Scandinavian Journal of Medicine & Science in Sports

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Effects of 8 Weeks of Moderate- or High-Volume Strength Training on Sarcoplasmic Reticulum Ca^{2+} Handling in Elite Female and Male Rowers

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Received: 5 September 2024 | **Revised:** 7 January 2025 | **Accepted:** 8 January 2025

Funding: This study was supported by the Wenner-Gren Foundation. Team Denmark by the means of Novo Nordisk Foundation. The Swedish Olympic Committee.

Keywords: Ca^{2+} release | Ca^{2+} uptake | muscle plasticity | sex difference | strength training

ABSTRACT

While acute exercise affects sarcoplasmic reticulum (SR) function, the impact of resistance training remains unclear. The purpose of the present study was to investigate SR Ca^{2+} handling plasticity in response to moderate- and high-volume strength training in elite rowers. Twenty elite male ($n = 12$) and female ($n = 8$) rowers performed three weekly strength training sessions for 8 weeks and were randomly allocated to either perform 3 sets (3-SET) or progressive increase from 5 to 10 sets (10-SET) of 10 repetitions during the training period. Skeletal muscle biopsies were collected before and after the intervention period and analyzed for SR vesicle Ca^{2+} handling, SR related proteins, and myosin heavy chain (MHC) composition. Muscle strength was determined by isometric midthigh pull (IMTP). Training increased both the overall SR Ca^{2+} release (19%) and uptake rates (34%), with no differences between groups. SR protein analysis revealed a high variability but suggests an increase of RYR1 and SERCA1, while SERCA2 decreased, corroborating changes in SR function, with no differences between groups. Regardless of training volume, a 9% higher relative MHCIIa proportion and a 7% decrease in the MHCI isoform was observed. There was an overall 8% increase of IMTP. Males exhibited higher SR Ca^{2+} uptake and release rates compared to females, likely explained by a higher proportion of MHCII. These findings suggest that 8 weeks of moderate- or high-volume strength training enhances SR vesicle Ca^{2+} uptake and release rates in elite male and female rowers, accompanied by a shift toward a larger proportion of MHCIIa fiber type.

1 | Introduction

Upon neural activation, skeletal muscle function is dependent on the series of events termed excitation-contraction and relaxation (E-C-R) coupling. E-C-R coupling is initiated by sarcolemmal and t-tubule depolarisation leading to a rapid Ca^{2+} release from the sarcoplasmic reticulum (SR) into the cytoplasm by passive diffusion through the ryanodine receptor Ca^{2+} release channels (RyR1) located in the SR terminal cisternae. This initiates muscle contraction by the cross-bridge cycling and subsequently, Ca^{2+} ions are actively resequestered into the SR through the SR Ca^{2+}-ATPase (SERCA), leading to muscle relaxation. Hence, SR Ca^{2+} release and reuptake are key determinants of muscle function and power production. Accordingly, changes in Ca^{2+} handling of individual skeletal muscle fibers cause either a force depression or improved muscle function (1).

Experiments conducted on individual muscle fibers have consistently demonstrated that fatiguing contractions are associated with decreased tetanic intracellular Ca^{2+} concentrations [1, 2]. To this end, direct measures of the SR vesicle Ca^{2+} release rate have demonstrated a reduced release acutely following fatiguing strength-, high-intensity-, and aerobic exercise in both untrained [3–6] and well-trained individuals [7–9]. Similarly, reductions in SR vesicle Ca^{2+} uptake have been observed following acute exercise in untrained individuals [4, 5, 10, 11], while results for well-trained individuals are equivocal [7–9]. Together, it is evident that acute exhaustive exercise can impair SR Ca^{2+} handling capacity in both untrained and trained individuals, contributing to impaired muscle function and performance. However, less is known about the effects of training, and especially strength training, on SR function and the expression of related proteins in well-trained athletes.

As maintenance of SR Ca^{2+} handling capabilities is crucial for muscle function and performance, improvements in the SR Ca^{2+} regulation with training could serve as a preventive mechanism postponing impaired muscle function and in turn improve performance. Still, only a few studies have examined changes in SR Ca^{2+} handling with exercise training. High-intensity interval training (HIIT) increases the SR vesicle Ca^{2+} release rate in already highly trained endurance athletes [7], while sprint interval training and strength training have been shown to increase SR vesicle Ca^{2+} release rate in untrained individuals [12, 13]. Moreover, strength training has been shown to increase both SR vesicle Ca^{2+} uptake [13] and SERCA activity in untrained individuals [14]. In line with an increased SERCA activity with exercise training, 12 weeks of strength training in untrained men has been shown to attenuate the exercise-induced decrease in SERCA activity [15]. However, knowledge about potential adaptations in the SR Ca^{2+} handling in response to training (e.g., high-volume strength training) in athletes is lacking.

Many athletes dedicate large proportions of their training time toward strength training, with the general goal of improving their rate of force development (RFD), maximal voluntary contractile ability (MVC), and performance [16]. Due to its pivotal role in muscle function and implication in fatigue, improvements in SR Ca^{2+} handling in response to strength training may contribute to increased muscle performance. Accordingly, data from rodents has suggested that RFD is to some extent dependent on the SR vesicle Ca^{2+} release rate [17]. As SR function is clearly dependent on muscle fiber type, MHCII fibers have greater SR Ca^{2+} kinetics and Ca-ATPase contents as compared with MHCI fibers [18–22] an increased MHCII proportion may in turn allow for greater contractile power outputs. Strength and power training has been shown to increase the individual MHCII fiber volume and the proportion of MHCII fibers in well-trained athletes [16], and thereby potentially increasing the muscle SR Ca^{2+} handling. Similarly, 20 bouts of 10-s all-out sprints, performed three times per week for 5 weeks, have also been shown to increase intrinsic SR volume [12]. Of note, it has been documented that the protein expression of SERCA1 and 2 is unchanged following high-intensity (4 × 4RM, 3 times per week for 8 weeks) strength training in young males [23], indicating that low-volume strength training may not have an effect on SERCA expression. Together, it remains uncertain whether SR Ca^{2+} handling undergoes adaptations in response to strength training among elite athletes, and the role of moderate- vs. high-volume strength training volumes is unclear. Consequently, it is also not well understood if potential training adaptations are contingent upon the distribution of MHC isoforms. Furthermore, little is known about possible sex differences in SR Ca^{2+} handling and the role of MHC distribution herein.

Muscle strength is a key determinant of 2-km rowing performance [24] and in the pursuit of hypertrophy and increased maximal muscle strength, very high volumes of heavy strength training (e.g., 10 sets of 10 repetitions in multijoint exercises) are anecdotally performed periodically in some successful elite rowing environments. Evidence suggests a positive dose–response relationship between strength training volume and increases in muscle mass [25]. Although a plateau in the muscle hypertrophy response has been observed by four to six sets per exercise [26], performing additional sets may further enhance muscle mass gains in resistance-trained individuals [27]. Incorporating such high-volume strength training in elite rowers could therefore be an effective strategy to increase lean body mass, maximal strength, and muscle function (e.g., SR Ca^{2+} handling) compared to more conventional heavy strength training with lower volumes (e.g., 3 sets of 10 repetitions).

