types (type 1, type 2A and type 2X) to a certain degree respond to strength training (Holm et al., 2008). One could, therefore, expect that the type of strength training affects the outcome and changes in the MHC-expression. Consequently, the changes in the muscles' phenotypic profile may affect fiber size (hypertrophy/atrophy) or, as mentioned, the fiber type composition (fast-to-slow or slow-to-fast switch) (Green et al., 1999, Pette and Staron, 1997), according to the various tasks demanded by the muscle.

2.1.2 Muscle fiber types

Architectural differences in the muscle are based on characteristics such as the speed of which the fiber exerts an action potential, producing a single contraction and relaxation cycle (i.e. twitch). In humans, this corresponds to the three main groups of muscle fibers; type 1, type 2A and type 2X. This division ensures a separation of slow and oxidative type 1 fibers from fast and glycolytic type 2X fiber (Staron, 1997, Schiaffino and Reggiani, 1994). The adjustment of a fiber type-specific muscle is defined by a stepwise transition of fibers composed of a combination between two fiber types (Pette and Staron, 1997, Neunhauserer et al., 2011), thereby placing type 2A fibers in an intermediate position between type 1 and type 2X. Consequently, muscle fibers meet the demands by harmonizing the size and/or metabolic properties accordingly (Allen et al., 1999), modifying the muscle fiber's phenotypic profiles (Hoppeler and Fluck, 2002, Coffey and Hawley, 2007).

Usually, strength training does not affect MHC 1, while the percentage of MHC 2A increase as MHC 2X decrease (Hather et al., 1991, Staron et al., 1994b). Adams et al. (1993) showed changes in fiber type composition after 19 weeks of heavy strength training, presumably reflected by a change in gene expression. Following the training intervention, there was a percentage decrease in type 2X fibers associated with an increase in type 2A and type 1 fibers. The same observation was seen in another study (Fry et al., 1994a), though no significant rise in type 2A and type 1 was detected, in contrast with quadriceps studies.

Despite that the changes in strength did not correlate with the changes in the MHC content (i.e. fiber type composition), strength training caused alternations in the contractile protein profile (Jurimae et al., 1996) and thus hypertrophy.

2.1.3 Motor units

Muscle fibers are controlled by motor units where one motor unit comprise of a motor neuron and the muscle fiber that it innervates (Buchthal and Schmalbruch, 1980). The electrical properties of the motoneuron strongly covary with the mechanical properties of their muscle units. Hence, Burke et al. (1974) were able to identify three types of motor units: slow, fast fatigable and fast fatigue resistant. However, discussing the properties of motor units referred to humans, they adopted the terms low- and high-threshold motor units (Heckman and Enoka, 2012). Upon a muscle contraction, motor units were recruited following a size principle in an orderly manner (Carpinelli and Fitness, 2008). The size principle was first proposed by Henneman (1985) and dictates that, during a given movement, the smallest motor units are recruited first. The recruitment is dependent on the effort of the activity, and as force production requirements increase, the larger motor units are eventually engaged (i.e. an inverse relationship between firing rate and recruitment threshold) (Lee et al., 2013, De Luca and Hostage, 2010). In that sense, the firing of previous recruited motor units is greater than the firing of later recruited once. Since firing pattern is essential for muscle fiber activation, firing in response to loading during exercise is of increasing importance. Further, it seems as if it is important to acknowledge the effects of certain stimulation patterns during exercise on muscle fiber architectural specificities.

Heavy loading seems to be required to fully recruit higher threshold motor units, as they are responsible for promoting maximal muscular adaptations (Schoenfeld et al., 2014a). Thus, ensuring preferential activation of the distinct motor units according to the exercise (Sale, 1987). Recommendations are predicated on the belief that complete motor unit activation is necessary to accomplish optimal improvements in strength and hypertrophy (Kraemer and Ratamess, 2004). Yet, the perception may be different as suggested by Schoenfeld et al. (2017) that complete motor unit recruitment would ultimately be the result during strength training at lower intensities (30% 1 RM), granted that training was performed to momentary muscular failure. Additional studies support this view, concluding with the remark that heavy loads are not always necessary (Carpinelli and Fitness, 2008). Overall,

these results suggest that even low-load strength training can recruit sufficient muscle activation, eliciting strength gains and hypertrophic response.

2.2 Strength training

Aerobic endurance training and heavy strength training represents the opposites of the adaptations continuum required by the muscle (Coffey and Hawley, 2007). Regular exposure to heavy strength training will result in increased maximal muscular strength (Abe et al., 2000). In making this statement, it must be noted that when it comes to strength training, studies display different suggestions to how it most profitable should be performed (Medicine, 2009). Regardless, resistance exercise intensity is operationally defined as the percentage of maximal strength (% of 1 repetition maximum [%1RM]) or the number of repetitions (xRM) used for a particular exercise (Fry, 2004). Thus, the ability to adapt positively to increasing training load is dependent on the progression of the training program (Kraemer and Ratamess, 2004). To prevent injury and overtraining, careful considerations are required.

2.2.1 Strength training with high- and low-loads

A general approach has been that strength training should be performed with sufficient training resistance (≥ 70 % of 1RM) to maximize training-induced muscle strength and hypertrophy (Medicine, 2009), with even higher loads to maximize strength (>80% of 1RM) (Campos et al., 2002b, Peterson et al., 2004) or maximal hypertrophy (>90-95% of 1RM) (Fry, 2004). Such strength training is defined as high-intensity strength training as opposed to low-intensity strength training (<70% of 1 RM). Until recently, heavy strength training has been believed to be the only optimal way to elicit favorable improvement in muscle hypertrophy and strength (Campos et al., 2002b, McDonagh and Davies, 1984). Burd et al. (2012) proposed in a review that lower intensities, when performed to volitional failure, could provide a comparable muscle fiber activation typically seen when performing high-intensity strength training (Morton et al., 2016b, Martin et al., 2018, Ahtiainen et al., 2005, Elliott et al., 2002). That way, he opened for speculations that low intensities (30% of 1RM) until muscular failure could bring about the same degree of training-induced muscle hypertrophy as high-intensity strength training.

Moreover, Laurentino et al. (2008) tested the effects of vascular occlusion in combination with low-intensity strength training, but this did not augment hypertrophy or strength as opposed to regular high-intensity strength training. As occlusion cause additional stress to the muscle (Manini and Clark, 2009), some researchers hypothesized that occlusion at low intensities (20% of 1RM) could cause increased muscle strength and muscle size as well (Kubo et al., 2006). Other studies have also provided evidence that low-load strength training with vascular occlusion increase both muscle size and muscle strength compared to those typically seen after conventional high-load strength training (Abe et al., 2006, Takarada et al., 2002, Moore et al., 2004). High-loads can therefore no longer be considered the exclusive driver of resistance exercise-induced changes in muscle strength and hypertrophy.

Several studies have conducted experiments investigating hypertrophic and strength benefits regarding high- vs. low-load strength training, and the conclusions along the studies differ. Whereas some suggests an identical increase in muscle mass (i.e. hypertrophy) and muscle strength with both training modalities (Schoenfeld et al., 2015a, Morton et al., 2016a), others conclude that they are only comparable in regard to muscle hypertrophy and not strength (Mitchell et al., 2012b). Morton et al. (2016b) showed that well-trained men performing high- (8-12 repetitions at 75-90% of 1RM) or low-load (20-25 repetitions at 30-50% of 1RM) unilateral strength training for 12 weeks induced similar skeletal muscle hypertrophy. Evidence of no significant strength difference between the two legs was also presented. Contrary results from a similar study comparing high- and low-intensity strength training did not agree with their findings, concluding that high-intensity (80-85% of 1RM) strength training resulted in greater strength gains and hypertrophy compared to low-intensity (50-70% of 1RM) strength training (Vinogradova et al., 2013).

The majority present evidence suggesting that low-load strength training to volitional failure induce an equal increase in both hypertrophy (Ogasawara et al., 2013) and also, if not a more efficient, increase in muscle protein synthesis as opposed to high-load strength training (Burd et al., 2010). Interestingly, Schoenfeld et al. (2017) concluded in a review that increases in 1RM favored high-load strength training. Additionally, evidence of a trend toward high-load being slightly greater regarding muscle hypertrophy was presented, while no difference between the two training modalities was detected testing isometric strength. They are supported by the study of Schmidtbleicher (1987) which carried out a comparative study consisting of one group completing 7 sets of 1-3 repetitions at 90-100% of 1RM and a second

group completing 3 sets of 12 repetitions at 70% of 1RM. The strength increases were seemingly similar for both groups, though the high-repetition group displayed a greater CSA. The overall results may indicate that prescriptions for great hypertrophy and maximum strength may differ. Therefore, it is important to acknowledge the individual variance in training progression, and training volume and intensity must be modified each individual and their starting position (Kraemer and Ratamess, 2004, Kraemer et al., 1998). As it seems that hypertrophy can occur performing both training modalities, perhaps one could expect fiber type-specific changes as well.

Several studies have investigated differences in induced hypertrophic responses caused by different loads and whether potential differences are fiber type-specific. Some studies suggest that performing low-load strength training to volitional failure may emphasize type 1 muscle fiber hypertrophy more than high-load strength training (Ogborn and Schoenfeld, 2014, Grgic et al., 2018), which preferentially induce hypertrophy of type 2 muscle fibers (Netreba et al., 2013, Vinogradova et al., 2013). That said, as briefly mentioned, other studies indicate that low-load strength training is equally effective in promoting muscle fiber growth as high-load strength training, assuming that training is carried out to muscular failure (Morton et al., 2016b, Martin et al., 2018). The current literature covering the topic show conflicting results and do not brought enough evidence to draw a firm conclusion regarding the adaptations in the different fiber types when exposed to various loading modalities. Further research is therefore needed.

2.3 Satellite cells

2.3.1 Characteristics

The satellite cell was first described by Mauro (1961) when examining the skeletal muscle fiber of a frog, providing the first electron microscopy description of these unfamiliar cells. The satellite cell was named after its peripheral association between the myofiber (sarcolemma) and the extracellular matrix (basal lamina) of the muscle fiber (Figure 2.1A). To begin with, they were considered myogenic progenitors. Later, their ability to self-renew to maintain the satellite cell pool or differentiate upon muscle regeneration characterized them as myogenic stem cells (Dumont et al., 2015a, Collins et al., 2005). It is, however, important to appreciate that satellite cells and muscle-derived stem cells (e.g. muscle side population (SP)) represent distinct cell populations (Seale et al., 2000, Chen and Goldhamer, 2003). In healthy tissue, satellite cells collaborate with mesenchymal stem/stromal cells (MSCs) (Figure 2.1B) and $PW1^{+}/Pax7^{-}$ interstitial cells (PICs) to achieve an efficient regenerative process (Ferrari et al., 1998, Saito et al., 1995). Mesenchymal progenitors present an important stem cell population and contribute in promoting the satellite cell-dependent myogenesis (Uezumi et al., 2014) but in concern of changes in the muscle following strength training, satellite cells are more studied.

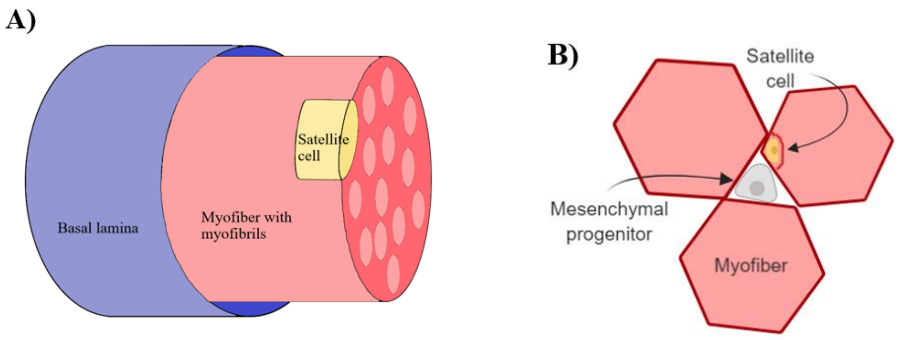

Figure 2.1 | Satellite cell localization in the skeletal muscle fiber.
(A) The satellite cells are located between the sarcolemma (myofiber) and the basal lamina (extracellular matrix) of the muscle fiber. The mesenchymal progenitor is enclosed by the basal lamina. (B) A schematic representation of the satellite cell location in its peripheral niche, quiescent and "ready to alert" whenever activated by a proper stimulus (e.g. stress of muscle fiber). *Figures adapted from (Skuk, 2013).*

The satellite cells are a heterogeneous population normally found in a "ready to alert"-quiescent state. Here, they will only sporadically fuse with one another to compensate for muscle turnover caused by daily wear and tear (Yin et al., 2013). In this resting state, the

satellite cells generally express transcription factors such as the transcription factor paired box 7 (Pax7) (Dumont et al., 2015a). They are characterized by a low rate of metabolism and mitotic activity (Rocheteau et al., 2015a, Schultz et al., 1978). Their low transcriptional activity, on the other hand, is invaluable during the activated state. Moreover, they have a relatively high nucleus-to-cytoplasm ratio with few organelles (Hawke and Garry, 2001). From a biological perspective, an optimal distribution of nuclei with respect to minimizing transport distances to and from cells are highly beneficial. These morphological features are consistent with the arguments that satellite cells are normally transcriptionally less active and rather quiescent. Thus, satellite cells are fundamental as they can fuse with the muscle fiber to donate their nucleus and contribute to hypertrophy and increased protein synthesis when necessary (Moss and Leblond, 1970, Moss and Leblond, 1971).

2.3.2 Satellite cell activation and myogenesis

Satellite cells are activated as a result of stimuli from several factors (e.g. physiological stress from exercise or injury) or growth factors during development (Hawke and Garry, 2001). This can cause the adult mammalian skeletal muscle to undergo a process of myogenesis (i.e. growth). Seale et al. (2000) demonstrated the importance and unique requirement of the transcription factor Pax7 regarding satellite cell specification, as Pax7 deficient mice presented complete absence of myogenic satellite cells. Today's understanding is that Pax7-positive satellite cells provide an essential role in adult regenerative myogenesis (Sambasivan et al., 2011), and are crucial to obtain a normal function of the satellite cells in the skeletal muscle (von Maltzahn et al., 2013a).

As opposed to muscle fibers which contain differentiated cells, satellite cells can undergo mitosis (figure 2.3). Thus, they are crucial for the regeneration of the muscle after trauma (physical stress) (Hawke and Garry, 2001). The satellite cell activation is defined with them reentering the cell cycle from their quiescent state (Dumont et al., 2015a) before they then proliferate and differentiate to form new daughter cells. A perception is that satellite cells can divide symmetrically and asymmetrically upon cell cycle entry (Figure2.2) and that these options rely on the physiological condition of the cell. The proliferation involves symmetric division, which is suspected to be the primary mechanism for self-renewal involving the formation of a subpopulation of new, identical and quiescent satellite cells (Moss and Leblond, 1971). An asymmetric division which gives rise to two identical daughter cells, one

daughter cell which will actively maintain the satellite cell pool, while the other will fuse with the muscle fiber to form new myonuclei (Kuang et al., 2007). They represent a subpopulation already committed to becoming myogenic progenitors.

