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Role of CaMKII and sarcolipin in muscle adaptations to strength training with different levels of fatigue in the set

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Abstract

Strength training promotes a IIX-to-IIA shift in myosin heavy chain (MHC) composition, likely due to changes in sarcoplasmic [Ca^{2+}] which are sensed by CaMKII. Sarcoplasmic [Ca^{2+}] is in part regulated by sarcolipin (SLN), a small protein that when overexpressed in rodents stimulates mitochondrial biogenesis and a fast-to-slow fiber type shift. The purpose of this study was to determine whether CaMKII and SLN are involved in muscle phenotype and performance changes elicited by strength training. Twenty-two men followed an 8-week velocity-based resistance training program using the full squat exercise while monitoring repetition velocity. Subjects were randomly assigned to two resistance training programs differing in the repetition velocity loss allowed in each set: 20% (VL20) vs 40% (VL40). Strength training caused muscle hypertrophy, improved 1RM and increased total CaMKII protein expression, particularly of the δ_D isoform. Phospho-Thr^{287}-CaMKII δ_D expression increased only in VL40 (+89%), which experienced greater muscle hypertrophy, and a reduction in MHC-IIX percentage. SLN expression was increased in VL20 (+33%) remaining unaltered in VL40. The changes in phospho-Thr^{287}-CaMKII δ_D were positively associated with muscle hypertrophy and the number of repetitions during training, and negatively with the changes in MHC-IIX and SLN. Most OXPHOS proteins remained unchanged, except for NDUFB8 (Complex I), which was reduced after training (−22%) in both groups. The amount of fatigue allowed in each set critically influences muscle CaMKII and SLN responses and determines muscle phenotype changes. With lower intra-set fatigue, the IIX-to-IIA MHC shift is attenuated.

KEYWORDS

exercise, fatigue, human, myosin heavy chain, skeletal muscle, velocity-based training

1 | INTRODUCTION

The amount of fatigue allowed during resistance training determines the structural and phenotypic changes elicited by a strength training program.[1] In this study, a higher level of fatigue (ie greater reduction in strength, velocity, and power) by performing repetitions closer to muscle failure was associated with greater muscle hypertrophy, a IIX-to-IIA myosin heavy chain (MHC) switch,[1] and a lesser improvement in jumping performance despite each repetition being executed at the maximal intended velocity. However, we did not address the molecular mechanisms explaining these differences in muscle adaptation.

Experiments involving genetic manipulation, denervation, and immobilization indicate that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a principal regulator of muscle phenotype.[2,3] CaMKII regulates the expression of the transcription factor coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), which is a critical regulator of mitochondrial biogenesis.[4] Besides this, CaMKII has been implicated in the regulation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB),[5,6] which is stimulated by high-intensity exercise[7] and muscle metabolism.[8-10] Nevertheless, previous evidence on the role of CaMKII in the regulation of muscle phenotype comes from extreme experimental models and information using physiological models in humans is lacking.[2] CaMKII has been implicated in muscle hypertrophy in animal models.[11-13] CaMKII is encoded by four genes (α, β, γ, and δ) and more than 40 splice variants have been reported[14] of which, the isoforms β_M, γ, δ_A, and δ_D are expressed in skeletal muscle.[15-19]

Although increases of resting CaMKII γ/δ expression and phosphorylation status have been reported after sprint training,[20] no study has determined the effect of strength training on basal total expression and phosphorylation state of CaMKII isoforms in human skeletal muscle. Moreover, it remains unknown whether differences in CaMKII phosphorylation specific to the level of fatigue could be implicated in the IIX-to-IIA MHC changes elicited by most strength training programs.[21] The latter could be the case, since it has been shown that CaMKII is activated by Ca^{2+}-calmodulin binding and autophosphorylation,[2] particularly in response to Ca^{2+} transients elicited by prolonged contractions[22] or repeated briefs contractions.[23] In turn, CaMKII activation may stimulate PGC-1α expression and mitochondrial biogenesis.[22]