This study was designed to investigate changes in SR Ca^{2+} handling and related proteins in response to 8 weeks of strength training in elite to elite female and male rowers. Specifically, changes in these outcomes were examined following a period of moderate- vs. high-volume strength training volumes.

2 | Material and Methods

2.1 | Ethical Approval

All participants were fully informed of any potential risks associated with the experiments before written and verbal consents were obtained. The Ethics Committee of Southern Denmark approved the present study protocol (Project ID S-20220335), and all experiments were conducted in accordance with the standards of the Helsinki Declaration.

2.2 | Participants and Matching

Twenty rowers were recruited for this study, comprising 12 males and eight females. Inclusion criteria were (1) a minimum of four weekly training sessions of rowing, (2) a minimum of 1 year of competitive rowing experience, and (3) regularly performing strength training, i.e., a minimum of two times per week. Rowers were not included if they had conducted high-volume strength training (more than six sets per exercise per session) within the past 6 months. Fifteen of the participants had a current or past affiliation with the Danish national rowing team, at the junior, under 23, or senior level, competing at the international level. Following pretests, the rowers were matched based on sex, performance, and training volume before being randomly allocated to either moderate-volume strength training (3-SET, $n = 10$) or high-volume strength training (10-SET, $n = 10$).

2.3 | Training Intervention

The training intervention has been described in detail elsewhere [28]. Briefly the intervention was conducted during the early general preparation period of the rower's annual season (i.e., 1 week following the national championships). It consisted of an 8-week training period and was structured into two 3-week blocks with a recovery week following each block. During the training intervention, the rowers engaged in three weekly strength training sessions, with a minimum of 36 h between each session. These sessions consisted of three primary exercises involving muscles highly involved during the rowing movement: leg press, trap-bar deadlift and bench pull, and one of three single secondary press exercises alternately: flat barbell bench press, incline barbell bench press or standing barbell shoulder press. During the first 3-week block the 10-SET group progressively increased their volume from five sets of 10 repetitions (5 × 10) to 10 sets of 10 repetitions (10 × 10) by incorporating an additional set for each primary exercise per session. As such, by the sixth session (final session of week 2), the 10-SET group were conducting 10 × 10 repetitions. In the second training block, the 10-SET group carried out all strength training sessions as 10 × 10 repetitions. The 3-SET group conducted all strength training sessions during both blocks as three sets of 10 repetitions (3 × 10). Both groups were instructed to execute the final three sets of the primary exercises with zero reps in reserve (RIR), i.e., to failure. The 10-SET group was additionally instructed to perform the first three sets with two RIR, and the following four sets with one RIR, resulting in an equal number of total sets to failure in both groups. During the recovery weeks, both groups performed three sets of three repetitions with three RIR. The secondary exercises were always performed as 3 × 10 with approximately three RIR. Rest periods between sets were 90 s throughout the training period. Immediately following all strength training sessions, all participants consumed 20 g of whey protein. Additionally, all participants received dietary guidelines for daily energy consumption, in accordance with the American College of Sports Science guidelines (1.7 g kg bm^{-1} day^{-1} of protein, 6-10 g kg bm^{-1} day^{-1} of carbohydrates, 20%–35% of total calories from fats).

During the training period, the matched pairs conducted identical endurance training programs, which ensured that the overall average training volume and intensity of training were identical between the two groups. As the intervention was carried out in the early part of the general preparation phase, the endurance training plans were characterized by low training volumes for all endurance activities. All endurance training was recorded using TrainingPeaks software for analysis (285 Century PI, Louisville, CO 80027), and categorized as either low intensity (< 84% HR_{max}), moderate (84%–89% HR_{max}), or high intensity (> 89% HR_{max}), in accordance with training zones used by the Danish Rowing Federation.

2.4 | Analytical Procedures

The participants visited the laboratories four times; twice before the 8-week intervention and twice after the intervention. Participants visited the laboratories at the same time of day before and after the training period and refrained from intense exercise for 24 h before all visits. All laboratory visits were separated by a minimum of 36 h. On laboratory visits one and three, muscle biopsies were obtained, while on visits two and four performance trials were conducted. Before performance trials, the participants were asked to adhere to their habitual competition day diets. Participants were not permitted to consume caffeine or other ergogenic aids on test days.

2.5 | Muscle Biopsies

Using the Bergström needle technique with suction, 100–150 mg of muscle was obtained from the *m. vastus lateralis* of the participants' dominant leg before (pre) and after (post) the 8-week training intervention. Following local anesthetic, an incision was made through the skin and fascia from where the muscle biopsy was extracted. Muscle tissue was placed on filter paper upon an ice-cooled Petri dish, blotted, and dissected free from fat and connective tissue. The biopsies were separated into five parts, with two parts being used in the present study, while the others were stored for later analysis in companion studies. One part was frozen in liquid nitrogen and stored for later immunoblot analysis. The other part was manually homogenized with a Potter–Elvehjem glass-homogenizer (Kontes Glass Industry, Vineland, NJ) in an ice-cold buffer containing 300 mM sucrose, 1 mM EDTA, 10 mM NaN_3, 40 mM Tris base, and 40 mM L-histidine at pH 7.8. The homogenization was performed in a weight-to-volume ratio of 1:10. Homogenate was divided into parts of 25–100 μL, frozen in liquid nitrogen, and stored at −80°C until further analyses were performed.

2.6 | SR Vesicle Ca^{2+} Uptake and Release

The fluorescent dye technique was used to determine Ca^{2+} uptake and release rates in SR vesicles in whole homogenates, which has been described in detail elsewhere [9, 12]. Briefly, [Ca^{2+}] was determined by the fluorescent Ca^{2+} indicator Indo-1 (1 μM) (20 Hz, Ratiomaster RCM; Photon Technology International, Brunswick, NJ, United States). SR vesicle oxalate supported Ca^{2+} uptake was initiated by adding 2 mM ATP to a final concentration of 5 mM and Ca^{2+} uptake was recorded for 3 min, before [Ca^{2+}] reached a plateau (nadir), which was < 100 nM and not different between groups. The SR Ca^{2+} uptake rate (Tau) was defined as the time for free [Ca^{2+}] to decrease by 63%, and as uptake rates at 600 and 200 nM Ca^{2+}, respectively. Upon measurements of Ca^{2+} uptake, the SR Ca^{2+}ATPase was blocked with cyclopiazonic acid, in order to estimate vesicle leak, before SR vesicle Ca^{2+} release was initiated by addition of 4-chloro-M-Cresol (4-CmC) (5 mM). Raw-data for [Ca^{2+}] were mathematically fitted using monoexponential equations as previously described (Curve Fitting Toolbox version 1.1.1; The MathWorks, Natick, MA, United States) [9, 21]. Values obtained for SR Ca^{2+} uptake and release rates are relative and expressed as arbitrary units; Ca^{2+} g protein^{-1} min^{-1}. Tau is expressed reciprocal as s^{-1} mg^{-1} protein, i.e. higher value denoting a faster reuptake. The SR assays were performed in duplicates and averaged. Protein content in the muscle homogenate was measured in duplicates using a standard kit (Pierce BCA protein reagent no. 23225). The data are analyzed as paired pre- and post-values. Due to individual missing values, there are 8 pairs for the 10-SET group and 9 pairs for the 3-SET group, with 10–11 individual values for males and 6–7 individual values for females, respectively.