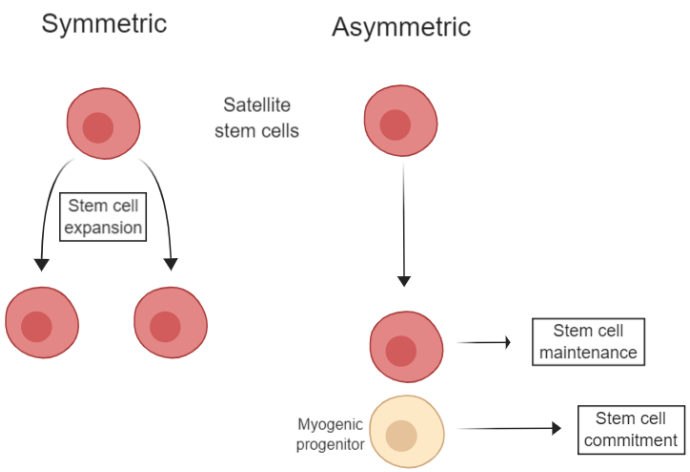

Figure 2.2 | Symmetric and asymmetric division.
Symmetric cell division gives rise to two identical daughter cells with the means to proliferate the satellite cell pool. This cell population will have a higher self-renewal capacity. Asymmetric cell division is a property of stem cells that gives rise to two daughter cells with different developmental fates. In this case, one satellite cell will ensure maintenance of the satellite cell pool while the other will commit to become a myogenic progenitor. *Figure adapted from Dumont et al. (2015b) and Berika et al. (2014).*

The proliferation process to form new myonuclei have been suggested to be necessary for efficient hypertrophic growth of the skeletal muscle (Schoenfeld, 2010, Snow, 1990b, Egner et al., 2016, Goh and Millay, 2017). There are, however, conflicting reports that challenge that this perception exists (Lee et al., 2012, McCarthy et al., 2011). Regardless, satellite cells are considered essential for optimal hypertrophy, in addition to repair and regeneration of damaged muscle fibers (Karalaki et al., 2009, Goh and Millay, 2017, Egner et al., 2016). Quiescent satellite cells display different gene expression profiles compared to activated, dividing cells (Motohashi et al., 2014). The earliest molecule associated with satellite cell activation is phosphorylated p38 MAPK (Jones et al., 2005). This protein kinase is followed by myogenic regulatory factor MyoD, which can be expressed by myogenic cells although not in quiescent cells or myofibers (Kanisicak et al., 2009, Yablonka-Reuveni and Rivera, 1994). Shortly after activation, MyoD directly regulates the expression of yet another molecule, Cdc6, to allow cell cycle entry (Zhang et al., 2010).

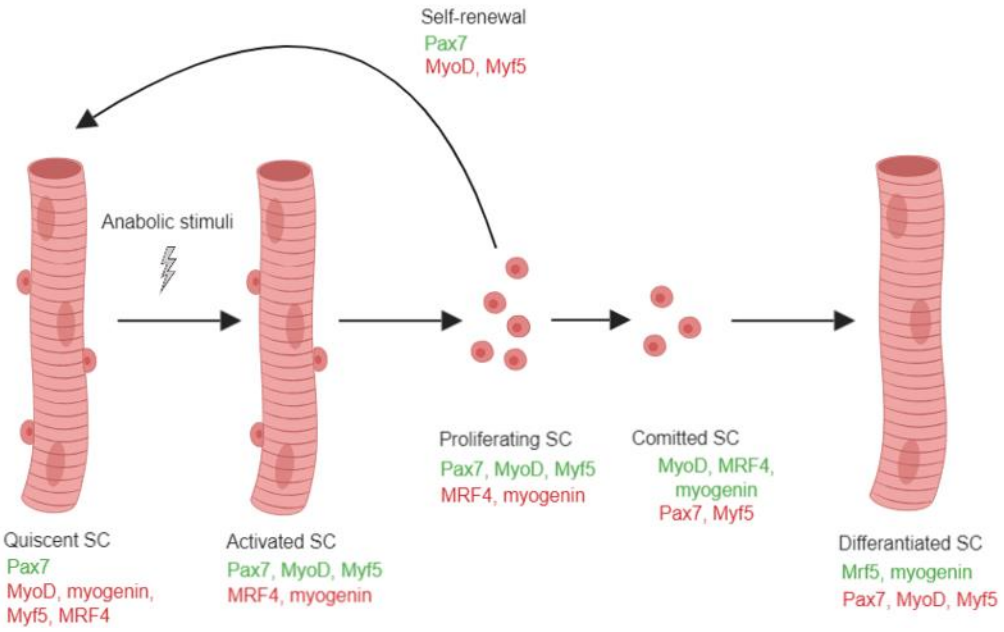

Figure 2.3 | Satellite cell progression in response to exercise.
Following strength training, the up- and downregulation of Pax7 and the MRFs determine the progression through the myogenic program causing triggering of morphological and functional changes. The upregulated proteins are represented in green, while the downregulated proteins are colored red. The process is described in more detail in the main text. *Figure adapted from Snijders et al. (2015a) and Bazgir et al. (2017).*

Over the centuries, researchers have tried to display the details regarding satellite cell activation, but there are still uncertainties and more to reveal. Some growth factors and pathways have been suggested to be central to different stages of activation. Growth hormones are known as a performance-enhancing drug. It is well-characterized to improve the amount of circulating insulin-like growth factors 1 (IGF-1) and increasing the proliferation, thus triggering the formation of myoblasts (myogenic precursor cell) (Fu et al., 2015). IGF1 and IGF2 are known to be important mediators in the anabolic pathway of the skeletal muscle by activating numerous cascade reactions when bound to their receptors. This binding can cause an increase in proliferation through MAP kinases or differentiation and hypertrophy through phosphorylation of Akt which activates mTOR and p70S6 kinase (Fu et al., 2015). By stimulating the Akt/mTOR pathway, IGF1 consequently downregulates the transcription factor FOXO which have shown to induce cycling of satellite cells (Machida et al., 2003, Schiaffino and Mammucari, 2011). Moreover, a study by Musaro et al. (2001) on transgenic embryos displayed that local overexpression of IGF1 may activate satellite cells. Obviously, the pathways are compound yet the regulatory factors encoding for muscle growth has been defined.

The MRFs are a family composed of four members: myogenin, MRF4, MyoD and, Myf5. They are all regulatory factors important for muscle growth occurring in the muscle after strength training. Psilander et al. (2003) demonstrated an alternation in myogenin, MyoD, and MRF4 following strength training, indicating that these genes may be fundamental in regulating hypertrophy. Quiescent satellite cells display different gene expression profiles compared to active, proliferating satellite cells, whereas MyoD expression absent in quiescent satellite cells (Fu et al., 2015). Activation of satellite cells also seems to be influenced by the release of nitric oxide (NO) produced by dying fibers, triggering the release of hepatocyte growth factor (HGF). Elevated levels of NO at the injury site, indicates that NO is required for normal muscle repair post injury (Rigamonti et al., 2013, De Palma and Clementi, 2012). A calcium influx and HGF from the extracellular matrix occur in the disrupted muscle to directly activate the unprotected satellite cells (Wozniak and Anderson, 2007, Allen et al., 1995b).

2.3.3 Effects of strength training on satellite cells and myonuclei

Several studies have over the years studied the role of satellite cells during muscle growth and degradation. The major conclusion is that depletion precipitate less functional myofibers (Egner et al., 2016, Finnerty et al., 2017). Thus, strength training is postulated to be a stimulus required to activate satellite cells (Hawke, 2005), inducing transcriptional and translational changes in the skeletal muscle. Various models have discussed the need for satellite cells to achieve a good hypertrophic response (Rosenblatt et al., 1994, O'Connor and Pavlath). Conflicting results have further, over the years, questioned the recruitment of satellite cells and the addition of myonuclei after a period of strength training. Previous research has shown an increase in fiber type 2-associated satellite cells in response to strength training in both elderly (Verdijk et al., 2009a) and younger individuals (Nederveen et al., 2017).

When arriving at the site of interest, the satellite cells fuse together and/or with existing myofibers. A natural expectation would be a subsequent increase in myonuclei concurrent with increasing satellite cell number. Still, the presented results are conflicting, some indicating that the addition of myonuclei is fundamental for muscle fiber hypertrophy in both young and elderly individuals (Allen et al., 1995a). Leenders et al. (2013) postulated that

traditional, prolonged strength training (60-80% of 1RM) increased satellite cell and myonuclear content, in addition to muscle mass and strength in both elderly men and women. A newer study by Snijders et al. (2016) observed the same accompanied increase in satellite cells and myonuclei during the hypertrophic response. Additionally, they presented evidence that an increase in the myonuclear domain does not drive skeletal muscle fiber hypertrophy. Yet, some studies have demonstrated a hypertrophic response deprived of an increase in myonuclei (Kadi et al., 2004). Keeping that in mind, upon a hypertrophic response, the muscle fibers may be short of newly incorporated satellite cells as the need for supplementary is dependent on the training stimulus.

Insufficient training stimulus may only serve for renewing and replenishing of the already present satellite cell pool (Mackey et al., 2011). Analyzing changes in the satellite cell pool in relation to different phases of a strength training program, Damas et al. (2018) proposed that extensive muscle fiber growth over the limits of which pre-existing myonuclei can contribute with enough support, new satellite cells are required to supply additional myonuclei to both fiber types. So, during prolonged strength training, an increase in both myonuclear and satellite cells number would be expected considering the training stimulus is adequate. Accordingly, Kadi et al. (2000) presented evidence confirming that the acquisition of additional myonuclei per fiber during muscle hypertrophy is necessary to support the enlargement of multinucleated muscle cells. That way, maintaining the cytoplasmic volume per nucleus (i.e. the nuclear domain) (O'Connor and Pavlath, 2007). In response to 10 weeks of strength training, the cross-sectional area increased in coincidence with a significant increase in both myonuclei (~70%) and satellite cell number (46%). Thereby indicating a positive correlation between an increasing concentration of myonuclei with a correspondingly higher number of satellite cells.

The distribution of satellite cells in fast and slow skeletal muscle fibers have previously been investigated, with the perception that satellite cells occur more frequently in slow muscle fibers (Hawke and Garry, 2001), as they are first recruited during muscle activity. Certain studies have, however, found a higher number of satellite cells in the fast muscle (type 2X) in mice and suggested an unequal distribution of satellite cells in the various muscle types (fast vs. slow) (Gibson and Schultz, 1982b). Other studies have revealed no difference between type 1 and type 2 fibers, apart from a decrease in type 2 associated satellite cells with increasing age (Verdijk et al., 2014). Even so, Kadi et al. (2006b) provided

new information that the satellite cell distribution in human vastus lateralis was not dependent on fiber type composition. Meaning that a firm conclusion is not to be made at this point.

2.3.4 Identification of satellite cells

(1) Immunohistochemistry

Immunohistochemistry is an excellent detection technique determining the tissue distribution of an antigen (protein) of interest (Duraiyan et al., 2012), and their exact position. Immunohistochemical markers for satellite cells are monoclonal and polyclonal antibodies selectively identifying antigens located on the satellite cell. The principle was discovered in the early 1930s, but the first study was reported first in 1941 by Coons et al. (1941). Further, expansion and development of the immunohistochemical technique have led to the discovery of the antibody against Pax7. Additionally, antibodies against neural cell adhesion molecule (NCAM) and M-Cadherin (calcium-dependent adhesion glycoproteins) have also been used in studies on both mice and humans (Kinney et al., 2017, Marti et al., 2013). Using antibodies against NCAM, satellite cells in their quiescent, proliferating and activated state was stained (Hawke and Garry, 2001). Staining with NCAM also cause the synapses in the muscle nerve cells to be stained (Covault and Sanes, 1986) and may present challenges during analysis. M-Cadherin is expressed in quiescent, proliferating and activated satellite cells (Marti et al., 2013, Cornelison and Wold, 1997). Pax7 is expressed uniformly in quiescent as well as active, proliferating satellite cells, and downregulated when the cells commit to muscle differentiation (Zammit et al., 2006). Distinctly from their quiescent progenitors, the myoblasts (proliferating progeny) co-express Pax7 and MyoD. The phase in which Pax7 is downregulated along with an induction of myogenin is marked as the entering of the differentiation phase (Danoviz and Yablonka-Reuveni, 2012).

Though electron microscopy opened a new world of possibilities when it came to the identification of specific cells located in a tissue of interest, the method offers challenges. The analysis is considered extremely time-consuming and the tissue sample is remarkably small compared to traditional histological methods.

(2) Biopsy analysis

Over the years, a number of studies have been practicing different biopsy techniques to study muscle fiber type composition, satellite cell number and cross-sectional area (Costill et al., 1979, MacDougall et al., 1980, Friedmann-Bette et al., 2010). The proportions of studies performed on untrained subjects are usually more abundant compared to studies on well-trained subjects. Since it seems like untrained subjects often adopt higher gains in muscle mass and/or strength during shorter training interventions, muscular responses are easier observed and thus more favorable studies to conduct. Regardless of this, the gathering of tissue samples enables the use of immunohistochemistry which can contribute to a better understanding of how strength training affects factors such as satellite cell number, fiber type distribution and myonuclei.

2.4 Strength training-induced muscle hypertrophy

An increase in muscle mass and muscle cross-sectional area (CSA) is usually associated with a process of muscle fiber hypertrophy (Chesley et al., 1992), in contrast to hyperplasia whereas the fiber CSA increases due to the addition of new myofibers (Kelley, 1996). Generally speaking, it is well known that strength training induces hypertrophy and increased CSA and muscle mass in both humans (Bellamy et al., 2014, Charette et al., 1991, Tesch, 1988) and other animals (Bagby et al., 1972, Barany and Close, 1971, Heck et al., 1996). Hypertrophy is often seen in relation with strength training as a result of an imbalance between the protein degradation and protein synthesis, where the protein synthesis exceeds the rate of protein degradation (Goldberg, 1968, Pallafacchina et al., 2013).

As some research has considered hypertrophy a slow process usually requiring 6-7 weeks before demonstrating whole muscle hypertrophy (Goreham et al., 1999, Green et al., 1999), some researchers have observed hypertrophy already after 20 days of heavy strength training (Seynnes et al., 2007). Despite, DeFreitas et al. (2011) wrote that significant hypertrophy was present after 3-4 weeks of high-intensity strength training, observing an increase in CSA already after two training sessions. It should be noted that excesses in results are likely affected by differences in training stimulus (i.e. some only performed one exercise; Seynnes et al. (2007)) and various exercises.

Muscle CSA and fiber type composition are both important factors concerning muscle characteristics. Curiously, studies have shown an increase in muscle CSA and satellite cell number in *vastus lateralis* superior to observations in upper extremities when an equal number of sets were performed (Rønnestad et al., 2007, Hanssen et al., 2013b). This provides indications of adaptive differences between upper and lower body strength. The predicted improvement in strength and hypertrophy are, however, expected at different time points following strength training. Additionally, studies investigating hypertrophic responses between men and women are limited. Early attempts studying the efficiency of strength training in women relative to men showed an absent muscle hypertrophy (Ivey et al., 2000), posited by the low androgen production in women. Anyhow, newer evidence suggests that the relative percentage change in strength and hypertrophy between men and women are similar (Cureton et al., 1988). Although there are physical differences between men and women (e.g. smaller myofibers), strength training seems to increase fiber size and strength in a similar manner in women as men when presented to the same exercise stimulus (Lemmer et al., 2000, Staron et al., 1994a)

2.4.1 Myonuclear involvement in muscle hypertrophy

In response to the hypertrophic process, adult myofibers require the formation of new myonuclei to sustain the increasing myonuclear domain (Allen et al., 1999). Fusing myonuclei causes the enlargement of preexisting fibers. A rodent study demonstrated the relation between hypertrophy and myonuclei in response to strength training (Figure 2.3) (Bruusgaard et al., 2010). Prior to any major increase in muscle volume, the hypertrophic response caused the addition of new myonuclei. Still, research on mice reveals that an increased number of nuclei in larger fibers contributes differently as to the nuclear domain (Bruusgaard et al., 2003). The nuclei seemingly repulse each other intending to obtain an optimal distribution of nuclei with respect to minimizing transport distances. Further, these newly acquired myonuclei were, besides already existing myonuclei, retained in the muscle during atrophy. Myonuclei, therefore, seem apoptotic resistant to degradation after subsequently disuse and are not lost (Bruusgaard and Gundersen, 2008).