Another mechanism that could explain muscle phenotype changes is a reduction in expression of the protein sarcolipin (SLN).[24] Sarcolipin is a small protein expressed only in skeletal and cardiac muscle,[25] which regulates the maximal activity and thermodynamic efficiency of the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA).[26] Animal experiments indicate that SLN could have a role in exercise performance and muscle phenotype.[27,28] Mice overexpressing SLN (Sln^{OE}) have superior resistance to fatigue during electrically induced contractions than their wild-type (WT) counterparts.[27] Likewise, compensatory muscle hypertrophy elicited by overloading of the *plantaris* muscle by bilateral surgical excision of the *soleus* and *gastrocnemius* muscles (synergist ablation) is associated with increased SLN mRNA[29] and protein[24] expression in mice. However, the effects of strength training on SLN protein expression remain unknown. Therefore, the principal aim of the present investigation was to determine whether basal CaMKII phosphorylation in skeletal muscle is modified depending on the level of fatigue elicited during a strength training program. A secondary aim was to ascertain whether the changes in muscle mass (hypertrophy),[1] MHC composition,[1] and mitochondrial protein expression are associated with the changes in CaMKII phosphorylation. Finally, we sought to identify whether SLN expression in skeletal muscle is increased by strength training and if it associates with muscle hypertrophy, as previously reported in rodents.

We hypothesized that CaMKII phosphorylation would increase more in subjects training with greater fatigue (reduction in power) allowed during each set, stimulating a IIX-to-IIA shift in MHC expression, muscle hypertrophy, and the expression of mitochondrial proteins. We also hypothesized that training with more fatigue would be associated with a higher increase in SLN expression.

2 | MATERIALS AND METHODS

2.1 | Participants

This study is an extension of our previous study (see Pareja-Blanco et al[1]). The characteristics of the two groups analyzed, experimental design and general procedures have been reported previously.[1] Twenty-four young and healthy men volunteered to participate in this study. Their initial one-repetition maximum (1RM) strength for the full (deep) squat (SQ) exercise was 1.41 ± 0.19 kg body mass^{-1}. All subjects were physically active sports science students with a resistance training experience ranging from 1.5 to 4 years (1-3 sessions/wk) and were accustomed to performing the SQ with a correct technique. Subjects were randomly assigned to one of two groups only differing in the magnitude of repetition velocity loss allowed within each set during training: 20% (VL20; n = 12) or 40% (VL40; n = 12). Consequently, training volume was larger for VL40 than VL20 (310.5 ± 42.0 vs 185.9 ± 22.2 total repetitions, respectively).

Two subjects from the VL40 dropped out, one of them due to an injury not related to the training intervention. All subjects were informed about the experimental procedures and potential risks before they provided their written informed consent. The study was approved by the institutional review committee of

Pablo de Olavide University and was performed in accordance with the Declaration of Helsinki. Subjects refrained from taking drugs, medications, or dietary supplements during the study and had not been on medical treatments or taken supplements for at least two months before the start of the study.