2.7 | MHC Composition

MHC composition was determined from homogenate using gel electrophoresis as described previously [29] and modified for humans [12]. In brief, muscle homogenate (80 μL) was mixed with 200 μL of sample-buffer (10% glycerol, 5% 2-mercaptoethanol and 2.3% SDS, 62.5 mM Tris and 0.2% bromophenol blue at pH 6.8.), boiled in water for 3 min, and loaded with two different quantities of protein (0.03 and 0.04 mg) on an SDS-PAGE gel [6% polyacrylamide (100:1 acrylamide: bis-acrylamide), 30% glycerol, 67.5 mM Tris-base, 0.4% SDS and 0.1 M glycine], with pre and post samples loaded next to each other at the same gel. Gels were run at 80 V for at least 42 h at 4°C and MHC bands were made visible by staining them with Coomassie. The gels were scanned (Linoscan 1400 scanner; Linoscan Heidelberg, Germany) and MHC bands were quantified densitometrically (Phoretix 1D, nonlinear; Phoretix International Ltd., Newcastle, UK) as an average of the two clear MHC bands, i.e., for each subject and time point.

2.8 | Determination of Total Protein Concentration

A fresh batch of cold homogenization buffer (10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES (pH 7.5), 1% Igepal, 20 mM β-glycerophosphate, 2 mM Na_3VO_4, 10 mM NaF, 2 mM PMSF, 1 mM EDTA (pH 8), 1 mM EGTA (pH 8), 10 $\mu g \cdot ml^{-1}$ Aprotinin, 10 $\mu g \cdot ml^{-1}$ Leupeptin and 3 mM Benzamidine) was prepared and 80 μL added per 1 mg of freeze-dried and dissected muscle tissue. Samples were homogenized 2×2 min at 28.5 Hz in a TissueLyser (Qiagen TissueLyser II, Retsch GmbH, Haan, Germany). Afterward, samples were rotated end over end for 1 h at 4°C, the steel ball removed, and samples sonicated (Branson Digital Sonifier) 2×10 s at 10% amplitude, based on (Vigelso et al., 2015; Thomassen et al., 2023). Total protein concentration in the homogenate used for immunoblotting was determined in triplicate with a standard BCA kit (Millipore). 15 μL of the samples were diluted with 60 μL of ultrapure water (Milli-Q Reference system, Millipore) to keep the concentration within the linear range of the calibration curve (0.2 to 2 $\mu g \mu L^{-1}$). The protein concentration of each sample was adjusted by a 6×Laemmli buffer (7 mL 0.5 M Tris-base, 3 mL glycerol, 0.93 g DTT, 1 g SDS and 1.2 mg bromophenol blue) and ultrapure water to reach equal concentrations (2 $\mu g \mu L^{-1}$) before electrophoresis.

2.9 | Immunoblotting and SDS-Page

Equal amount of total protein was loaded from the same subject on the same of two gels (4%–15% TGX Stain-Free, Bio-Rad) for each round of analysis together with two protein markers (Precision plus all blue or Precision plus dual color, Bio-Rad) and three human skeletal muscle standard samples, obtained as a pool of all samples included in the experiment. Proteins were separated by SDS-page gel electrophoresis (max 150 V for 1–2 h). In one analysis, the total protein amount loaded in each lane was determined as the stain-free signal by 5 min of UV light incubation of the gels before a digital picture was obtained (ChemiDoc MP Imaging System, Bio-Rad) and the protocol continued. The gels were cut horizontally and the same parts of two gels (e.g., ~70–160 kDa and ~160 kDa and all proteins above) were placed on one PVDF membrane for each protein of interest and then the proteins were semidry transferred to the membrane (6–12 V for 2 h). The empty parts on the membrane were blocked with either 2% skimmed milk or 3% BSA in Tris-based saline with 0.1% Tween-20 (TBST) before overnight incubation with primary antibody. The membrane was washed in TBST, incubated for 1 h in HRP conjugated secondary antibody at room temperature, and washed 3×15 min in TBST before the bands were visualized with an enhanced chemiluminescent reaction (Immobilon Forte Western HRP substrate, Millipore) and signals recorded with a digital camera (ChemiDoc MP Imaging System, Bio-Rad). Densitometry quantification of the immunoblotting band intensities was done using Image Lab version 4.0 (Bio-Rad) and determined as the total band intensity adjusted for the background intensity. A five-point standard curve was used to confirm that the loaded amount of samples was capable of determining differences between samples by the signal intensity being on the linear and steep part of the standard curve. The average of the triplicate human standard sample signal loaded across each gel was used for normalization of all samples on the gel to allow for semiquantitative comparisons across gels and subjects. Listed are the primary antibodies used and their size of migration. RyR1: MA3-925, ThermoFisher Scientific, 565 kDa; SERCA1: MA3-912, ThermoFisher Scientific, 100 kDa; SERCA2: SC-376235, Santa Cruz Biotechnology, 100 kDa. HRP conjugated goat anti-rabbit (4010–05, SouthernBiotech), goat anti-mouse (P0447, DAKO Denmark) and a rabbit anti-goat (P0449, DAKO Denmark) were used as secondary antibodies. Immunoblotting of the SR proteins was conducted only on a subset of 13 subjects, i.e., 6 (10-SET) and 7 (3-SET), due to the unavailability of homogenates that were paired pre- and post-training.

2.10 | Isometric Midthigh Pull

Maximal isometric strength was assessed using the isometric midthigh pull (IMTP). Testing was conducted using an Olympic style 20 kg barbell (Eleiko Sport Group Company AB, Halmstad, Sweden), fixed to an IMTP rack (Kairos Strength, USA), which allowed for adjustment of the bar height to the individual participants' midthigh position. A force plate (AMTI R6-5-1000, Watertown, MA) was situated under the bar. Following 5-min of light warmups on a bicycle, participants stood unshod on the force plate and were weighed. Participants conducted two warmup attempts pulling at approximately 50% and 75% of maximum effort, respectively. Following this, participants had three attempts to generate their highest possible amount of isometric force. Each attempt was followed by 120 s rest. During all attempts participants were fixed to the bar using lifting straps. Between attempts, participants were allowed to step off the platform and either lightly walk around the laboratory or sit on a chair. Thirty seconds before the next attempt, participants were refixed to the bar using the lifting straps. Bodyweight was subtracted from the force plate raw data, and the best of the three maximum attempts was chosen for analysis.

2.11 | Statistical Analysis

Data were tested for normal distribution using a Shapiro–Wilk test. Training effects were tested using a two-way ANOVA with

repeated measures (group × time) and baseline comparisons between sexes were performed using an unpaired *t*-test. Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software LLC, San Diego, CA 92108, USA). The significance level was set at $p < 0.05$. For some of the parameters full sample size is not available and indicated in text with number of data (*n*) presented in the figure and table texts. Values are presented as mean ± SD, with exercise training and sex differences presented as mean and 95% confidence intervals in square brackets.