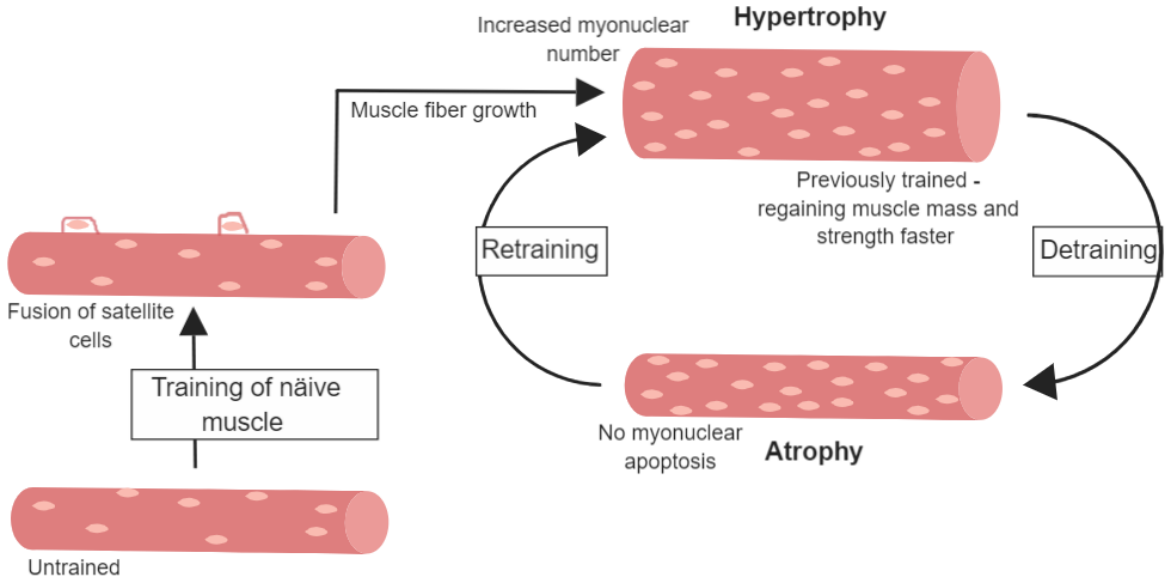

Figure 2.3 | Proposed model of cellular memory and myonuclear addition following exercise. In response to a hypertrophic process following resistance exercise in untrained muscle fibers, new myonuclei are recruited through fusion satellite cells. This occurs prior to the muscle fiber growth. As myonuclei are not lost during detraining, the elevated myonuclear number are preserved when the muscle mass is decreased, leading to a small fiber with high myonuclear density. Thus, when returning to training, increased muscle mass is gained easier. This permanently elevated number of myonuclei demonstrates a cellular memory mechanism, suggesting the maximal size the muscle fiber has ever had at a point. *Figure adapted from Gundersen (2016).*

2.4.2 Effects of strength training on fiber CSA

The increase in size following strength training is caused by the addition of sarcomeres and an increase of noncontractile elements (e.g. fat). Strength training is an effective stimulus to improve muscle protein balance, primarily by stimulating protein synthesis (Phillips et al., 1997). Wilkinson et al. (2008) demonstrated a post-exercise increase in both myofibrillar and mitochondrial protein synthesis in human skeletal muscle after strength training, with increasing specificity as the muscle increased its strength. These results are in analogy with studies examining the post-exercise acceleration of muscle protein turnover and amino acid transport (Biolo et al., 1995, Cuthbertson et al., 2006).

Indications show that there is likely an upper limit to how large CSA the muscle fibers may obtain (Mitchell et al., 2012a, Schoenfeld, 2013). However, some evidence suggests that production of more muscle fibers (hyperplasia) can occur in some animals and under certain conditions (i.e. chronic stretch, strength training), as a hypertrophy-compensatory process (Chalmers et al., 1992). For evident reasons, studies regarding hypertrophy versus hyperplasia relating muscle CSA is only issued indirectly. Anyhow, questions concerning the maximal

degree of hypertrophy achievable through strength training in well-trained subjects are somehow debated and the information is scarce. In an eight-week comparative study performed in resistance-trained, young men, high-intensity strength training (3-5 repetitions, ~90% of 1RM) were tested opposed to high-volume strength training (10-12 repetitions, ~70% of 1RM) (Mangine et al., 2015). It appears that high-intensity strength training stimulated greater improvements in 1RM bench press but produced a similar magnitude in hypertrophy as high-volume strength training. Others submit evidence suggesting that strength training should be performed to the highest intensity of effort (i.e. muscular failure) and, that way, recruit the maximal number of motor units and muscle fibers possible (Gondin et al., 2005). This was exemplified in a study separating active, but untrained women into two distinct groups; total-body training (2-8 RM) and upper-body training (8-12 RM). After 24 weeks of training, both groups showed increased hypertrophy with no significant difference between the groups (Kraemer et al., 2004). Due to good hypertrophy responses but a low number of studies conducted on trained individuals, more studies are desirable.

2.5 Summary

Current research concludes that the constant process of production and degradation in the muscle determines whether there will be a net gain (hypertrophy) or a net loss (atrophy) of muscle mass. Strength training induces muscle hypertrophy, a process in which satellite cell (i.e. myogenic precursor cells) activation is required to contribute in the addition of new myonuclei. Thereby, supporting the increasing nuclear domain.

Muscle fibers adapt according to the stress in which it is exposed to. As fiber type 2 is more vital for explosive strength, provided by activation of high-threshold motor units recruited by increasing effort of activity, the content of fast twitch (type 2) fibers are expected to be higher in strength-trained individuals as opposed to type 1 fibers. Thus, due to its high velocity, type 2X fibers are more suitable with respect to strength training, compared to its subgroups (MHC 1 and MHC 2A).

Conflicting suggestions have been introduced concerning the most efficient load regarding a maximal increase in muscle strength, some assuming high intensity has been required to yield complete motor unit recruitment. Previously, strength training was presumably performed at high resistance, usually ≥70% of 1RM, to elicit favorable

improvement in muscle hypertrophy and strength. Subsequent research has caused the interpretation high-intensity contractions can no longer be considered the exclusive driver of resistance-exercise induced changes. Consequently, evidence illustrates that adult skeletal muscle exposing to different loading modalities is associated with similar responses concerning an increase in muscle strength and CSA.

3 Aim

The main object of this study was to elucidate how unilateral high (90-95% of 1RM) or low load (40-60% of 1RM) strength training may contribute to hypertrophy and strength outcomes, and how this may or may not be reflected by changes and/or differences in muscle fiber cross-sectional area, satellite cells, and myonuclear number in well-trained individuals.

4 Method

This master thesis was implemented as part of a collaboration project performed at GIH, Stockholm, Sweden. The project description was already predetermined, and the present findings are based on the work conducted at GIH. The project started at the beginning of 2017, where the training intervention was carried out. Several tests (e.g. 1RM) were done to measure hypertrophy and force development before muscle biopsies were gathered. From there, the samples were sent to NIH for further analysis.

Ethics and approval

Before entering the study, all volunteers had to answer a health assessment chart, in addition to signing a written informed consent. Here, they received information about the study purpose, experimental procedure and possible risks associated with the study. The protocol was approved by the Swedish ethical review authority (reference: 2016/2159-31) and was performed in accordance with the Helsinki declaration. Participants and all information were handled confidentially, including the data which were kept only available for the leaders of the study. Participants were closely informed that participation was voluntary, and they could withdraw from the study at any time. Ethics was approved by local review board, Etikprövningsnämden Stockholm. The trial number was registered as 2016/2159-31.

4.1 Participants

A total of 16 subjects volunteered to take part in the study, recruited through different forms of social media, as well as ads placed at the university (GIH). Descriptive data of the participants in the study are presented in table 4.1. During the project, a couple (n=2) of participants disrupted the study due to injury caused by activities outside intervention. Altogether, 14 healthy, well-trained men (n=11) and women (n=3) aged between 20-35 years completed the study.

Table 4.1 | Subject characteristics at baseline.
Differences in weight and length between the sexes. Values are given as mean ± standard deviation.

	n = 14	***Men (n=11)***	***Women (n=3)***	
Age, years	26.4 ± 4.4	26.7 ± 4.4	25.3 ± 5.1	26.4 ± 4.4
Weight, kg	79.9 ± 10.7	83.4 ± 9.4	67.2 ± 2.0	79.9 ± 10.7
Height, cm	179.4 ± 7.6	181.7 ± 6.6	169.8 ± 0.3	179.4 ± 7.6
BMI	24.9 ± 2.8	25.3 ± 3.1	23.3 ± 0.7	24.9 ± 2.8

To increase the eligibility of the study, the inclusion criteria were set to a minimum RT experience of two years of lower body strength training prior to the study. This, including one weekly session of the lower body. They also had to be able to participate during entire training intervention and restrict other sorts of exercise. At baseline, 1RM measurements revealed ranges between 46,7-87.1 kg in leg extension. Throughout the training period, the subjects were asked to refrain from other strength training activities involving the lower body. Beyond that, the subjects were allowed to do strength training on the upper body, as well as endurance exercises. Endurance exercises could not cause an increase in endurance exercise volume and intensity if performed during the project.

The subjects had to be able to participate in two scheduled training sessions (Monday and Thursday) per week. To control for confounding physiological factors, their legs were randomly assigned to different unilateral training protocols, consisting of either high-load (HL) or low-load (LL) strength training.

Protein supplementation was given considering some research suggests that it may help enhance the hypertrophic response and assist muscle adaptation to prolonged resistance exercised (Cintineo et al., 2018, Pasiakos et al., 2015, Snijders et al., 2015b). For that reason, subjects were provided with a protein drink containing 27 g of high-quality whey protein dissolved in 300 ml water following each session.

4.2 Study design

Data utilized in this master thesis was gathered in collaboration with the Swedish school of sport and health science (GIH), in which they investigated the effect of muscular hypertrophic adaptations in high- and low-load training modalities. The study continued for a

total of 11 weeks, where the first eight weeks was fully supervised training sessions two times a week (Mondays and Thursdays) (figure 4.1). Data was collected over a period of 11 weeks, from January to March 2017. The intervention lasted from week two until week 10, with a total of 17 planned training sessions per participant. This included a deloading period, at week six, involving only one training session. In addition, training volume was lowered to one exercise and one set per leg, but intensity and number of repetitions remained unchanged. During the first week (week one), all participants performed baseline testing, whereas the load was adjusted to correspond to 90-95% of 1RM for the HL leg and 40-60% of 1 RM of the LL leg. Considering the subjects' previous experience with strength training, it was decided that additional accustoming to the strength training equipment was not necessary. The pre- and post-testing were arranged over one week at week one and week 11, involving both strength training and biopsies from each of the legs.

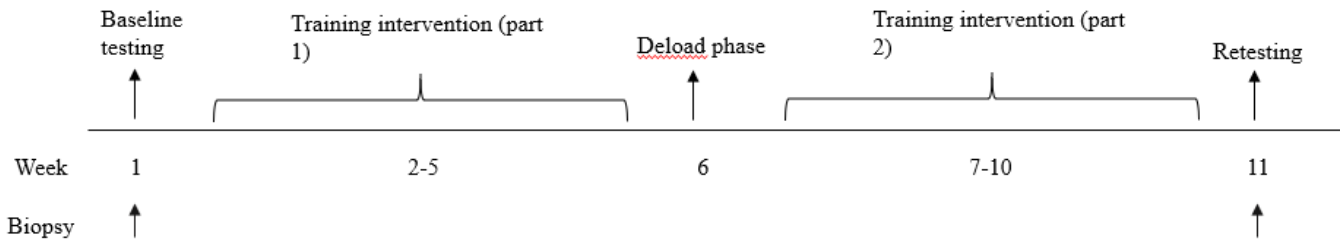

Figure 4.1 | Figurative representation of the training intervention.
Progression and strength training program for the eight weeks of intervention. The table presents the training protocol during the intervention period. The strength training exercises consisted of leg press and leg extension. As mentioned above, the intervention was interrupted by a deload week characterized by a decrease in the number of sets.

4.2.1 Strength training exercises

The subjects were conducted through the strength exercise leg press (LP; Cybex International, Medway, MA, USA) and leg extension (LE; Cybex International, Medway, MA, USA) in a unilateral fashion, as these exercises are considered technically easy to perform and associated with low-risk of injuries etc. Figure 4.2 demonstrates the performance of predetermined strength training exercises. A leg press repetition was considered valid and approved once the platform in which they kept their legs was concentrically pushed until full extension, and then eccentrically lowered to the starting position with a 90-degree angle at the knee joint. Leg press repetitions were correctly performed once the weight was pushed up causing a minimum of 160-degrees in the knee joint angle. To fit them as best as possible, the

equipment was individually adjusted according to each participant. Leg extension, on the other hand, was correctly performed when the resistance was pushed up to a minimum of 160-degree knee angle. The starting position was set to a little less than 90-degrees in the knee joint angle.

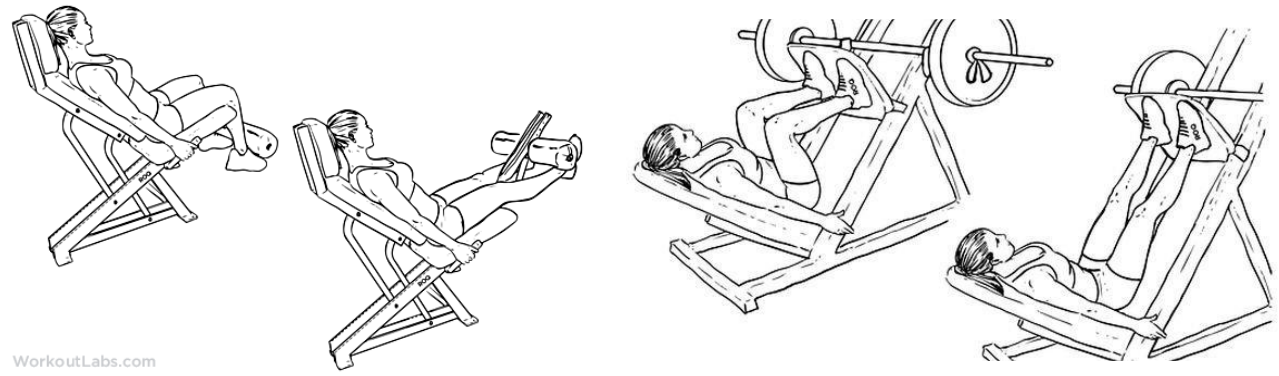

Figure 4.2 The implementation of the exercises.
Drawings demonstrate leg press and leg extension respectively, and the way the exercises were carried out.

4.3 Training protocol

Each training sessions started out with a general warm-up including five minutes cycling on an ergometer bike on an optional intensity, before specific warm-up was carried out in a leg press machine. The manual includes information that sets a good stage for practical learning. Additional warm-up in the strength training machines was required for the leg performing the HL protocol, whereas the leg performing the LL protocol completed the strength training at such low intensities that further warm-up was considered unnecessary. Volume-load was calculated by multiplying the number of repetitions with the load for each session, dividing it with the attended sessions (Morton et al., 2016b). The load was expressed in kg and was set to correspond to 3-5 reps (90-95% of 1RM) for the HL leg and 20-25 reps (40-60% of 1RM) for the LL leg (see table 4.2). If the implementation of the exercise deviated from the prescribed and desired repetition range, the load was adjusted accordingly in the following set. Thus, during the intervention, the load was adapted according to the individual progress to maintain the pre-determined percentage of 1 RM.