2.2 | Study design

Subjects trained twice a week (48-72 hours apart) during 8 weeks for a total of 16 sessions. A progressive resistance training program which comprised only the SQ exercise was used (see Pareja-Blanco et al[1] for a thorough description of the training program). Briefly, the two groups trained at the same relative intensity (%1RM), which was increased gradually from 70% to 85% of 1RM between the 1st and 16th training sessions) but differed in the maximum percentage velocity loss allowed in each exercise set (20% vs 40%, of the mean propulsive velocity [MPV] attained in the fastest repetition of the set). The 40% velocity loss limit for the SQ exercise allowed performing repetitions to, or close to, failure, while the 20% velocity loss limit is reached when the athlete has performed ~50% of the possible repetitions per set.[30] When the corresponding target velocity loss limit was exceeded, the set was finished. Consequently, the VL40 groups performed almost twice as many repetitions as the VL20 group (310.5 ± 42.0 vs 185.9 ± 22.2 total repetitions, respectively). All training sessions were supervised and carried out in the laboratory at the same time of the day (±1 hour) for each subject and under controlled environmental conditions (20°C and 60% humidity). Subjects were asked not to engage in any other type of strenuous physical activity, exercise training, or sports competition for the duration of the investigation. The use of any putative recovery treatments, nutritional, and anti-inflammatory supplements was prohibited during the study. Training compliance was 100% of all sessions for the subjects that completed the intervention. The two groups were assessed on two occasions: 48 hours before (Pre) and 48 hours after (Post) the 8-week training intervention. Both pre- and post-training testing took place in one session in the following order: a 20-m running sprint (data not reported here), countermovement vertical jump (CMJ), a progressive loading test in the SQ exercise and the fatigue test.[1] Five minutes after the SQ progressive loading test, the subjects performed the fatigue test described below.

2.3 | Fatigue test

This test was performed with the same absolute load (kg) at pre- and post-training measurements. This load corresponded to 60% of the 1RM attained at pre-training. During each repetition, volunteers were required to execute the eccentric phase in a controlled manner and performing the concentric phase at maximal intended velocity. Subjects were required to complete as many repetitions as possible until the MPV felt below 0.5 m s^{-1}. The following variables derived from this test were used for analysis: (a) the maximal number of repetitions and (b) the average MPV attained against the same number of repetitions to pre- and post-training. The MPV was defined as the average bar vertical velocity during the propulsive phase, which is defined as the portion of the concentric phase where acceleration is greater than −9.8 m s^{-2}.[31] Sprint, CMJ, and SQ progressive loading tests were performed as described previously.[1]

2.4 | Muscle biopsies, protein extraction, Western blotting, and MHC composition

Subjects reported to the laboratory after a 12 hours overnight fast, two days after the strength tests. The dinner preceding the biopsy day was standardized for pre- and post-training. After 10 minutes of rest in the supine position, the skin over the middle portion of the *vastus lateralis* (*VL*) muscle was anaesthetized with 2% lidocaine (2 mL). After that, muscle biopsy samples (80-160 mg) were obtained from the superficial region (2-3 cm depth) using the Bergstrom technique with suction. The leg to be biopsied was assigned randomly. The same leg was biopsied after training, 2 cm more proximal than the pre-training biopsy.

The same medical doctor performed all muscle biopsies, and great care was taken to standardize the site and depth of the sample. Upon collection, muscle samples were dried on a sterile gauze, carefully freed from visible blood, connective tissue and fat, and fractionated into two pieces. The first half was mounted on cork blocks with the use of Tissue-Tek OCT™ embedding medium and orientated so that myofibers could be cut transversely. Specimens were frozen by 10-15 seconds immersion in isopentane pre-cooled in liquid nitrogen. The other piece of the muscle biopsy was immediately frozen in liquid nitrogen. Both biopsy pieces were stored at −80°C until analyzed.

Whole skeletal muscle lysates were prepared as described previously[32] and total protein content quantified using the bicinchoninic acid assay.[33] In brief, ~10 mg of muscle was grinded by stainless steel balls during one minute in a Mikro-Dismembrator S (Sartorius) and immediately homogenized in urea lysis buffer (6 mol/L urea, 1% SDS) and 50× Complete protease inhibitor and 10× PhosSTOP phosphatase inhibitor cocktails (Roche). The concentration of protein was adjusted to 2.8 μg/μL in all muscle extracts. Then, the lysate was centrifuged for 12 min at 25 200 *g* at 16°C. The resulting supernatant was diluted with electrophoresis loading buffer (160 mmol/L Tris-HCl, pH 6.8, 5.9% SDS, 25.5% glycerol, 15% β-mercaptoethanol-bromophenol blue).