3 | Results

3.1 | Training, Muscle Strength, and Body Composition

Physical and performance characteristics of the included subjects are depicted in Table 1. The general training characteristics and effects of training on strength and body composition have been presented elsewhere [28] and the variables are therefore included only to characterize the training intervention and general training effects. The two groups displayed similar compliance with the strength training regimes (10-Set: 89% ± 14%; 3-SET: 89% ± 10%, $p < 0.99$). Total tonnage (sets × reps × weight) during the intervention increased in the 10-set groups from 7872 ± 2986 kg to 14 533 ± 5428 kg, and in the 3-set group from 3872 ± 1438 kg to 5138 ± 1465 kg. Both the total endurance training volume (10-SET: 6.1 ± 3.0 h week^{-1}; 3-SET: 6.0 ± 2.8 h week^{-1}, $p = 0.95$) and the endurance training intensity distribution were similar between groups (10-SET: zone 1: 4.9 ± 3.8 h week^{-1}, zone 2: 0.7 ± 0.6 h week^{-1}, zone 3: 0.6 ± 0.6 h week^{-1}; 3-SET: zone 1: 4.7 ± 3.6 h week^{-1}, zone 2: 0.8 ± 0.5 h week^{-1}, zone 3: 0.5 ± 0.4 h week^{-1}, $p = 0.80–0.95$).

There was an overall increase of 8% [4; 12] in isometric midthigh pull strength following the 8-week training period (10-SET: +12 kg [−3; 27]; 3-SET: +25 kg [10; 39], mean difference: −13 kg [−33; 8] in 10-SET vs. 3-SET, group × time: $p = 0.21$, overall time-effect: $p = 0.001$). In the first week, the 10-SET group completed all 10 × 10 sets (week 3), the trap-bar deadlift intensity for the 3-SET group and the last three sets of the 10 × 10 sets (10-SET group) were 45.2% ± 6.0% and 44.5% ± 10.2% of isometric midthigh pull strength, respectively.

There was a main effect of training on BM during the intervention period (+0.61 kg [−0.14; 1.07], $p = 0.03$), with no difference between the training groups. However, there were no main effect of training on lean body mass (+0.3 kg [−0.2; 0.8], $p = 0.42$) or fat mass (+0.3 kg [−0.2; 0.7], $p = 0.37$) during the training period.

3.2 | MHC Composition and Effects of Moderate- and High-Volume Strength Training

Regarding changes in the MHC composition following the training period, no clear differences were observed between training groups (Table 2). However, there was an overall increase in the MHCIIa isoform distribution (9% [5; 12], $p = 0.002$) accompanied by a decrease in the MHCI isoform (7% [−11; −3], $p = 0.0002$) as well as a tendency toward a decrease in the MHCIIx isoform (3% [−3; 0], $p = 0.06$) (Table 2).

3.3 | Changes in SR Ca^{2+} Function in Response to Moderate- and High-Volume Strength Training

No clear differences were observed between the 10-SET group and the 3-SET group with respect to training-induced changes in SR Ca^{2+} handling. Following the training period SR vesicle Ca^{2+} uptake rate was increased, as indicated by increased Tau value (1/Tau) by 25% [1; 49], 42% [3; 82] and 34% [12; 56] in the 10-SET group, the 3-SET group and overall, respectively (Figure 1A). In absolute values this corresponded to increases of 18 s^{-1} mg protein^{-1} [0; 37] (10-SET) and 27 s^{-1} mg protein^{-1} [3; 51] (3-SET) (mean group difference: −9 s^{-1}·mg protein^{-1} [−37; 19] in 10-SET vs. 3-SET, group × time: $p = 0.51$, time-effect: $p = 0.004$) (Figure 1A). In line with this, overall increases in SR Ca^{2+} uptake rates of 18% [7; 29] and 23% [3; 44] were observed at 600 and 200 nM Ca^{2+}, respectively (Figure 1B,C). The SR Ca^{2+} release rate was increased following the intervention period by 12% [1; 23], 25% [11; 38] and 19% [10; 27] in the 10-SET group, the 3-SET group and overall, respectively (Figure 1D). This corresponded to absolute increases of 0.14 μmol g protein^{-1} min^{-1} [0.00; 0.28] (10-SET) and 0.25 μmol g protein^{-1} min^{-1} [0.13; 0.38] (3-SET) (mean difference: −0.11 μmol g protein^{-1} min^{-1} [−0.27; 0.05] in 10-SET vs. 3-SET, group × time: $p = 0.17$, time-effect: $p = 0.0001$; Figure 1D).

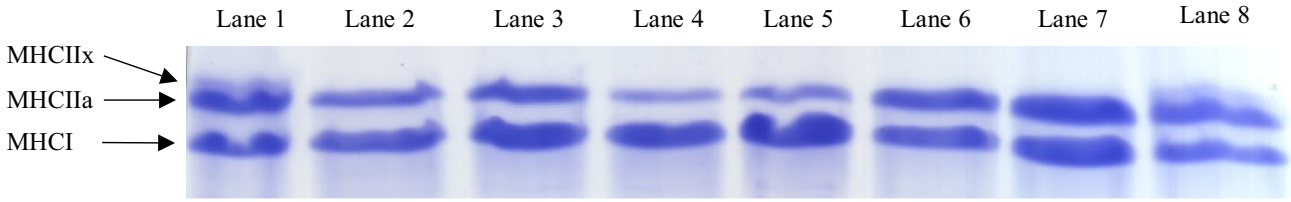
TABLE 1 | Subject characteristics.

	10-SET		3-SET		Group diff.
	Males (*n*=6)	Females (*n*=4)	Males (*n*=6)	Females (*n*=4)	*p*
Age (years)	22 ± 4	23 ± 4	21 ± 3	23 ± 4	0.57
Body mass (kg)	81.2 ± 7.4	71.5 ± 3.3	90.7 ± 10.7	74.1 ± 4.2	0.15
Height (cm)	184 ± 4	175 ± 5	193 ± 5	173 ± 3	0.22
VO_2peak (mL min^{-1} kg^{-1})	61 ± 3	46 ± 4	56 ± 6	48 ± 6	0.54
2k-TT (watts)	354 ± 35	235 ± 38	375 ± 53	261 ± 22	0.47
IMTP (kg)	255 ± 19	179 ± 37	299 ± 50	191 ± 65	0.28

Note: 10-SET, high-volume strength training, 5–10 sets of 10 repetitions; 2k-TT, 2 km ergometer rowing test; 3-SET, moderate-volume strength training, 3 sets of 10 repetitions; IMTP, isometric midthigh pull. Data are presented as mean ± SD.

TABLE 2 | Myosin Heavy Chain composition before and after strength training.