Following every session, training was initiated with leg press followed by leg extension. Apart from the deload week, the subjects performed three sets of leg press and three sets of leg extension for both legs. The legs were trained alternately with a recovery

period of two minutes between each set. To avoid any familiarization or crossover effects, the leg starting each training sessions was altered every week. The sessions lasted until all sets and repetitions were completed for the respective subjects. Each set was performed to volitional failure accompanied by strong verbal encouragement before and during each set, due to the mental challenges of maintaining the high intensity. All the test was conducted in the physiological labs at GIH, where the subjects were closely supervised by experienced strength trainers and members of the investigative team.

Table 4.2 Strength training protocol for the intervention.
The table presents the progression and training program during the intervention period for the high- and low-load leg. The strength training exercises consisted of leg press and leg extension. As mentioned above, the intervention was interrupted by a deload week characterized by a decrease in sets.
Warm-up: five-minute cycling on an ergometer bike on self-selected intensity.

Progression model for *M. vastus lateralis*		
Sessions/week	Both legs	2
Exercise/session	Both legs	2
Set/exercise	Both legs	3
Set/week	Both legs	12
Repetitions	HL reps/exercise	3-5
	HL reps/week	48
	LL reps/exercise	20-25
	LL reps/week	270
Load %	HL	90-95
	LL	40-60

4.4 Muscle sampling

Muscle biopsies were obtained from the *vastus lateralis* at least 48 hours before the pre-testing at week one, and 48 hours after the final tests at week 11. Prior to the procedure, aseptic precautions were taken, and the skin was disinfected using a surgical antiseptic solution, chlorhexidine. The area was locally anesthetized (2% Carbocain, AstraZeneca, Södertälje, Sweden) before a small incision was taken in the skin and the muscle fascia. The biopsy procedure was conducted utilizing a Weil-Blakely conchotome. This method has previously been reported by Henriksson (1979) as a semi-open muscle biopsy and is a safe procedure to obtain muscle samples from humans. The closed jaws of the sterile conchotome were inserted through the incision (Dietrichson et al., 1987). The jaws were placed parallel to the muscle fascicles. The jaws were opened and the conchotome twisted 180-degrees after it had been closed around the muscle sample. In general, one can obtain 50-150 mg of muscle tissue for each biopsy. As for the opposite leg, the same procedure was performed for all the participants. After the biopsy, the tissue was quickly cleaned for connective tissue, blood, and fat. The tissue was subsequently distributed for various purposes. The muscle with the largest surface area was selected and shaped to a perpendicular square using a razor blade before it was enclosed in an O.C.T. compound and mounted in an embedding medium (OCT Cryomount, Histolab Products AB, Gothenburg, Sweden). The sample was frozen in isopentane pre-cooled to its freezing point (-120˚C) in liquid nitrogen. The samples were then stored in a freezer (-80˚C) for further immunohistochemical analyzes.

4.4.1 Muscle tissue sectioning

To start with, the samples were first removed from the freezer (-80˚) and placed in a cryostat (CM1860 UV, Leica Microsystems; Nussloch, Germany) for 20 minutes along with the equipment (scalpel, brushes, tweezers) that were necessary for successful cutting. Furthermore, the tissue was attached to a cutter screw with OCT before it was mounted to the cutting head. The quality of the sample and the orientation of the muscle fibers were mapped by trimming the tissue piece. In this way, one could also achieve a clear cutting-surface and correct orientated muscle fibers. Each sample was cut with a thickness of 8 µm and mounted on SuperFrost Plus (Thermo Fischer/Gerald Menzel, Braunschweig, Germany) slides. All muscle samples from the same subject were placed on one single slide (figure 4.3). That is, in

this case, for each subject, four muscle biopsies, one from each leg before and after the training intervention.

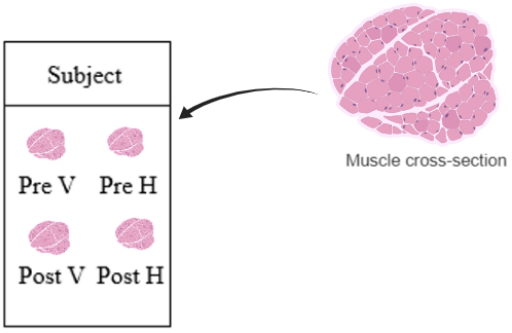

Figure 4.3 | Template of muscle tissue sample. The muscle samples from each subject were placed on a slide with two sections; one from the left leg (pre-V and post-V respectively) and one from the right leg before and after the training intervention (pre-H and post-H) (see figure 4.2). Since the legs were randomized, one could not tell from the sample which leg had performed HL or LL.

4.5 Immunohistochemistry

Antibodies have a variety of preferences to which protein structure they prefer to bind. An overview of the relevant primary and secondary antibodies used for identification of such protein structures in this project is given below (table 4.3). A total of three protocols for staining were used to identify satellite cells (Pax7), myonuclei (PCM1 and DAPI) and the muscle fiber type composition (MHC1). Winje et al. (2018) demonstrated in a study that pericentriolar material-1 (PCM1) specifically labeled all myonuclei in skeletal muscle of both rodents and humans, making it a good histological marker for labeling myonuclei in skeletal muscle. To determine the localization of the different nuclei, antibodies were also used for labeling the cell membrane (antibody against laminin or dystrophin).

Table 4.3 | Primary and secondary antibodies.
Overview of the primary and secondary antibodies used for staining and identification of specific proteins during the immunohistochemistry. A total of three protocols staining-protocols were used to identify the cells of interest (i.e. satellite cells (Pax7), myonuclei (PCM1 and DAPI) and the muscle fiber type composition (MHC1)).

Antibody	Binds to	Producer	Host organism	Dilution	Product number
Anti PAX7	Paired box transcription factor 7	DSHB	Chicken	1:20	Ab528428
DAPI	A-T rich regions in nuclei DNA	Invitrogen		Found in muting solution	P36931
Anti-dystrophin	Dystrophin in the cell membrane	Abcam	Rabbit	1:500	Ab15277
Anti-laminin	Laminin	DAKO	Rabbit	1:500	Z0097
Anti MHC1	Myosin heavy chain 1	DSHB	Mouse	1:500	BA-D5
Anti PCM1	Cytoplasm and centrosomes	Sigma	Rabbit	1:1000	B114638
MANDYS8(8H11)	Dystrophin	DSHB	Mouse	1:20	MANDYS8(8H11)
Anti mouse Alexa® 488	(Anti-) mouse	Invitrogen	Goat	1:200	A11001
Anti rabbit Alexa® 488	(Anti-) rabbit	Invitrogen	Goat	1:200	A11008
Anti rabbit Alexa® 594	(Anti-) rabbit	Invitrogen	Goat	1:200	A11012
Anti mouse Alexa® 594	(Anti-) mouse	Invitrogen	Goat	1:200	20110

4.5.1 Satellite cell labeling

The sections were incubated with a 4% formaldehyde and 0.05% Triton X-100 (T8787, Sigma Life Science, St. Louis, MO, USA) solution for 10 min (appendix 1). The samples were then washed with PBS (phosphate buffered saline, Sigma, P4417, USA) for 3x3 min before reincubated with serum-free protein block (X0909, Dako, Glostrup, Denmark) for 10 min at room temperature. Afterward, the sections were incubated with a primary antibody against laminin and Pax7 at four degrees overnight. The following day, sections were washed 3x3 min in PBS and then incubated for 60 minutes at room temperature with the secondary antibody consisting of Alexa 488 anti-mouse (Alexa fluor 594 goat anti-rabbit IgG) and Alexa 594 anti-rabbit (Alexa fluor goat anti-mouse IgG) diluted in 1% BSA (bovine serum albumin; A4503, Sigma Life Science) in PBS (524650, Calbiochem, EMD Millipore, Darmstadt, Germany). At ended incubation, the sections were washed 3x3 min before mounted with a mounting medium containing DAPI (Invitrogen, P36931) and covered with a cover glass (Marienfeld, 0107222, Lauda-Königshofen, Germany).

4.5.2 Labeling of myonuclei

Sections were collected from the freezer and placed at room temperature (see appendix 2). Briefly, sections were preincubated for 30 minutes with 2% BSA diluted in PBS-t, before incubated with the primary antibodies at four degrees overnight. The primary antibodies consisted of PCM1 (Sigma, B114638) and MANDYDS8 dystrophin (DSHB, MandyDS8) diluted in a solution of 5% BSA (bovine serum albumin, Sigma, A4503, USA) dissolved in PBS-t added 0,02% Igepal CA-630 (Sigma, I3021)). Following the overnight incubation, the sections were washed 3x5 minutes in PBS before incubated with the secondary antibodies (Alexa 488 conjugated anti-rabbit and Alexa 594 conjugated anti-rabbit) for one hour at room temperature. The secondary antibodies were diluted in 2% BSA dissolved in PBS. The sections were once again washed for 3x5 minutes in PBS before the cover glass was placed using a mounting medium containing DAPI.

4.5.3 Labeling of fiber types

Detailed staining protocol for fiber type see appendix 3. Fiber type CSA and distribution were stained with antibodies against myosin heavy chain 1 (BA-D5, DSHB, monoclonal) and anti-dystrophin (Abcam, rabbit polyclonal). Briefly, sections were blocked with 1% BSA in PBS with 0,05% Tween-20 (PBS-t) for 60 min at room temperature before incubated in primary antibodies overnight at 4 degrees. Next day, sections were washed 3x10 min in PBS-t and incubation in secondary antibodies for 60 min at room temperature. The secondary antibody consisted of Alexa 488 anti-mouse (Alexa fluor® αM goat anti-mouse IgG) and Alexa 594 anti-rabbit (Alexa fluor® 594 goat anti-rabbit IgG). At ended incubation sections was washed 3x10 min in PBS-t before being mounted with Prolong Gold antifade reagents with DAPI and covered with a coverslip. Fiber types showing negative stain against myosin heavy chain 1 (black/not stained) was quantified as type 2 fibers. Information about antibodies, see table 4.3.

4.6 Microscopy

4.6.1 Analysis

All images were visualized using light microscopy (Olympus BX61, Japan) with a fluorescence light source (EXFO X-Cite 120PC-Q, Ontario, Canada) using a 4x air (UPlanFL Nm 0,12 NA, Olympus) or a 10x air objective (UPlanFL N, 0,55 NA, Olympus). Micrographs were captured using a connected digital camera (DP72, Olympus). Fiji image processing software (released under the General Public License) was employed to inspect the images and quantify the number of satellite cells and myonuclei.

4.6.2 Quantification of satellite cells

For every sample, an overview picture was taken with a 4x air objective and later printed in paper form. This made it easier to locate the satellite cells and relate them to the respective fiber type. The samples were examined through a 10x objective in the microscope and illuminated by fluorescent light. That way, photo sequences were obtained with the light of different wavelengths to visualize and identify the different antibodies bound to the

respective proteins/structures in the sample. Positive PAX7 labeling was shown in the form of a bright green stain, whereas positive DAPI labeling was visible in the microscope as a blue stain.

Previously, in the lab at the Norwegian school of sports sciences, NCAM has been extensively used as a marker but has been associated with challenges to quantifying satellite cells with high accuracy. Satellite cells were included if an apparent overlap in shape, size, and location between PAX7 (green color) and the nuclei marked with DAPI (blue color) could be observed (figure 4.4). This was set as a criterion for defining each the satellite cells. Which muscle fiber the satellite cells were associated with, was determined using an antibody against laminin (red color) to label the basal lamina. The satellite cells have a unique anatomical location peripheral in the muscle fiber, between the sarcolemma and the basal lamina (Morgan and Partridge, 2003b). The muscle fiber type to which the satellite cells belonged to was determined by staining an equivalent sample from the same leg and the same subject with an antibody against myosin heavy chain 1 and dystrophin (table 4.3). The result is presented as the number of satellite cells per 100 fibers of each fiber type. Folds or other damages on the samples were stained again. Still, some parts were ruled out.

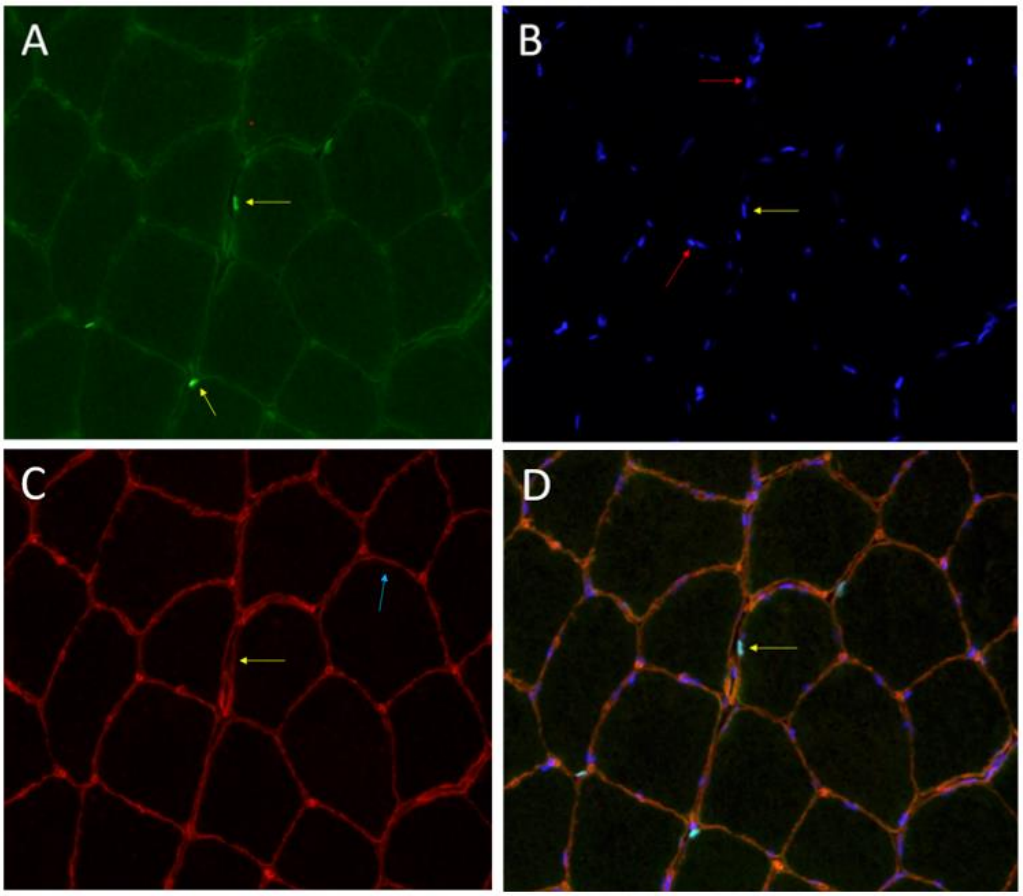

Figure 4.4 | Quantification of satellite cells by positive PAX7 labeling.
The biopsy samples were stained with specific antibodies and examined through the microscope. The images were later used for counting the total number of satellite cells. (A) The image illustrates staining of satellite cells with PAX7 (green). The yellow arrows indicate the position of the satellite cell, located in between the sarcolemma and basal lamina. (B) Yellow arrow points out the same satellite cells as identified in image A. The red arrows show the myonuclei, which is stained with DAPI (blue). (C) The basal lamina represents the membrane of the muscle fibers and are stained with laminin (red), illustrated with blue arrows. The yellow arrow is the same satellite cell identified in image A. Further, the image was merged (D) to easier quantify the satellite cells. An apparent overlap in shape, size and location between PAX7 (green color) and the nuclei marked with DAPI (blue color) led to the inclusion of the satellite cell. Finally, an equivalent sample from the same leg was stained (myosin heavy chain 1 and dystrophin) to determine the muscle fiber type in which the satellite cell was connected to (figure 4.5).