For Western blotting, the optimal amounts of protein loaded and antibodies were determined using a gradient of protein extracts ranging 2.5-30 μg and the linear relationship between the total protein loaded and the optical band density. Following assurance of linearity within this range, equal amounts of protein (5-15 μg) of each sample were electrophoresed with SDS-PAGE using the system of Laemmli[34] and transferred to Immun-Blot PVDF Membranes for Protein Blotting (Bio-Rad Laboratories). For SLN protein expression determination, two samples (pre- and post-training) from each subject (independently of the group) were run in duplicate together with two control samples (prepared from healthy human skeletal muscle). In the case of phospho-Thr^{287}-CaMKII, total CaMKII, and oxidative phosphorylation (OXPHOS) proteins, two samples (pre- and post-training) were run together with three control samples. All gels included two protein ladders (prestained protein standards All Blue; Bio-Rad Laboratories). Samples from the same subject were loaded onto the same gel with two or three control samples for quality check. The membranes were blocked for one hour in 4% bovine serum albumin or 5% nonfat dry milk diluted in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) (BSA or Blotto blocking buffers). Antibodies were diluted in BSA (phosphorylated and total CaMKII and SLN) or Blotto blocking buffer (OXPHOS) at a 1:2000 concentration and incubated overnight at 4°C. After incubation with primary antibodies, the membranes were incubated with an HRP-conjugated antibody (diluted 1:5000 in Blotto blocking buffer in all instances) for 1 hour at room temperature and subsequent chemiluminescent visualization with Clarity™ Western ECL Substrate (Bio-Rad Laboratories) using a ChemiDoc™ Touch Imaging System (Bio-Rad Laboratories). Protein ladders were captured under white light immediately following chemiluminescent imaging with the membranes positioned in the same position. Finally, densitometry band quantification was performed with the Image Lab© software 5.2.1 (Bio-Rad Laboratories). All membranes were stained with Reactive Brown 10 (Sigma-Aldrich) to control for differences in loading and transfer efficiency,[35] and the bands captured with the ChemiDoc™ Touch Imaging System and quantified with the Image Lab 5.2.1 software. Since loading was homogeneous in all membranes, no further corrections were performed. MHC composition was assessed as previously reported in the same extracts used for Western blotting.[1] Identification of CaMKII isoforms was based on their electrophoretic mobility (β_M: 72.7 kDa; δ_A: 60.0 kDa; γ: 56.0-62.2 kDa; and δ_D: 58.4 kDa), their predicted molecular weights (MW) and their reactivity with the antibodies for the total and phosphorylated forms of the enzyme, and an antibody specific for the δ isoforms.[15,19] The band showing the lowest MW in the membranes was considered as δ_D, the band appearing immediately above δ_D, which had a somewhat higher MW, was considered as γ (which was not marked by the antibody specific for the δ isoforms), the small thin band with a slightly higher MW just above the γ band was considered as δ_A band, and the band with the highest MW was considered as the β_M isoform. The results are reported and analyzed for each CaMKII isoform band separately or for combinations of bands, as done by others.[20,36-38]

The corresponding catalogue numbers of primary antibodies were as follows: anti-phospho-CaMKII (Thr^{287}), no. 12716 and anti-CaMKII, no. 4436, were purchased from Cell Signaling Technology. Anti-sarcolipin, no. ABT13 was purchased from EMD-Millipore. The anti-OXPHOS antibody was an optimized premixed cocktail antibody (total OXPHOS human antibody cocktail, no. ab110411) purchased from Abcam which targets 5 oxidative phosphorylation proteins: Complex I subunit NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8 (NDUFB8) (no. ab110242), Complex II subunit succinate dehydrogenase [ubiquinone] iron-sulfur subunit (SDHB) (no. ab14714), Complex III subunit cytochrome b-c1 complex subunit 2 (UQCRC2) (no. ab14745), Complex IV subunit cytochrome c oxidase subunit 2 (COX-II) (no. ab110258), and ATP synthase F1 subunit alpha (ATP5A) (no. ab14748). A CaMKII δ isoform-specific antibody (anti- CaMKII delta isoform no. A010-55AP; Badrilla) was used to distinguish between the γ and δ isoforms. The secondary HRP-conjugated goat anti-rabbit (no. 111-035-144) and goat anti-mouse (no. 115-035-003) antibodies were purchased from Jackson ImmunoResearch.