	Pre	Post	Change (%)	Males (pre), *n*	Females (pre), *N*
MHCI (%)					
10-SET	54 ± 7	48 ± 5	−5 [−11; 0]	5	3
3-SET	55 ± 10	47 ± 6	-8 [−14; −2]	6	4
Overall	54 ± 8	47 ± 5	−7 [−11; −3]	51 ± 8	60 ± 5
MHCIIa (%)					
10-SET	43 ± 9	50 ± 5	7 [2; 12]		
3-SET	41 ± 8	51 ± 6	10 [4; 15]		
Overall	42 ± 8	50 ± 5	9 [5; 12]	46 ± 7	36 ± 4
MHCIIx (%)					
10-SET	3 ± 5	2 ± 2	−2 [−4; 1]		
3-SET	4 ± 4	3 ± 2	−2 [−4; 1]		
Overall	4 ± 5	2 ± 2	−2 [−3; 0]	3 ± 4	5 ± 5



Note: 10-SET: high-volume strength training, 5–10 sets of 10 repetitions ($n = 8$); 3-SET: moderate-volume, 3 sets of 10 repetitions ($n = 10$). Data is presented as mean ± SD and mean changes and 95% CI. Separate data from males and females represents the MHC composition at baseline (Pre). Lower part depicts representative bands of MHC analysis, with internal standards of all three MHC isoforms at lanes 1 and 8, and lanes 2–3 (pre), 4–5 (post) and 6–7 (pre, and post on right hand side of same gel, not show), the two bands (0.03 and 0.04 mg protein) of each subject and condition. Pre and post are run next to each other on the same gel.

3.4 | Changes in SR Ca^{2+} Related Proteins in Response to Heavy Strength Training

Training-induced changes in the expression of proteins involved in SR Ca^{2+} regulation was in general uncertain with no clear findings (Figure 2). However, the results show a small decrease (−8% [−29; 12]) in the abundance of SERCA2 (10-SET: −12% [−42; 19]; 3-SET: −5% [−42; 32], mean difference: −6% [−50; 37] in 10-SET vs. 3-SET, group × time: $p = 0.89$, time-effect: $p = 0.08$) (Figure 2A). Larger, but still uncertain, changes were observed with SERCA1 + 28% [0; 57] (10-SET: 27% [−24; 77]; 3-SET: 30% [−16; 75], mean difference: −3% [−63; 57] in 10-SET vs. 3-SET, group × time: $p = 0.96$, time-effect: $p = 0.17$) (Figure 2B), and RyR1 + 36% [−23; 94] (10-SET: 49% [−91; 190]; 3-SET: 24% [−29; 77], mean difference: 25% [−97; 147] in 10-SET vs. 3-SET, group × time: $p = 0.89$, time-effect: $p = 0.46$) (Figure 2C).

3.5 | Sex Differences at Baseline

Compared to female rowers ($n = 7$), the male rowers ($n = 11$) displayed a lower percentage of MHCI (50.6% ± 7.8% vs. 59.7% ± 5.2%, mean difference −9% [−2; −16], $p = 0.02$), with a concomitant higher percentage of MHCIIa (46.0% ± 6.6% vs. 35.5% ± 3.6%, mean difference + 12% [6; 18], $p = 0.002$) ($n = 7$). No sex difference was found for MHCIIx (males: 3.1% ± 4.4% vs. females: 4.5% ± 4.5%, mean difference −1% [−6; 4] in males vs. females, $p = 0.57$).

There was also an apparent, but not statistically significant, sex difference in SR vesicle Ca^{2+} handling. Although Tau appeared similar between sexes (males: 67.8 ± 11 s^{-1} mg $protein^{-1}$ vs. females: 68.7 ± 9 s^{-1} mg $protein^{-1}$, mean difference: −1 s^{-1} mg $protein^{-1}$ [−12; 10], $p = 0.86$) (Figure 3A), males had numerically higher SR Ca^{2+} uptake rates at 600 nM Ca^{2+} (males: 2.32 ± 0.50 μmol g $protein^{-1}$ min^{-1} vs. females: 2.16 ± 0.39 μmol g $protein^{-1}$ min^{-1}, mean difference: 0.16 [−0.34; 0.66], $p = 0.50$) (Figure 3B) and 200 nM Ca^{2+} (males: 0.76 ± 0.18 μmol·g $protein^{-1}$·min^{-1} vs. females: 0.64 ± 0.19 μmol·g $protein^{-1}$·min^{-1}, mean difference: 0.12 μmol g $protein^{-1}$ min^{-1} [0.09; 0.32], $p = 0.24$) (Figure 3C). A similar pattern was observed for the difference in the SR Ca^{2+} release rate between the male and female rowers (males: 1.23 ± 0.18 μmol g $protein^{-1}$ min^{-1} vs. females: 1.13 ± 0.21 μmol g $protein^{-1}$ min^{-1}, mean difference: 0.11 μmol g $protein^{-1}$ min^{-1} [−0.09; 0.31], $p = 0.25$) (Figure 3D).

An apparent, but uncertain pattern of a higher expression of SERCA1 and lower SERCA2 and RyR1, were found in male versus and female rowers. Observed expressions were SERCA2 (males: 0.96 ± 0.29 vs. females:1.09 ± 0.21, mean difference: −0.13 [−0.46; 0.19], $p = 0.38$) (Figure 4A); SERCA1 (males: 0.96 ± 0.31 vs. females: 0.75 ± 0.31, mean difference: 0.21 [−0.16;