4.6.3 Fiber type distribution

Fiber specific CSA and fiber type distribution were analyzed using a micrograph obtained with a 4x objective and calculated using TEMA software (CheckVision, Hadsund, Denmark). Muscle fiber type 1 was observed as MHC1-positive cells (green) and the basal lamina was stained with dystrophin (red) (figure 4.5). Further, the fiber CSA and fiber type composition were calculated. Muscle fibers at the outer edge of the sample were excluded

from the analysis, in addition to fibers with abnormal shape (i.e. long or uneven cell membranes). The CSA area is presented as μm^2.

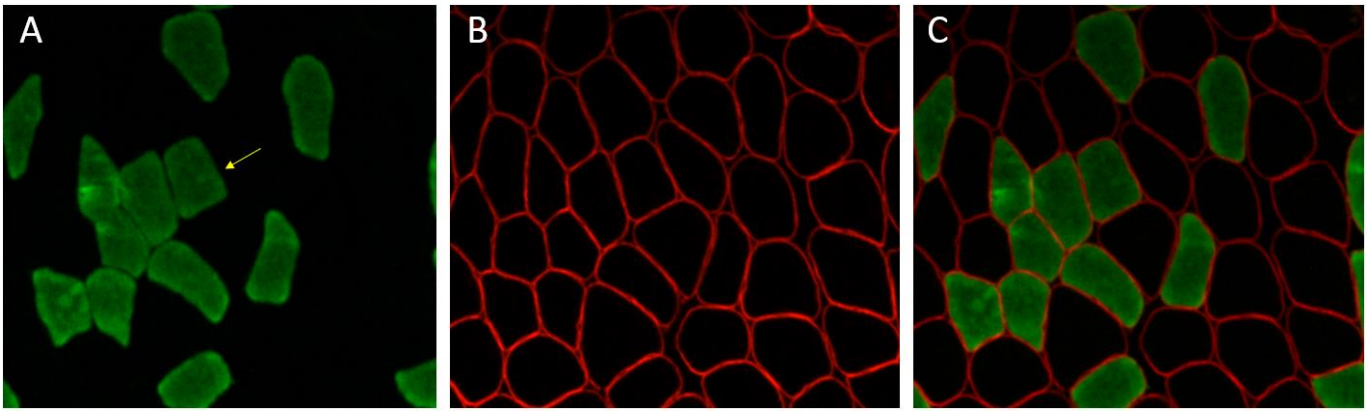

Figure 4.5 | Illustration of muscle fiber type staining.
Image illustrations of small muscle fiber sections from overview images used to analyze muscle fiber type and muscle fiber CSA. Staining with MHC1 (A) are presented in green, whereas the yellow arrow points out a single muscle fiber type 1. The black cells, on the other hand, are either fiber type 2A or 2X. Dystrophin (B) was used to visualize the basal lamina. When merging the two images together (C), staining (dystrophin and MHC1) allowed a greater perception of the distribution of type 1 and type 2 fibers in the muscle.

4.6.4 Quantification of myonuclei

Two pictures where obtained from each sample after examining them through a 10x objective. Myonuclei were quantified by counting 50 type 1 and 50 type 2 fibers. Next, these images were combined with those from the fiber type analysis. As for the satellite cells, a myonucleus was included if both the labeling of PCM1 and DAPI had the same shape, size, and co-localization (figure 4.6). Only then it would be counted and included in further analysis. The results will be presented as the number of myonuclei per type 1 and type 2 fibers separately.

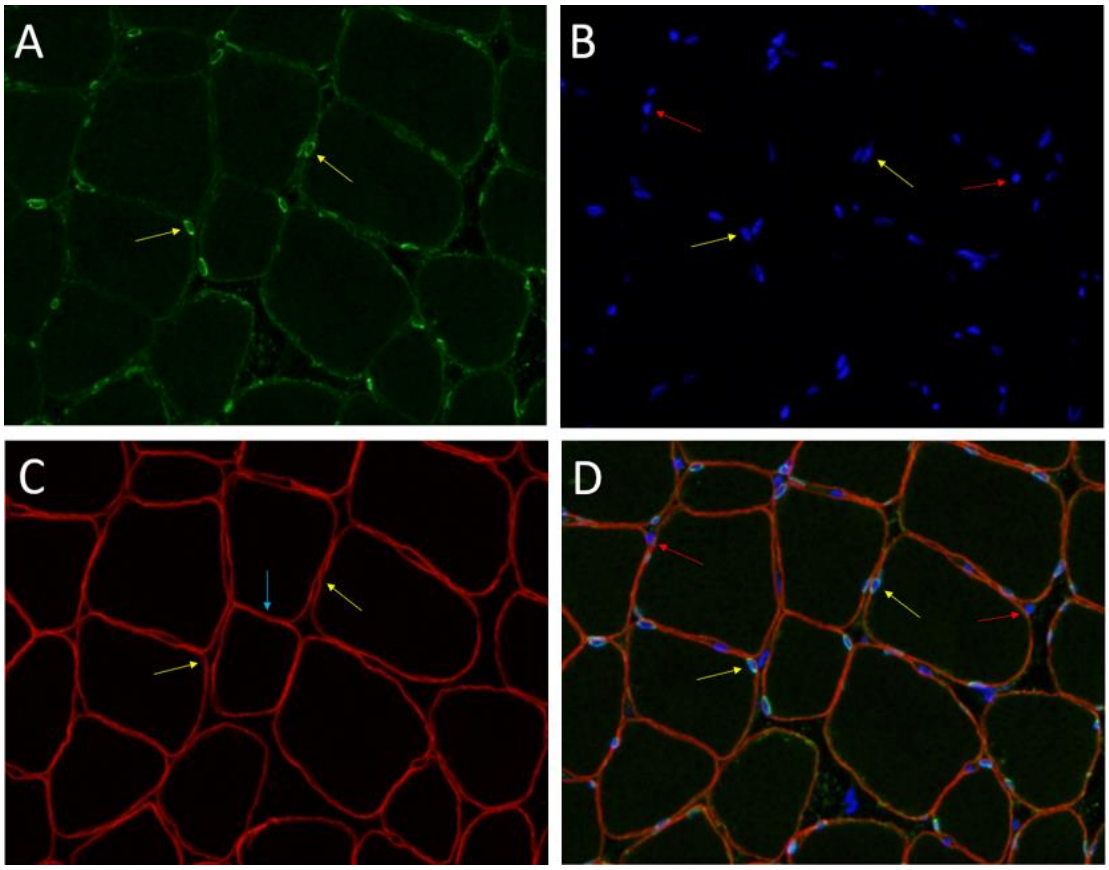

Figure 4.6 | Quantification of myonuclei by positive PCM1 labeling.
The biopsy samples were all stained with specific antibodies, in this case DAPI, dystrophin and PCM1, before examined through the microscope. The images were later used to count the myonuclear number associated with each muscle fiber. Some of the PCM1 positive myonuclei (green) is illustrated by the yellow arrows. The yellow arrows are represented in image B as well, but in addition, staining with DAPI led to the visualization of all the nuclei present in the muscle (blue). The basal lamina was stained with dystrophin (C). Lastly, an overlay image (D) was used to detect all the nuclei belonging to the counted muscle fibers. Nuclei positive for both DAPI and PCM1 was accounted as myonuclei.

4.7 Statistical analysis

All values are presented as individual variation, means and standard deviation (SD) if not otherwise is stated. In this study, statistics were used to investigate differences between and within the protocols. Since analysis for paired comparisons (paired t-test) equivalence to ANOVA, it was encouraged to use a 2-way ANOVA to investigate the effects of training and differences between the training protocols. If the statistical test showed an interaction effect, a Holm-Sidakś multiple comparisons test was performed. The analysis was set to involve correlation between the number of satellite cells and myonuclei corresponding to the different fiber types (type 1 and type 2) before and after training intervention, and comparisons between the legs in which had performed the high- and low-load training. Significant level

was set to $p < 0.05$ for all measures, meaning that levels below indicated no correlation between the training modalities. Calculations were performed in Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

5 Results

5.1 Baseline measurements and descriptive characteristics

A total of 14 subjects completed the study (table 4.1), each subject with one leg performing HL and the other LL. Overall adherence to training during the intervention was high, with a participation rate of 95.8% of those who completed the study. The study included both men and women, and since the number of female participants was low (n = 3) as opposed to men (n = 11), analysis comparing the two sexes were not performed. At baseline, there was no difference in either CSA, fiber distribution (%), number of satellite cells, nor the number of myonuclei between the HL and LL leg (table 5.1). The strength in 1RM leg press and leg extension is listed as well.

Table 5.1 | Descriptive characteristics of muscle variables at baseline.
Baseline values of 1RM strength in leg press and leg extension, fiber CSA and distribution (%), the number of satellite cells (per 100 muscle fiber) and the number of myonuclei (per fiber) for each protocol and are based on a total of 14 participants. There was no difference between the HL and LL leg. Values are given as means ± standard deviation for type 1 and type 2 fibers separately.

	HL	LL
Strength (1RM, kg)		
Leg press	170.9 ± 43.8	169.8 ± 48.2
Leg extension	68.4 ± 13.7	70.4 ± 13.4
Cross-sectional area		
Type 1 (μm^2)	5373 ± 962	5016 ± 1064
Type 2 (μm^2)	6639 ± 1186	6715 ± 1268
Fiber distribution (%)		
Type 1	47.1	43.1
Type 2	52.9	56.9
SC per 100 fiber		
Type 1	10.2 ± 2	11.2 ± 4
Type 2	13.5 ± 5	14.5 ± 8
Myonuclei per fiber		
Type 1	3.3 ± 0.7	3.6 ± 0.8
Type 2	4.1 ± 0.9	4.1 ± 0.8

5.2 Training effects

The average total training volume per session for the HL and the LL leg was higher in the LL leg, with 2671 ± 678 kg for the HL leg and 9289 ± 2839 kg for the LL leg ($p < 0.001$) (Figure 5.1). The same difference in training volume was observed between legs for each exercise ($p < 0.001$). In total, the training volume for HL leg in leg press was 1937 ± 553 kg and 7615 ± 2501 kg for the LL leg ($p < 0.001$). Leg extension training volume was 798 ± 210 kg for HL leg and 1893 ± 458 kg for the LL leg ($p < 0.001$).

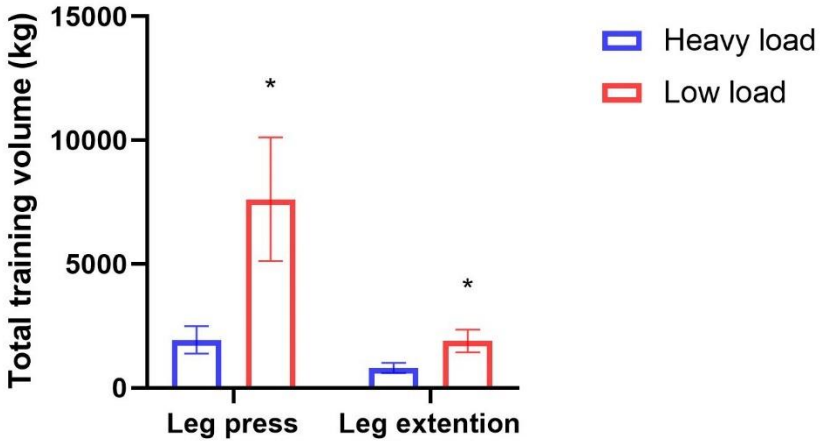

Figure 5.1 | Average training volume per strength training exercise.
The average total training volume per session was higher in the LL leg in comparison to the HL leg. The same variation in training volume was seen between the exercises for each leg, while the training volume for the LL leg was significantly higher ($p < 0.001$) than the LL leg for both leg press and leg extension. Training volume is by multiplying the load with the number of repetitions for each session. The values are given as mean (±SD) values and * indicates the significant difference in average volume for each exercise between the legs.

5.2.1 Muscular strength

Following the intervention, no significant difference in strength increase was noted between the legs ($p = 0.88$, figure 5.2). The maximal leg press strength increased significantly and equally great for both HL (170.9 ± 43.8 kg to 205.6 ±49.8 kg; $p < 0.001$) and LL (169.8 ± 48.2 kg to 205.5 ± 50.3 kg; $p < 0.001$). As for leg extension, no difference was observed in the LL leg (70.4 ± 13.4 kg to 68.5 ± 14.3 kg; $p > 0.05$), whereas a significantly increase in strength was seen for the HL leg (68.4 ± 13.7 kg to 74.3 ± 15.9 kg; $p < 0.05$). Still, post-values revealed no significant difference between legs.

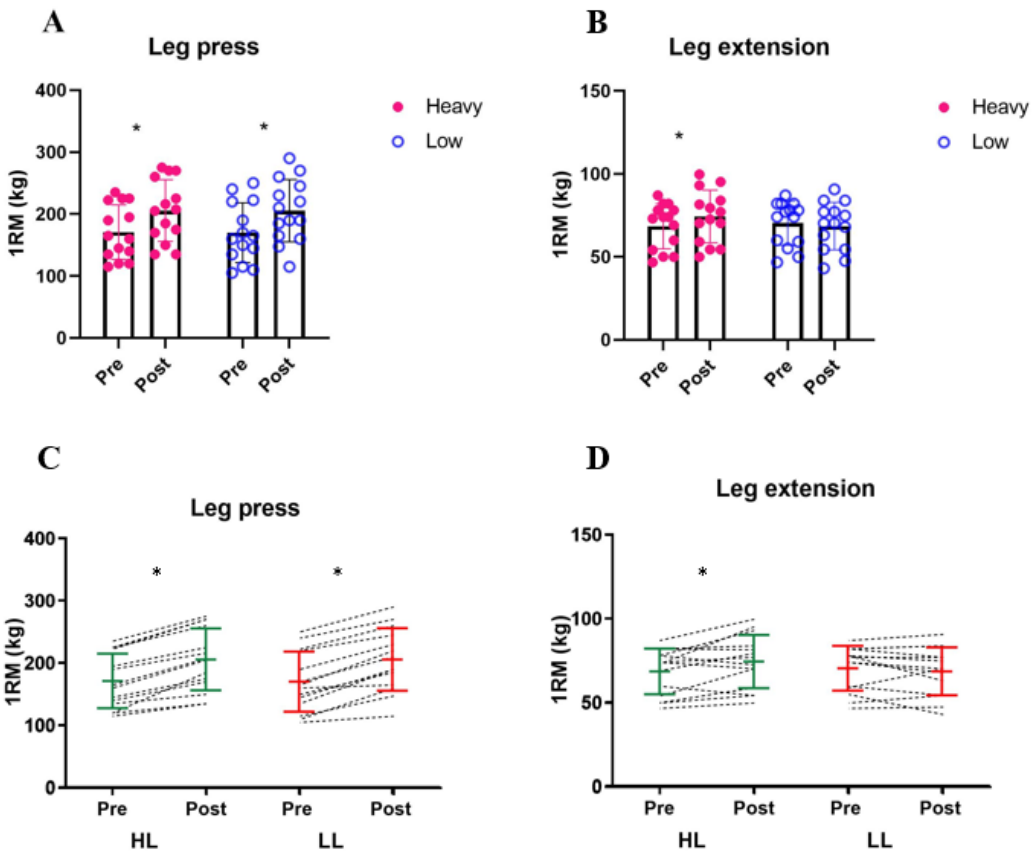

Figure 5.2 | Increases in 1RM leg press and leg extension (kg) for the HL and LL leg. Change in 1 RM strength in *vastus lateralis* after unilateral leg press and leg extension for both HL (pink) and LL (blue), prior to (pre) and after (post) eight weeks of training. (A) For the leg press exercise, both legs showed a significantly (*) greater increase in maximal strength than the corresponding pretraining values in both the HL and LL leg ($p < 0.001$). Additionally, the HL protocol in leg extension (B) resulted in a significant increase in strength ($p < 0.05$). The stippled lines show individual lines in strength following the training intervention are presented for leg press (C) and leg extension (D). No significant difference was noted between the legs (HL and LL) after the intervention. Each dot represents a participant (A, B). The values are expressed as kilograms (kg)given as mean (±SD) values and * indicates the significant increase in 1RM strength. N = 14.