2.5 | Muscle volume and cross-sectional area determination

Magnetic resonance imaging 1.5-T scanner (General Electric, Milwaukee, Wisconsin, USA) was used to determine the total volume of the quadriceps muscle of both extremities as previously described.[1] The muscle cross-sectional area was determined using adenosine triphosphatase (ATPase) histochemical analysis, microscope visualization, and image analysis of fiber boundaries as described in detail elsewhere.[1]

2.6 | Statistical analyses

Descriptive values are presented as means ± standard deviations (SD). The normality of distribution of the variables was assessed with the Shapiro-Wilk test and, when necessary, data were transformed logarithmically before analysis. Homogeneity of variance was checked with the Levene's test. Data were analyzed using a repeated-measures ANOVA including one within-subjects factor (time, with two levels: pre- and post-training) and one between-subjects factor (training program, with two levels: VL20 and VL40). Associations between variables were examined by Pearson's correlation

analysis. Statistical significance was set at $P < .05$. All statistical analyses were performed using SPSS software version v.18 for Windows (SPSS Inc).

3 | RESULTS

The effects of the strength training program have been reported previously.[1] Briefly, both groups had similar improvements in 1RM, but the enhancement in jumping height performance was higher when training with less fatigue in the set (VL20) than when training close to failure (VL40). Training close to failure elicited greater hypertrophy of *vastus lateralis* and *intermedius* (+9%) than training with less fatigue (+3.4%). MHC-IIX percentage was reduced in VL40 but not in VL20.[1] Likewise, training close to failure elicited greater increase of *vastus lateralis* CSA at the level where the muscle biopsies were taken (+11.0%, from 29.3 ± 4.4 to 32.5 ± 5.1 cm^2, pre- and post-training, respectively, $P < .001$) than training with less fatigue (+0.7%, from 26.6 ± 2.9 to 26.8 ± 3.6 cm^2, pre- and post-training, respectively, $P = .67$) (time effect $P < .001$, group × time interaction $P < .001$).[1]

After training, the total expression of CaMKII protein, which includes all the isoforms detected, was increased by 20% (time effect $P < .001$, group × time interaction $P = .70$) (Figure 1A, B). This effect was due to a 40% higher expression of the CaMKII δ_D isoform after training (time effect $P < .001$, group × time interaction $P = .45$) (Figure 2D), while no statistically significant changes were observed in the expression of the other isoforms (Figure 2A-C).

After training, phospho-Thr^{287}-CaMKII protein expression was 20% higher than before training when all isoforms were analyzed conjointly (time effect $P = .055$, group × time interaction $P = .039$) (Figure 1C,D). Nevertheless, the Thr^{287}-CaMKII δ_D was increased by 89% in VL40 while it remained unchanged in VL20 (time effect $P = .01$, group × time interaction $P = .024$) (Figure 2H). No statistically significant changes were observed in protein expression of the Thr^{287}-CaMKII β, δ_A, and γ isoforms (Figure 2E-G), nor in the corresponding fractional phosphorylation ratios. The fractional phosphorylation of δ_D isoform was reduced by 27% in VL20, while it increased not significantly in VL40 (time effect $P = .74$, group × time interaction $P = .048$) (Figure 3). No significant changes