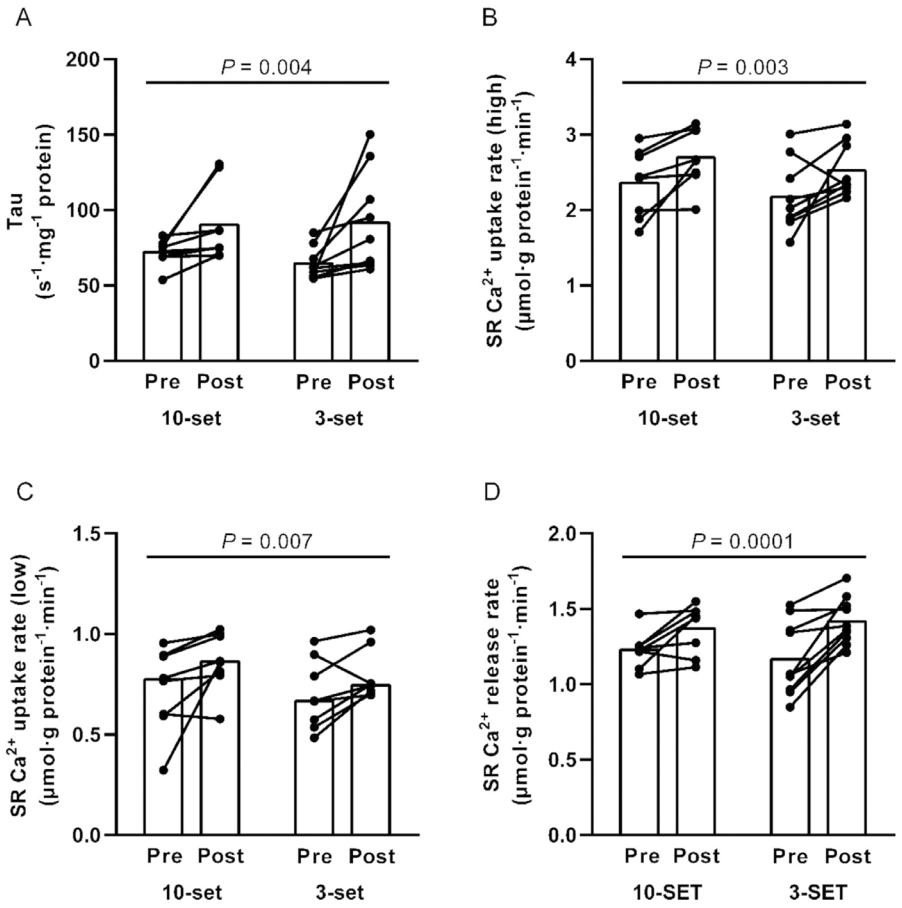


FIGURE 1 | Effects of 8 weeks of strength training on SR vesicle Ca^{2+} uptake and release rates. Changes in SR vesicle Ca^{2+} uptake, estimated as (A) Tau^{-1}, i.e., reciprocal time for free [Ca^{2+}] to decrease by 63%, with increased value denoting higher uptake rate (B) uptake rate at 600 nM Ca^{2+}, (C) uptake rate at 200 nM Ca^{2+} and (D) changes in SR vesicle Ca^{2+} release rate, following 8 weeks of either high-volume strength training (10-SET) or moderate-volume (3-SET). Data are presented as means and individual paired values. $n = 8$ for 10-SET and 9 for 3-SET, except for uptake low 3-SET ($n = 8$).

0.58], $p = 0.24$) (Figure 4B); RyR1 (males: 0.98 ± 0.39 vs. females: 1.14 ± 0.41, mean difference: -0.16 [-0.64; 0.33], $p = 0.49$) (Figure 4C). Together, this points to a seemingly, although not statistically different, higher SR Ca^{2+} uptake, likely explained by a higher MHCII proportion.

4 | Discussion

Here we investigated adaptations in SR Ca^{2+} handling, SR proteins and MHC composition following 8 weeks of strength training in a group of elite male and female rowers. Following the training period, both groups increased their maximal isometric midthigh pull strength. Regardless of strength training volume (3-SET and 10-SET) the rowers improved the SR Ca^{2+} release rate by 19% and the SR Ca^{2+} uptake rate (tau) by 32%, with no differences between groups. Concomitantly, the MHC composition shifted toward an increased percentage of the MHCIIa isoform. The changes in SR proteins were uncertain, but in line with an increase in the volumetric proportion of MHCII fibers, it suggests relevant increases in SERCA1 and RYR1 and a decrease in SERCA2. Furthermore, the results point to a seemingly higher SR Ca^{2+} uptake and release rates in males, compared to female rowers, likely explained by a higher MHCII proportion, in line with higher SERCA1 and lower SERCA2 expressions in the male rowers.

4.1 | Training Induced Changes in SR Function, SR Related Proteins, and MHC Composition

The results of the present study support the notion of SR plasticity in response to training, demonstrating increased Ca^{2+} uptake and release in SR vesicles after 8 weeks of strength training. This finding aligns with previous research, demonstrating a 12% increase in SR vesicle Ca^{2+} release and an 11% increase in Ca^{2+} uptake rate following 11 weeks of moderate-volume resistance training in untrained men (two to five sets of 10RM) [13]. Similarly, 12 weeks of resistance training improved SR Ca^{2+} uptake and Ca^{2+}-ATPase capacity in elderly women, though it did not alter relative fiber-type areas or SR function in young women [14]. Previous studies from our laboratory have shown a 9% increase in SR Ca^{2+} release rate following 5 weeks of sprint interval training in untrained males (12), and a 10% increase following 4 weeks of high-intensity interval training in highly trained male cyclists and triathletes [7, 12]. However, neither of the high intensity exercise training studies observed changes to the SR Ca^{2+} uptake rate [7, 12]. Similarly, 7 weeks of sprint

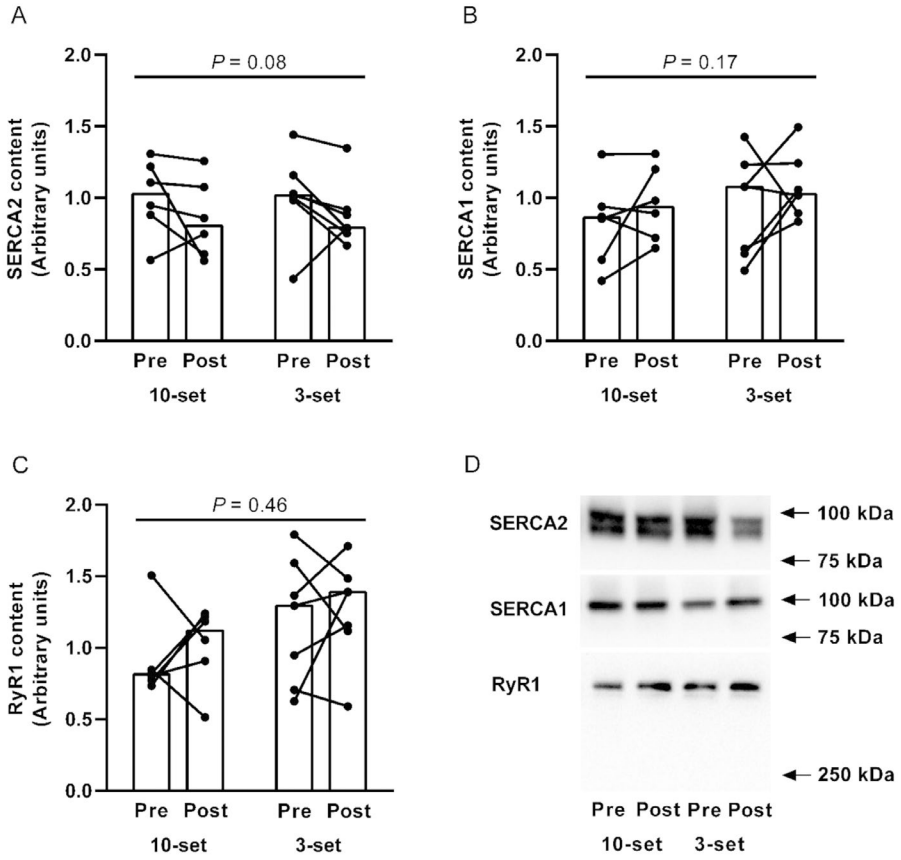


FIGURE 2 | Effects of 8 weeks of strength training on SR Ca^{2+} related proteins. Changes in m. vastus lateralis content of (A) sarcoplasmic reticulum Ca^{2+}-ATPase 2, (B) sarcoplasmic reticulum Ca^{2+}-ATPase 1, and (C) ryanodine receptor 1 following 8 weeks of either high-volume strength training with 5–10 sets of 10 repetitions (10-SET, $n = 6$): Or moderate-volume with 3 sets of 10 repetitions (3-SET, $n = 7$). Data are presented as means and individual paired values Immunoblotting of the SR proteins was conducted on 6 (10-st) and 7 (3-SET) paired pre- and post-training. (D) Representative bands of SERCA2, SERCA1, and RyR1.