The percentage change in strength between the high- and low-load leg in the leg press exercise at baseline was tested performing a paired t-test, but the comparison did not reveal any significant difference ($p = 0.543$). Overall, the strength in HL increased by 9 ± 11% (95% CI, 2.66-15.6; $p = 0.543$) whereas LL decreased by 2 ± 11% (95% CI, -8.94-4.0) (figure 5.4). No significant difference at baseline was calculated (these figures are not included in this thesis, as they can be related to figure 5.3C and D).

During the immunostaining, a couple of samples showed areas of freeze-damage. However, these areas were excluded from the analyses. Unfortunately, one of the samples were excluded on this basis due to the challenges caused when analyzing this sample. Some

of the samples had a low number of fibers (type 1/type 2 fibers < 50), so that the number of counted myonuclei differed slightly among the subjects.

5.2.2 Cross-sectional area

After eight weeks of strength training, there were no change in CSA of type 1 fibers for HL (5376 ± 1033 µm^2 vs. 5226 ± 1190 µm^2) or LL (4913 ± 1050 µm^2 vs. 5066 ± 1039 µm^2) leg (figure 5.3A and C). The same was observed in the type 2 fibers for HL (6643 ± 1298 µm^2 vs. 6845 ± 1224 µm^2) and LL (6684,36 ± 1325,43 µm^2 vs. 6851,55 ± 1336,60 µm^2) leg (figure 5.3B and D).

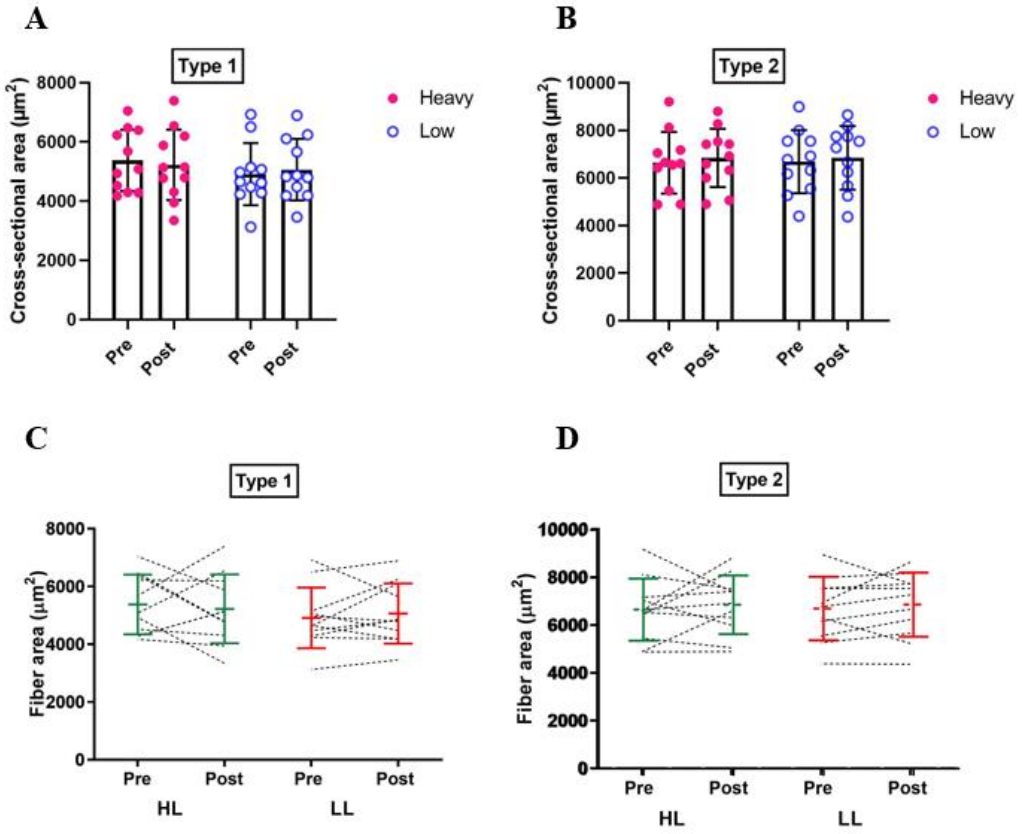

Figure 5.3 | No significant increase in CSA (µm^2) in type 1 and type 2 fibers.
CSA (µm^2) in type 1 fibers (A) and type 2 fibers (B) for the HL (pink) and LL (blue) leg prior to (pre) and after (post) the training intervention. Each dot represents a subjects' given value at the various times. (C) (D) Individual lines in fiber area (µm^2) for type 1 and type 2 fibers, respectively. Each dot represents a participant. The values are expressed as micrometers (µm^2) and given as mean (±SD) values. N = 14.

5.2.3 Satellite cells

Eight weeks of high- and low-load strength training showed no changes in the number of satellite cells in type 1 (figure 5.4A and C) or type 2 (figure 5.4B and D) fibers for the HL or LL leg. In type 1 muscle fibers, the number of satellite cells for the HL leg was unaltered with 10.3 ± 2.3 satellite cells per 100 muscle fibers at baseline to 13.3 ± 5 satellite cells after the training intervention. Satellite cells per 100 muscle fiber in the LL leg was unchanged from baseline values of 11.1 ± 3.7 satellite cells (pre) to 13.1 ± 6.7 satellite cells (post) during the eight-week training intervention. Per 100 type 2 muscle fibers, the number of satellite cells for HL and LL was unchanged from 13.7 ± 4.9 satellite cells per 100 fibers at baseline to 16.0 ± 5.2 (post) after eight weeks of training, and 14.9 ± 7.2 (pre) to 16.6 ± 9.1 (post) respectively.

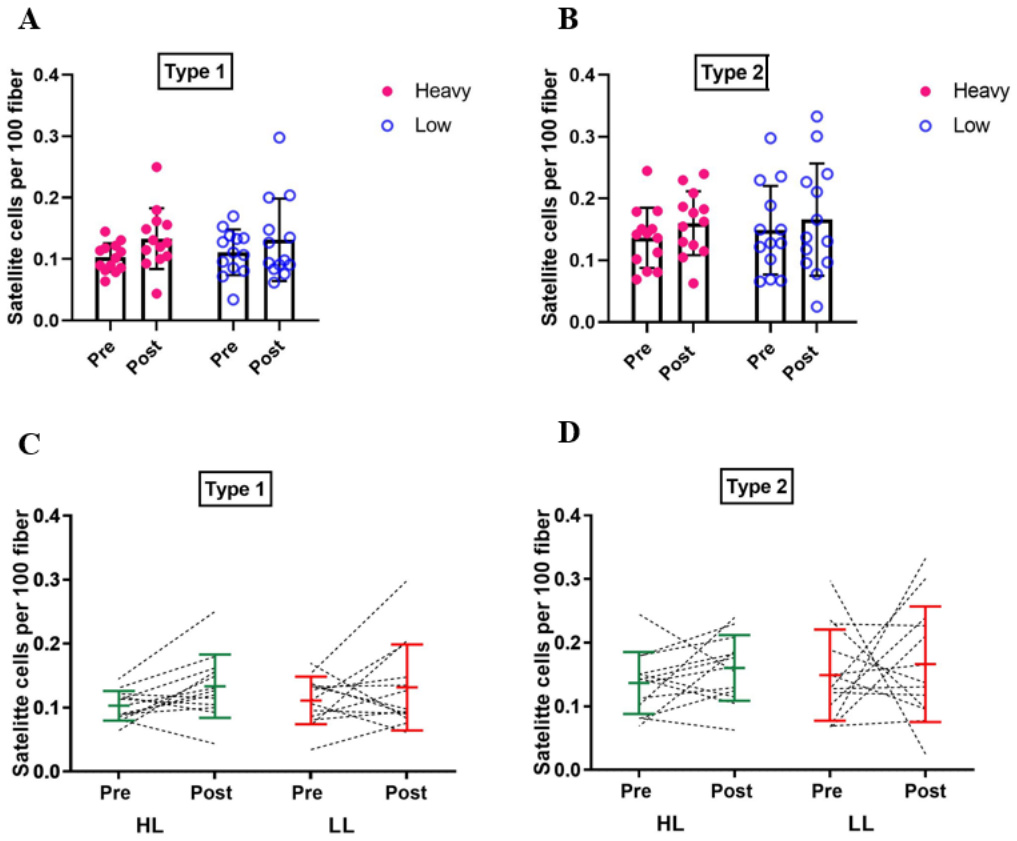

Figure 5.4 | No increase in the number of satellite cells per 100 muscle fiber.
Mean satellite cell distribution in type 1 and type 2 fibers prior to (pre) and after (post) the training intervention. The results are grouped, representing the change in satellite cell number in type 1 (A, C) and type 2 (B, D) fibers after HL (pink) and LL (blue) strength training. (C, D) Individual lines for both legs and fiber types. There was no significant difference between the protocols or the fiber types after the training intervention. Each dot represents a participant. The values are expressed as satellite cells per type 1 muscle fiber and given as mean (±SD) values. N = 14.

As opposed to the mean values, two subjects differed greatly in satellite cell number at baseline and after the training intervention (HL: 14.5 to 25.0; LL: 15.3 to 19.8). As for the type 2 fibers, the individual variance was to a much greater extent scattered, both for baseline and post-values. Additionally, some of the subjects with the highest satellite cell number at baseline experienced a decreased satellite cell number following intervention (e.g. subject one; HL: 24.5 to 11.5; subject two; LL: 29.8 to 2.5).

The satellite cell content in both HL and LL was combined and analyzed by a paired t-test. The HL and LL protocol resulted in no significant increase in type 2 fibers ($p = 0.395$), contrary, the satellite cell number increase significantly in type 1 fibers ($p = 0.05$). Comparing the HL and LL leg for type 1 and type 2 fibers, the change in satellite cell number was not significant ($p = 0.184$).

5.2.4 Myonuclei

After the training intervention, the number of myonuclei was unchanged from 3.3 ± 0.7 (pre) to 3.3 ± 0.6 (post) for the HL in type 1 muscle fibers (figure 5.5A and C), and from 4.1 ± 0.9 (pre) to 3.9 ± 0.9 (post) for type 2 fibers (figure 5.5B and D). As for the LL leg, the myonuclear number in type 1 fibers was unchanged giving 3.6 ± 0.7 (pre) to 3.3 ± 1.0 (post) (figure 5.5A), and 4.1 ± 0.8 (pre) to 4.0 ± 1.0 (post) myonuclei per fiber type 2 (figure 5.5B).

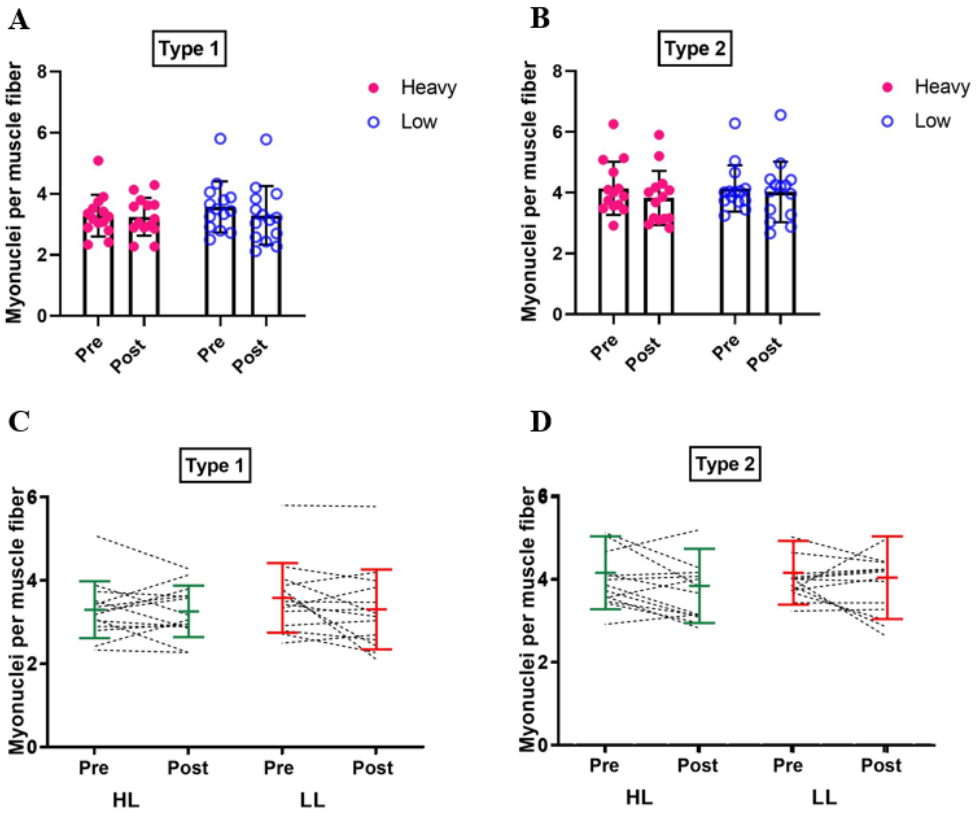

Figure 5.5 | No increase in the number of myonuclei.
The mean myonuclear number per muscle fiber represented in both type 1 (A) and type 2 (B) fibers. The number of myonuclei remained unchanged after eight weeks of HL and LL strength training. (C) Individual variation in myonuclear number in type 1 fibers. (D) The individual lines in myonuclear number in type 2 fibers. Each dot represents a participant. The values are expressed as myonuclear number per muscle fiber type 1 and given as mean (±SD) values. N = 14.

6 Discussion

Several studies have investigated the strength and hypertrophic response following high- and low-load strength training. To our knowledge, no studies have investigated cellular responses (i.e. the number of satellite cells and myonuclei) in human skeletal muscle exposed to strength training with either high-loads (3-5 reps) or low-loads (20-25 reps). Thus, the aim of this study was to investigate cellular adaptations in the human skeletal muscle caused by two different loading modalities during strength training. In short, comparing the legs performing high- and low-loads, the training resulted in changes in strength while there was no significant difference in either fiber specific CSA, satellite cells nor myonuclear number. However, while 1RM leg press increased equally in the high- and the low-load leg, only the high-load leg increased significantly in 1 RM leg extension.

6.1 Training effect on muscular strength

6.1.1 Increased muscular strength

Eight-weeks of high- and low-load strength training to volitional failure resulted in a significant increase in muscular strength measured as 1RM in the leg press, acknowledging no significant difference between the two loading modalities (Figure 5.2). A significant increase in strength gains was also observed for the high-load protocol in leg extension, while the low-load protocol led to no significant changes in 1RM leg extension. Compared to this study, research on untrained individuals have shown a rapid and early increase in strength following strength training (Abe et al., 2000, Abe et al., 2006, Adams and Haddad, 1996). Based on Kraemer et al. (2002), muscular strength increases are found to be approximately 40% in untrained individuals, in contrary to 16% in trained, 10% in well-trained and 2% in elite athletes over periods ranging from 4 weeks to 2 years. Relative to the present study, our subjects were considered well-trained which gives us reason to believe we could expect an increase in muscle strength close to trained or advanced individuals. Thus, this is in line with our findings, as muscle strength increased within roughly 10-20% for all the 1RM exercises except for the low-load leg extension.

Our findings are to some degree compatible with the study of Morton et al. (2016b). Comparing the effects of different loading zones on strength improvements in resistance-trained men, high- and low-load demonstrated the same consistent increase in strength, at least for 1RM leg press, when training was performed to volitional failure. Contrary, a majority of studies investigating strength gains in well-trained individuals have concluded that high-load training is more beneficial than low-load training in regards to strength increases (Ahtiainen et al., 2003, Ahtiainen et al., 2005, Allen et al., 1999). The degree of motor unit recruitment has been studied during muscle contraction and have been postulated to be dependent on the effort of activity (Bamman et al., 2007). Moreover, to produce further neural adaptations due to full motor unit recruitment, a load of >60-70% of 1RM has been recommended to maximize muscular strength and hypertrophy in untrained individuals. Instead, individuals accustomed to strength training should train with loads >80% of 1RM (Bagby et al., 1972, Bamman et al., 2007). Still, when sets have been performed to volitional failure, even low-loads will result in full motor unit recruitment (Sale, 1987). It seems as high-threshold motor units are recruited lastly, at the end of a failing reaching set (Henneman, 1985). This may explain the current findings, proposing that the training caused activation of a large range of motor units, ensuring full muscle activation in both legs. Basically, the time under load was quite similar but lifting low-load may induce more stress of the type 1 fibers, though the results showed no fiber type-specific differences (Ogborn and Schoenfeld, 2014). High-load strength trained are also better accustomed to lifting high-load, such as 1RM, and may for that reason have adapted a better technique during strength testing.

Schoenfeld et al. (2015a) presented a study investigating different loading zones in well-trained men. The high-load group was set to do 70-80% of 1 RM and the low-load group was set to do 30-50% of 1 RM, hence somewhat lower in comparison to the present study (90-95% 1 RM; 40-60% 1 RM respectively). The training volume for the low-load group was three times higher compared to the high-load group which is more or less similar to our low-load protocol. Surprisingly, the high-load strength training in Schoenfeld's study was superior to low-load when aiming for maximal strength adaptations. Furthermore, generally accepted recommendations are divergent in contrast to present findings, in which profess that maximal improvements in strength are achieved solely by heavy strength training beyond ≥ 70% of 1 RM (Allen et al., 1995b, American College of Sports, 2009). These recommendations are based upon studies concluding that loads corresponding to >70% of a subjects' 1RM have led

to greater increases in muscle strength (Campos et al., 2002b, Kalapotharakos et al., 2004, Peterson et al., 2004), with even higher loads (>80% of 1RM) are necessary to increase further neural adaptations and strength in experienced lifters (Häkkinen et al., 1985).

The results from leg press showed that both training modalities led to a similar increase in strength although the subjects' pre-training status in the current study was better. To put it in perspective; in a review, Häkkinen (1985) introduced the differences in muscle strength increases between groups differing greatly in the level of their pretraining strength (see figure 2 in ref.). He presented documentation of trainability status of the subjects at a given time and maximal strength increases between trained and untrained individuals. The percentage change in muscle strength occurring over the 24 weeks of training showed a remarkable difference favoring the untrained individuals. This suggests that strength increases (i.e. progression) tend to slow down with developing strength status.

Purposeful discrepancies in total strength training volume (repetitions x load) between protocols were created by the experimental design, leaving an unmatched total of volume lifted. On average, the low-load protocol included a significantly higher volume in contrast to the high-load leg (Figure 5.2). Yet, post-values of muscle strength revealed no significant difference in increased strength between leg press and leg extension. A similar study by Schoenfeld et al. (2014c) tested volume-equated strength training in well-trained individuals. Investigating strength gains performing 3 sets of 10 RM or 7 sets of 3 RM with leg press, leg extension and barbell back squat, the high-load protocol produced a superior increase in maximal strength, despite both protocols led to the same increase in muscle size (study supported by (Burd et al., 2012, Burd et al., 2010). This observation is consistent with previous studies on volume-matched strength training in untrained individuals, whereas high-load was better regarding a maximal strength increase (Burke et al., 1974, Campos et al., 2002a). Consequently, such volume-matched situations indicate that high-load is more efficient considering muscle strength and may prevent low-load protocols to reach the same degree of muscular failure. The unmatched volume situation in the current study, however, represent a full muscle activation in the low-load protocol, reaching complete muscular failure. And by that, given the chance to produce comparable results as the high-load protocol.

6.1.2 Training effect on cross-sectional area

No significant increase in fiber cross-sectional area

The individual variance in fiber CSA differed greatly between some of the subjects (e.g. mean CSA: 3419µm^2 vs. 10425µm^2) at baseline (see Table 5.1). The training period of eight weeks led to no difference in fiber CSA between the leg performing high- or low-load strength training (Figure 5.3). The overall CSA did, however, increase in type 2 fibers when both legs were pooled and analyzed together. Assuming the training was performed to volitional failure, our study, along with others (Mangine et al., 2015, Schoenfeld et al., 2015a, Schoenfeld et al., 2014b, Ahtiainen et al., 2003, McCall et al., 1996), show that strength training with repetition ranges from 3-35 with an intensity of 30-95% of 1RM respectively, yields an approximately similar response in CSA in the lower extremities of well-trained individuals. To our knowledge, only Schoenfeld et al. (2016a), who completed a study on already resistance-trained individuals, have reported a better hypertrophic response with the moderate-to-high load (8-12 repetitions) if the sets were performed to volitional failure. Both groups were set to perform an equal number of sets, which corresponds to more than a doubling of the volume in the group doing 8-12 repetitions in comparison to the high-load group. The authors suggested that the doubling in volume may have been the reason for the significant differences in hypertrophy responses of the moderate group (hypertrophy-type protocol). Besides, in a newly published study, Schoenfeld et al. (2019) evaluated strength training volume on muscular adaptations in trained men. The training volume was set to low-, moderate-, and high-volume strength training before muscle hypertrophy was evaluated. The results showed a dose-response relationship between strength training volume and hypertrophy, with increasingly greater hypertrophic gains achieved with higher training volumes. Moreover, the distinct training volume showed no differential effect on the 1RM tests (1RM squat and 1RM bench press) and they propose that "strength training volume enhances muscle hypertrophy but not strength in trained men". In comparison, their findings indicate that the low-load protocol should induce greater hypertrophy set against the high-load protocol. Secondly, the training volume enhanced only hypertrophy and not strength.

It is well documented that strength training leads to muscular adaptations such as hypertrophy in both trained and untrained individuals (Staron et al., 1991, Ahtiainen et al., 2003, Franco et al., 2019). The present results showed that there was no change in muscle size for both training modalities. Similar studies have presented evidence that high- and low-load

strength training leads to hypertrophy (Schoenfeld et al., 2015a). Interestingly, the lack of adaptations found in this study was somewhat unexpected and contradicts with what Burd et al. (2010) reported after analyzing muscle protein synthesis following high- and low-load leg extension. On the contrary, low-load strength training (30% of 1RM to failure) was suggested to be superior to triggering hypertrophy, as opposed to high-load (90% of 1RM). Mitchell et al. (2012a) reported training-induced hypertrophy in subjects performing strength training under three conditions, differing in contraction intensity (30% or 90% 1RM) or contraction volume (one/three sets of repetitions). Nevertheless, the training intervention in Mitchell's study was set to 10 weeks (in comparison to eight weeks in the current study) and the subjects were short of strength training experience (i.e. untrained).

The conflicting results may be explained by the characteristics of the exercises (e.g. complexity) and training frequency. The idea of a dose-response relationship would indicate that a higher weekly training frequency could produce increased metabolic stress and mechanical tension when training is performed to volitional failure (McLester et al., 2000, Schoenfeld et al., 2015b). This suggests that the hypertrophic response is not failure-dependent, and there are likely other variables affecting the hypertrophic response and strength increase. Strength training has been suggested to affect the architectural composition of the muscle (Blazevich and Giorgi, 2001, Aagaard et al., 2001, Alegre et al., 2006). The internal arrangement is a highly organized structure and has a great influence on the muscle's contractile properties (Burkholder et al., 1994, Lieber and Fridén, 2000b). Especially eccentric strength training has been suggested to affect the fascicle length by regulating the number of sarcomeres in one series and to which degree they have been stretched (Lieber and Fridén, 2000a). Thus, longer fascicles have a higher number of sarcomeres and are more favorable for power production. Previous research has shown significant gains in fascicle length prior to CSA (Seynnes et al., 2007) proposing that the discrepancy between increased strength and CSA in the present study may be explained by changes in muscle length. The length-tension relation is explained by the sliding of the contractile filaments (i.e. actin and myosin) during a muscle contraction, and to which extent they overlap (Gordon et al., 1966). A greater number of sarcomeres provide a greater amount of active cross-bridges and, hence, a higher force production (Cormie et al., 2011). This can potentially lead to training-induced adaptations provided by the muscle by optimizing the length for increased force production in relation to the work in which it is exposed to (Brughelli and Cronin, 2007). It is, however, important to acknowledge several of the findings have been more apparent in untrained

individuals (Kawakami et al., 1995, Kim et al., 2015, Matta et al., 2011) compared to trained individuals (Blazevich and Giorgi, 2001, Nimphius et al., 2012). By that, it is challenging to know for sure what has caused an increase in strength and not CSA.

The results from measuring CSA showed no significant difference in fiber type composition. However, previous research has published observations showing great variance in the skeletal muscle, suggesting a non-random muscle fiber distribution (Lexell et al., 1983). Likewise, the literature presents many studies reporting a relation between loading to which the muscle is exposed to and fiber-type specific CSA (i.e. hypertrophy). Several of the studies indicate that heavy strength training (80-85% of 1RM) causes the preferential increase in fast twitch muscle fibers (i.e. type 2) (Netreba et al., 2009), of which low-load strength training to a greater extent stimulates endurance-resistance type 1 fibers (Dons et al., 1979, Russell et al., 2003). However, this is not comparable to the present study as both type 1 and type 2 fibers showed the same degree of hypertrophy. Given the fatigue-resistant nature of type 1 fibers, it seems logical to conclude that the increased time-underload associated with low-load training is necessary to fully stimulate these fibers. This hypothesis is supported by Vinogradova et al. (2013) who found that 8-10 weeks of strength training-induced preferential increases in CSA of fast-twitch fibers with loads of 80–85% 1RM, whereas training at 50% 1RM produced greater increases in slow-twitch fiber. These discrepancies may just as well be a consequence of the experimental design and training more than the character of the muscle fiber, depending on the stress the muscle fibers have been exposed to. Further, this may be important to consider, as studies comparing strength training with different loads demonstrate a similar trend (Schoenfeld et al., 2015a) as in our study; namely, that the increase in type 1 and type 2 fibers are not significantly different.

6.1.3 Training effect on satellite cells and myonuclei

No significant increase in the number of satellite cells or the myonuclear number

Data collected from the subjects showed that the initial training period did not cause significant changes in satellite cell number or the myonuclear number in type 1 or type 2 fibers for either of the legs after eight weeks of high- and low-load strength training (Figure 5.5 and 5.6). Anyhow, when the values from both legs were combined, it resulted in a significant increase in type 1 fibers ($p = 0.05$). Yet, when combining both fiber types or just type 2 fibers, no significant increase in satellite cell number was given. The present results are

strengthened by studies demonstrating an absent increase in satellite cell number between legs performing high- and low-load strength training (Mackey et al., 2011). Further, the myonuclear number per muscle fiber at baseline was not significantly different between the legs (see Table 5.1). Studies of untrained subjects have shown a lower myonuclear number at baseline (Hanssen et al., 2013a, Kadi et al., 1999b), which is consistent if one is to assume that the nuclear domain theory is correct (i.e. increasing myonuclear number with increasing strength).

Several studies have investigated increases in strength and CSA in well-trained individuals after strength training with various loads. Nevertheless, few studies have documented the effects on cellular changes (e.g. changes in satellite cells and myonuclei). Satellite cells demonstrate high plasticity in the context of strength training, meaning that they are not necessarily to be recruited immediately to supply more myonuclei. In addition, there are consistent findings showing a concurrent increase in satellite cell number in untrained individuals and rodents in response to different loads (Egner et al., 2016, Umnova and Seene, 1991, Nederveen et al., 2017, Darr and Schultz, 1987, Snow, 1990a), and its role in muscle growth and regeneration caused by stress/injury during muscle activation (e.g. strength training) has become more apparent (Guitart et al., 2018, Relaix and Zammit, 2012).

Taken together with previous research, the absent increase in satellite cell number and the myonuclear number was to some extent expected, as no increase in CSA was shown. Several studies indicate that satellite cells are important for hypertrophy of muscle fibers. They seem to be a major contributor during post-exercise recovery, undergoing symmetric division and proliferation (Dumont et al., 2015a, Morgan and Partridge, 2003a). This has been reported in several studies (Nederveen et al., 2017, Petrella et al., 2008) in which a prolonged strength training period (i.e. 16 weeks) led to a remarkable increase in the satellite cell pool. Additionally, authors have observed a significant increase in satellite cells after a single bout of strength training (Crameri et al., 2004, Snijders et al., 2015a).

It appears to be a positive correlation between the myonuclear number and the CSA in both trained and untrained individuals (Kadi and Thornell, 2000, Kadi et al., 1999a). This strengthens our results and may partially explain the absence of additional myonuclei following the present training intervention, and it is plausible to assume that increased CSA would have led to such changes. The underlying hypothesis of a myonuclear domain ceiling states that the myonuclear domain can increase to a given limit before further recruitment is

needed (Qaisar and Larsson, 2014). Not to disable further expansion of fiber CSA, the addition of myonuclei are need for increased transcriptional activity. Nevertheless, if this is not necessary, the already existing myonuclei will be able to increase their protein synthesis to elicit moderate levels of fiber hypertrophy (i.e. support enhancement in cytoplasmic volume) (Kadi et al., 2004). This has been documented in several studies, for instance, Conceicao et al. (2018) who reported an increase in CSA and satellite cells regardless of an increase in myonuclei

Fiber type-specific measures revealed no indications of fiber type-specific differences in satellite cells. With its mixed fiber type composition, a study by Kadi et al. (2006a) revealed no such thing as a fiber type-specific satellite cell distribution in *vastus lateralis*, supporting the current findings. It should, however, be mentioned that the study was conducted on only five untrained individuals, leaving the results with scarce significant validity. Conflicting results based on rodent studies have indicated that the number of satellite cells are muscle specific and varies with fiber type (Rocheteau et al., 2015b). Particularly that a higher number of satellite cells have been associated with type 1 (slow muscle) fibers in the soleus muscle in rats (Gibson and Schultz, 1982a). If we're to assume the existence of a similar fiber type-specific relation in adult human skeletal muscle as in rodent, the expectation would, in this case, be a fiber-specificity opposed to type 2 fibers, considering the nature of strength training. Further studies are anyways needed to measure incidents of a potential fiber specificity following strength training with different loads, before drawing any conclusions.

6.2 Limitations

Before attempting to draw any conclusions, there are a few limitations worth considering. Probably the most obvious limitation of this study was what seems to be an insufficient training intervention. It can look as if the length and amount of training performed did not cause the expected response to the training. Most likely this is due to the pre-training status of our subjects. Further studies should take into consideration the subjects total training volume before setting up the experimental design. Perhaps, that way, the training stimuli will be good enough to hopefully induce more distinct changes in muscle architecture. Additionally, comparing our results with previous research have been challenging as research on high-/low-load strength training is performed mainly on untrained individuals (Bellamy et

al., 2014, Bruusgaard et al., 2010). It should also be taken into consideration that some of the abovementioned studies practice different analytic methods when studying the effects of various strength training loads.