training reduced Ca^{2+}-ATPase capacity but not Ca^{2+} uptake in sedentary type 1 diabetics and controls [30]. Together, these findings suggest that strength and HIIT training generally increase SR vesicle Ca^{2+} release rates, while effects on SR Ca^{2+} uptake rates and Ca^{2+}-ATPase capacity are equivocal. Endurance training appears to have no or negative effects on SR Ca^{2+} uptake and ATPase properties, with no studies having measured SR Ca^{2+} release properties. Specifically, Ca^{2+}-ATPase capacity was reduced after 6 days of low-intensity endurance training in six untrained men and after 10 weeks of single-legged submaximal cycling in 10 untrained men [5, 31]. Additionally, Ca^{2+}-ATPase content remained unaltered following 6 weeks of moderate-intensity endurance training in 39 trained men [22]. As the present intervention included both aerobic training and strength training, it cannot be excluded that the aerobic training, at least in part, contributed to the change in SR function. However, the strength training intervention was conducted just after a competition period, and the volume and intensity of aerobic training was therefore reduced compared to pre intervention period, which together argues for an effect of strength training on the SR function.

Since the SR function measurements are conducted in vitro under constant conditions, the observed changes in SR vesicle Ca^{2+} release rate depend directly on the number of RyR channels, the average opening time of these channels, and the electrochemical gradient of Ca^{2+} across the SR membrane. This assumes the conductance of the RyR channels remains constant. Similarly, the increase in SR vesicle Ca^{2+} uptake rate is directly dependent on the number of SERCA pumps, their pumping rate, and the Ca^{2+} gradient across the SR membrane. Importantly, muscle fiber types influence both SR Ca^{2+} content and handling. In rodents, type II fibers exhibit a faster SR Ca^{2+} release rate and a higher total amount of RyR1channels per muscle volume [18], as well as a greater peak myocellular [Ca^{2+}] during SR Ca^{2+} release [19] compared to type I fibers. Additionally, type II fibers in rats [32] and rabbits [33] have approximately 2–7 times greater SERCA expression (SERCA1) compared to the SERCA expression in type I fibers (SERCA2). In humans, SERCA1 is exclusively expressed in type 2 fibers [34]. Also, the relative distribution of fast-twitch fibers correlates with the Ca-ATPases content, revealing 2.2–2.4 times more ATPases in type II fibers. Furthermore, the maximal uptake rate and SR Ca^{2+} uptake capacity is higher in type II fibers [20, 35]. The improvements in SR Ca^{2+} handling observed in the present study were evident in both the 3-set and 10-set groups and coinced with an increased proportion of the MHCIIa isoform. Hence, the increase in the proportion of the MHCIIa isoform may explain the observed improvements in SR Ca^{2+} kinetics. Given that type 2 fibers have an assumed four times higher SR Ca^{2+} release rate,

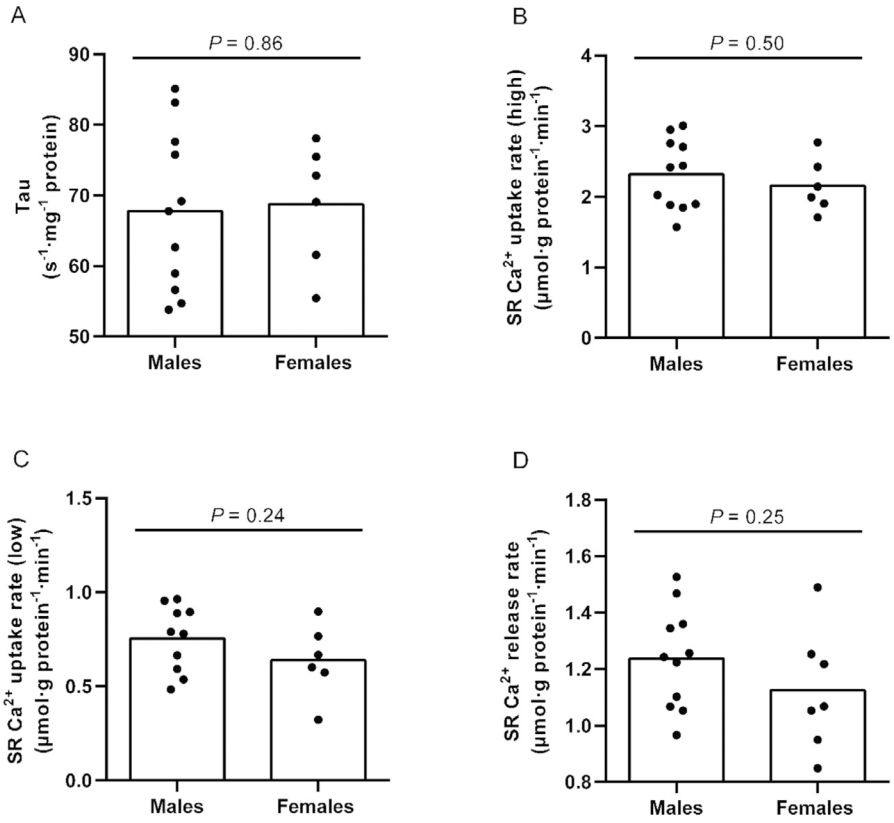


FIGURE 3 | SR vesicle Ca^{2+} uptake and release rates in male and females. Individual and mean values of (A) Tau^{-1}, i.e. reciprocal time for free $[Ca^{2+}]$ to decrease by 63%, with increased value denoting higher uptake rate (B) uptake rate at 600 nM Ca^{2+}, (C) uptake rate at 200 nM Ca^{2+}, and (D) SR vesicle Ca^{2+} release rate in elite male ($n = 10–11$) and female ($n = 6–7$) rowers. Data are presented as means and individual values.

most of the increase in SR vesicle Ca^{2+} release rate (67%) may be attributed to the increase in MHCII volume density, i.e., relative increase in type 2 fiber volume. In line with changes in MHC composition, numerical increases were observed in the abundance of RyR1 and SERCA1, while a numerical decrease was observed for the abundance of SERCA2 after the training period. However, these findings are somewhat uncertain, and indicated by 95% confidence intervals, possibly due to the low sample size of our western blots. Together, these findings suggest that the observed increase in SR vesicle Ca^{2+} regulation may be largely explained by an increased MHCII volumetric proportion, leading to a higher whole muscle content of SR and possibly Ca^{2+} regulatory proteins.

Notably, a previous study reported that 5 weeks of sprint training induced an increase in SR vesicle Ca^{2+} release rate, due to an enhanced fiber total SR contents, without changes in MHC distribution [12]. Thus, a fiber type-independent change in SR function and content may also partly explain the present increase in SR function. The change in MHC composition is consistent with previous results published by Liu and colleagues, who reported a 21% increase in MHCIIa and a 13% decrease in MHCI isoform in the m. vastus lateralis of highly trained rowers after 3 weeks of six strength training sessions per week [36]. Changes in MHC composition could result from either fiber type transformations (i.e., MHCI and MHCIIx to MHCIIa) or a relative increase in the volume of MHCIIa fibers due to hypertrophy. A MHCI to MHCIIa fiber transformation does not seem to occur following periods of high-volume strength training [37–39]. However, preferential hypertrophy of MHCII fibers, has been reported [40, 41]. Such specific hypertrophy of MHCIIa fibers, in combination with a MHCIIx to MHCIIa transformation, would explain the observed alterations in MHC composition following the training period.