Another conspicuous limitation is regarding unilateral training. A familiar phenomenon is whereby the exercise of one limb can induce significant improvements in the subsequent retest without evidence of measurable hypertrophy in the paired, untrained limb (i.e. cross-education) (Blaauw and Reggiani, 2014). The strength gains found in the contralateral limb have been investigated in both upper and lower extremities in several studies (Boivin, 2016). We cannot, therefore, exclusively eliminate the chance of a possible cross-education in the current study. It would be plausible to expect an increase in 1RM leg extension for both the high- and the low-load leg if the cross-education between the legs were very prominent. This requires further analysis are not taking into consideration here.

Third, a muscle biopsy sample only poorly represents the complete muscle under investigation and cannot say how the exercises have been performed (e.g. movement, load, repetitions). The depth in which the biopsies were taken along the muscle may have affected the results and could potentially limit the reliability of the study. This could likely influence the subject variation and may have reduced the chance of observing hypertrophy, if any.

In addition, it may be appropriate to point out the differences concerning satellite cell staining since some utilized Pax7 (Verdijk et al., 2007, von Maltzahn et al., 2013b), whereas others have used Neural cell adhesion molecule (NCAM/CD56) (Kadi et al., 2004, Olsen et al., 2006). NCAM is a reliable molecular marker for identifying satellite cells. However, as opposed to Pax7 which bind only a limited number of cells, NCAM can bind other substances in the muscle as well (e.g. myoblasts, developing and/or regenerative muscle fibers). Thus, there is no ensuring guarantee that satellite cells are stained exclusively. Studies have compared the two molecular markers, and while some have found an overall higher number of satellite cells using NCAM (~5%) (Lindström and Thornell, 2009), others have not (McKay et al., 2010). The lack of standardized methods for identifying cell types and nuclei in the muscle has probably led to conflicting results. To address this problem, Winje et al. (2018) discovered an antibody (PCM1) which specifically stain myonuclei. The authors recommended labeling with this PCM1, as it specifically labels myonuclei and myonuclei only. The protein was of value for the current study but also future studied, by expressing great specificity and accuracy.

Lastly, the subjects received no restrictions of suggested daily consumption of food during the training intervention. Therefore, we cannot rule out that the hypertrophic response may have been affected by individual energy intake. It should nevertheless be mentioned that they all received protein supply following exercise in an attempt to ensure adequate protein intake.

6.3 Conclusions

Apart from 1RM strength, there were no significant effects of eight-weeks of either high- or low-load training in the resistance-experienced subjects participating in this study. That said, the training led to no significant difference between the protocols, suggesting that both training modalities potentially can lead to similar cellular adaptations in the skeletal muscle. Thus, providing a good alternative to strength training for those who, for some reason, are prevented from participating in regular, heavy strength training.

Ultimately, this study shows that significant effects of high- and low-load strength training cannot be detected after eight weeks of training in well-trained individuals. A confounding variable is that both training modalities were carried out on the same subject, and cross-education cannot be entirely excluded. Anyhow, despite potential challenges and errors, the present results are supporting the hypothesis that high- and low-load may be equally good.

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Appendix 1

Merking mot satellittkjerner ved bruk av PAX7

Dag 1

Fersk løsning av PBS tilberedes ved tilsetting av én tablett (PBS) til 200 ml dH_2O. Lag gjerne til 1L hvis det er lite i kjøleskapet. Flasken med dH_2O og tabletter blir satt på spinner med en metallpinne.

Til fiksering trenger du:

- Lite begerglass
- 5 ml pipette og 2,5 µl pipette med pipettespisser
- Plastpipette
- Formaldehyd
- Triton X-100
- PBS
- 5 ml mikrotube
- Vaskebeholder klar til trinn 2
- Hjemmesnekra, gul beholder

1. Fiksering (trekkskap + labbfrakk!)
    a. I 4% formaldehyd skal 0,05% være triton X-100. det innebærer at det i 5 ml formaldehyd skal tilsettes 2,5 µl triton. Spinnes ned.
    b. Prøvene tas opp og merkes med lipidpenn når de er romtempererte, før de fikseres med fikseringsløsningen. Dette ble inkubert i trekkskap i 10 min.
        - Etter inkubering ble restene kastet i eget avfall merket for formaldehyd (nederst i skapet med nøkkel), men ble midlertidig under arbeidet puttet i et lite begerglass.
2. Vask (1xPBS)
    a. Vask prøvene 3x3 min med PBS på shaker.
3. Protein block

a. Etter vask legges prøvene i fuktkammer hvor de inkuberes med protein block i 10 min.
    - Protein blokkeringen står i kjøleskapet
    - Dryppes enkelt på

I mellomtiden, under inkuberingen, lages det primære antistoffet.

4. Primært antistoff
    a. Polyclonal Rabbit, Anti-Laminin finner man i kjøleskapet
    b. Pax 7 fra skuff fire i fryseskapet

I en løsning på 200 µl BSA brukes 20 µl Pax 7 og 2 µl laminin. Prøvene inkuberes med det primære antistoffet i fuktkammeret og settes i kjøleskapet over natten.

Dag 2

1. Vask (1xPBS)
    a. Vask prøvene 3x3 min med PBS på shaker.
2. Sekundært antistoff - lysfritt!

Sekundært antistoff hentet fra protokoll 1. Antistoffene blandes i forholdet 1-200 µl i 1% BSA før det mikses litt.

a. MHC: alexa fluor 488 (grønn)
    - Geit-anti-mus
b. Dystrofin: alexa fluor 594 (rød)
    - Geit-anti-kanin

Antistoffet inkuberes i fuktkammer i 60 min ved romtemperatur.

3. Vask (1xPBS) - lysfritt!
    a. Vask prøvene 3x3 min med PBS på shaker.
4. Montering av dekkglass (DAPI)

Appendix 2

Merking av myokjerner ved bruk av antistoff mot PCM1

Dag 1

1. Ta ut snitt fra fryseren og la de tempereres
2. Tegn rundt snittet ved bruk av lipidpennen
    a. La de tørke litt før neste steg
3. Blokker i 2% BSA løst i PBS i 30 minutter ved romtemperatur
4. Inkuber snittene i primært antistoff over natt ved 4 grader:
    a. Anti PCM1 1:1000
    b. Anti-dystrofin 1:20
        - Obs! Bruk kun MANDYDS8 mouse-anti-dystrofin
        - Antistoffet fortynnes i 5% BSA løst i PBS, som i tillegg er tilsatt 0,2% Igepal CA-630

Dag 2

5. Vask 3x 5 minutter i PBS
6. Inkuber i sekundært antistoff for 60 min ved romtemperatur. **Husk at det sekundære antistoffet må holdes lysfritt!**
    a. Goat-anti-rabbit 488 (grønn)
    b. Goat-anti-mouse 594 (rød)
        - Konsentrasjonen på begge antistoffene er 1:200
        - Antistoff ble fortynnet i 2% BSA løst i PBS
7. Vask 3x 5 minutter i PBS
8. Monter dekkglass med DAPI

Appendix 3

Merking mot MHC1 ved bruk av myosin heavy chain 1

Dag 1:

1. Ta ut prøvene fra fryseren og la de tempereres ved romtemperatur
2. Tegn opp en lipidbarriere rundt snittene ved hjelp av PAP-pennen
    a. La det tørke litt
3. Blokker med 1% BSA i PBS-t (0,05%) og inkuber i 60 min i romtemperatur. Dette gjøres i fuktkammer. Etter endt inkubering, ristes BSAen av.
4. Tilsett det primære antistoffet og la det inkubere over natt ved 4 grader i fuktkammeret.
    a. MHC I (BA-D5; DSHB; mus monoklonal): konsentrasjon **1:500** i 1% BSA i PBS-t (0,05%)
    b. Anti-dystrofin (Abcam; kanin polyklonal): **1:500** i 1% BSA i PBS-t (0,05%)
        - Obs! Antistoffene skal blandes i samme rør.

Dag 2

5. Vask snittene 3x10 min i PBS-t (0,05%)
6. Snittene ristes før de tilsettes sekundært antistoff i 60 min ved romtemperatur. **Husk at det sekundære antistoffet skal holdes lysfritt!**
    a. MHC I: alexa fluor (αM)488 (grønn) geit-anti-mus **1:200** i 1% BSA i PBS-t (0,05%)
    b. Dystrofin: alexa fluor 594 (rød) geit-anti-kanin **1:200** in 1% BSA i PBS-t (0,05%)
        - Obs! Sekundært antistoff skal blandes i samme rør!
7. Etter endt inkubering vasker du snittene 3x10 min i PBS-t (0,05%)
    a. Hold snittene lysfritt!
8. Monter dekkglass med DAPI
    a. Det mest optimale er å la snittene ligge til herding i noen timer eller over natta.

Appendix 4

Information till försökspersoner

Projekttitel:

Muskulära effekter av tung respektive lätt styrketräning.

Ansvariga:

Forskningshuvudman: Gymnastik- och idrottshögskolan (GIH).

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Bakgrund/syfte:

Större och starkare muskler är till nytta ur både en hälso- och prestationssynvinkel. Avgörande faktorer för att öka muskelmassan är belastning, träningsfrekvens och total träningsmängd. Nyligen publicerad forskning visar att träning med lätta vikter till utmattning kan generera samma träningseffekt som medeltung styrketräning. Det råder däremot oklarhet i om detta även gäller då tung styrketräning jämförs med lätt styrketräning till utmattning.

Syftet med den här studien är att undersöka huruvida lätt respektive tung styrketräning påverkar muskeltillväxt (hypertrofi), styrka och kraftutveckling i lårmuskulaturen samt olika muskelfibertyper i den yttersta lårmuskeln (vastus lateralis).

Vetenskapliga frågeställningar:

1.Hur påverkas hypertrofi, styrka och kraftutveckling i lårmuskulaturen till följd av tung respektive lätt styrketräning?

2. Hur påverkas hypertrofi, styrka och kraftutveckling i typ 1 respektive typ 2 muskelfibrer till följd av tung respektive lätt styrketräning?

Metod:

Under 10 veckor ska vältränade män och kvinnor utföra styrketräning under kontrollerade former på Gymnastik & Idrottshögskolan, GIH. Ena benet tränas med en hög belastning (ca 90% av max) och andra benet med en lättare belastning (ca 30% av max) där varje set utförs till utmattning. Träningen består av två övningar; unilateral (utförs med ett ben) benpress och unilateral benspark som anses tekniskt enkla och säkra att utföra. Före och efter träningsperioden utförs styrkemätningar och lårmuskulaturens storlek bedöms med hjälp av ultraljud. Utöver detta tas muskelprover (biopsier) både före och efter träningsperioden för att kunna studera effekterna på muskelfibernivå.

Vad är en biopsi?

En biopsi är ett invasivt ingrepp där man med hjälp av en tång tar ut en bit av lårmuskeln. Detta utförs under lokalbedövning. Det kan upplevas något obehagligt under ingreppet. Ingreppet utförs under strikt sterila förhållanden och risken för komplikationer är mycket låg. Med biopsi kan vi utläsa vilken typ av muskelfibrer som är dominant och hur stora respektive fiber är. Vi kommer även att kunna bedöma styrkan hos varje enskilt muskelfiber.

<u>Kunskapsvinster:</u>

Det är viktigt att förstå hur olika typer av träning påverkar muskeltillväxten så att den kan optimeras hos både idrottare, motionärer och patienter. Äldre och sjuka som inte kan träna med tung belastning kan ha nytta av att träna på lätt belastning som kan generera hypertrofi och ökad muskelstyrka. Från ett rehabiliteringsperspektiv kan detta ge förståelse för hur träningsrespons kan ske även på lätt belastning. Ur idrottsperspektiv kan detta ge förståelse för hur muskeltillväxt och styrka ska maximeras i syfte att öka prestationsförmågan.

<u>Hur går studien till?</u>

Studien är uppdelad i flera delmoment:

1. Första steget är att via telefonmöte informera och intervjua dig kring projektet. Anledningen till intervjun är att vi vill ha information angående din hälsa och träningsbakgrund för att du skall kunna inkluderas i studien.
2. Vid nästa delmoment kommer du att få fylla i en hälsoenkät, därefter mäter vi din maximala styrka i benpress och benspark. Vid ett separat tillfälle kommer vi även att ta muskelprov från yttre sidan av lårmuskeln på vardera ben, samt mäta muskeltjocklek i framsida lår med hjälp av ultraljud.
3. Efter dessa förberedande tester kommer du att genomföra styrketräningspass två gånger/vecka i 10 veckor. Träningen kommer att bestå av unilateral benpress och benspark där ena benet tränas med tung belastning och andra benet med lätt

belastning. Träningen kommer utföras på måndagar och torsdagar på GIH, varje pass tar ca 30 min.

4. Efter träningsperioden upprepas samtliga tester och prover som utfördes före träningen.

Vilka är riskerna?

Muskelbiopsi innebär att en liten bit muskelvävnad (0,05-0,10 gram) tas ut med en specialnål. Muskelbiopsi utförs efter lokalbedövning av huden och underliggande bindväv. Ett 4-5 mm långt snitt görs genom huden, genom vilket biopsinålen förs in och ett muskelprov tas ut. Själva ingreppet med biopsinålen är över på ett par sekunder. I allmänhet känns en muskelbiopsi som ett trubbigt slag mot benet. I vissa fall kan en skarpare smärta kännas, som går över så fort nålen tas ut. För att förhindra blodutgjutning i muskeln lägger vi ett lokalt tryckförband över biopsistället, som skall vara kvar under 1-2 timmar. Liksom vid alla hudsnitt kan en hudnerv skäras av med lokalt känselbortfall i huden som följd. Vid den här typen av biopsi är denna komplikation mycket ovanlig. I de fåtal fall där denna komplikation har ägt rum har allt normaliserats efter 6-12 månader.

Skötselinstruktioner vid muskelbiopsi: Under veckan före muskelprovtagning får du ej använda magnecyl eller någon annan medicin som innehåller acetylsalicylsyra (alvedon går bra). Två dygn (48 timmar) före testerna får du ej utföra något tungt fysiskt arbete (>30 min) eller dricka alkohol. Under veckan efter undersökningen skall du inte bada (p.g.a. infektionsrisk) och när du duschar skall du skydda området över biopsistället med plast. De inre vita långsmala plåstren skall du inte byta själv – de ramlar av efter ca 1 vecka.

Biobanksprover/hantering av data/sekretess:

Uppgifterna om din hälsostatus kommer endast att finnas tillgängliga för försöksledare och projektmedarbetare och kommer att förvaras så att inga obehöriga kan ta del av dessa. Du har rätt att ta del av dina registrerade uppgifter. Ändamålet med hälsoenkäten är att ge underlag för deltagande i studien. Proverna och undersökningsresultaten kommer att kodas. Endast försöksledare och medarbetare kan koppla provresultaten till namn. Dina resultat kommer att behandlas så att inte obehöriga kan ta del av dem. Vid publicering av forskningsdata kommer dessa inte kunna kopplas till dig som individ. Blod- och muskelprover fryses och förvaras vid -80˚C på Åstrandslaboratoriet vid GIH i väntan på analys. Proverna kodas löpande under försökets gång. Proverna är kodade och kan inte utan kodnyckel hänföras till en viss person. Proverna kommer att förvaras i en biobank registrerad vid Socialstyrelsen (se bif. underbilaga). Om proverna kommer att användas till projekt med andra frågeställningar kommer ny etisk prövning att genomföras och Du kommer ånyo att kontaktas för samtycke. Du har rätt att ta del av Dina resultat och få rättelse av evt. felaktiga personuppgifter (personuppgiftsansvarig se ovan).

Försäkring/ersättning:

Personskadeskyddsförsäkring tecknad av GIH gäller under studien. Ersättning per biopsitillfälle utgår med 500 kr (före skatt). Detta medför en total ersättning på 4x500 = 2000 kr om du deltar i hela studien.