4.2 | Sex Differences in MHC Composition, SR Ca^{2+} Function, and SR Related Proteins

A salient observation is that clear sex-based differences exist for many physiological parameters such as muscle mass [42], aerobic capacity [43] and hematology [28]. However, to the best of our knowledge, this study is the first to report on sex differences in SR Ca^{2+} dynamics and SR-related proteins between elite males and females. The greater proportion of MHCIIa expressed in muscles of male rowers aligns with most existing literature [44]. Since SR Ca^{2+} handling is largely dependent on the MHC composition and based on the higher relative amount of MHCII in males, SR Ca^{2+} handling would be expected to be higher among the male rowers. While there was a significant difference in MHC composition between sexes, no differences were however observed between male and female rowers for any measurements related to SR Ca^{2+} handling. In this regard, we cannot exclude the possibility that the difference in MHC composition was too small to reveal differences in SR Ca^{2+} handling between the sexes. More research is required to better understand possible sex differences in SR Ca^{2+} handling.

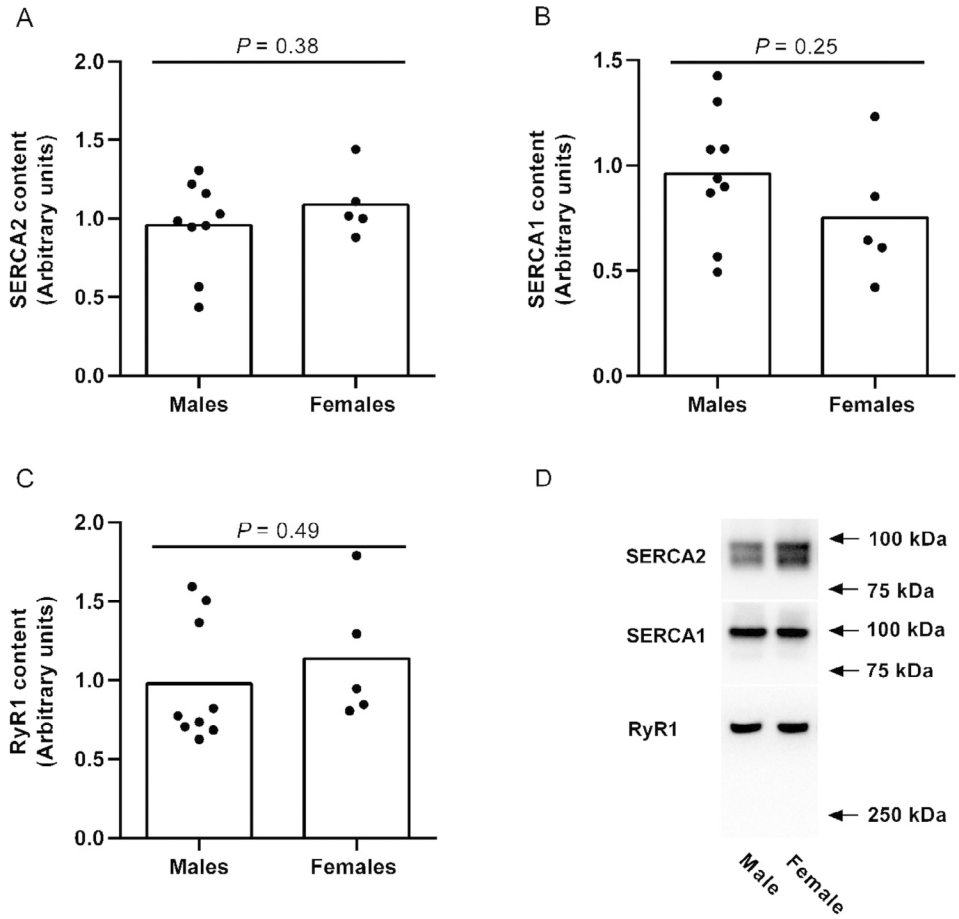


FIGURE 4 | SR Ca^{2+} related proteins male and females. Individual and mean values of (A) SERCA2, (B) SERCA1, and (C) RyR1 in *m. vastus lateralis* of elite male ($n = 9$) and female ($n = 5$) rowers. Data are presented as means and individual values. (D) Representative bands of SERCA2, SERCA1, and RyR1 in male and female.

4.3 | Limitations

A limitation of this training study is the lack of a control group conducting only endurance training in a similar manner and load as the 3-SET and 10-SET groups. However, the present study was designed to compare two strength training modalities, and their effects on SR function and -protein expression, in addition to possible baseline sex differences in SR Ca^{2+} handling properties in elite athletes. It cannot be excluded that the effects observed are due to other training stimuli, i.e., aerobic training. Accordingly, a design incorporating a control group would have strengthened the study but does not compromise the overall conclusions. Also, the intervention was conducted 1 week following the national ergometer rowing championships, where rowers aimed for maximal aerobic performance compared to pre intervention period.

The SR protein content findings were uncertain, and relevant differences between the groups or a main effect of training may have been overlooked, precluding any definitive conclusions about the effect of training on SR protein content. Although recruiting elite athletes for invasive studies requiring muscle biopsies is challenging, a larger sample size is therefore essential to confidently identify true effects of strength training on these proteins.

4.4 | Perspective

This study provides novel insights into the effects of an intensified period of strength training on muscle SR function in elite male and female rowers. The results show that strength training increased maximal SR vesicle Ca^{2+} release and uptake rates, which may also contribute to the greater muscle strength and power output observed following a period of strength training in trained individuals. Based on the present data, demonstrating similar outcomes in the matched 3-SET and 10-SET groups, one can conclude that resistance training with a lower volume (3-SET) is comparably effective at increasing MHCIIA, SR Ca^{2+} handling and muscle strength as a higher volume (10-SET). The practical implication of this is that with a short intense period of strength training outside regular endurance training 3-SET as efficient as higher volume (10-SET) training.

5 | Conclusion

In conclusion, 8 weeks of moderate- or high-volume strength training improved maximal strength and increased SR Ca^{2+} vesicle uptake and release rates, regardless of strength training volume (3×10 vs. 10×10 repetitions). The training also altered the MHC composition, toward a greater volumetric proportion of

the MHCIIa isoform. Collectively, we propose that the observed augmentation in SR vesicle Ca^{2+} regulation primarily results from an elevated volumetric representation of MHCII, leading to a greater overall content of SR and Ca^{2+} regulatory proteins.

Acknowledgments

This study was supported by Team Danmark by means from the Novo Nordisk Foundation, The Wenner-Gren Foundation, and The Swedish Olympic Committee. The authors would like to express their gratitude to all the rowers for their participation in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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