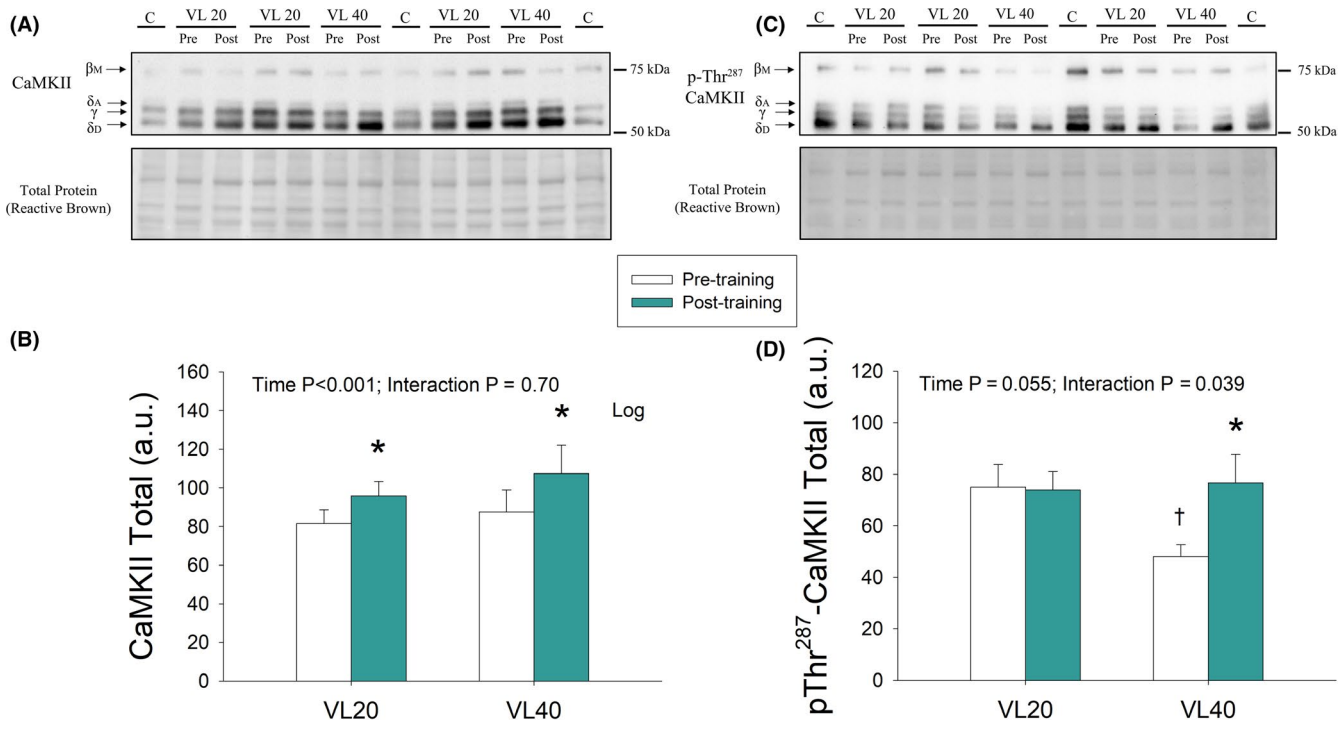

FIGURE 1 Skeletal muscle protein expression levels of total and phosphorylated CaMKII including all isoforms before (pre-training) and after (post-training) an 8-week velocity-based resistance training program using the full squat exercise in two groups of subjects allowing an intra-set repetition velocity loss of 20% (VL20, n = 12) or 40% (VL40, n = 10). (A) and (C): representative immunoblot images and total amount of protein loaded (Reactive Brown staining) of total CaMKII and phospho-Thr^{287}-CaMKII, respectively, from five participants in the study belonging to both training groups at pre- and post-training. A control human sample was run on triplicate on each gel as quality control. Estimated molecular weights are indicated on the right side of the blot. (B) and (D): protein expression levels of total CaMKII and phospho-Thr^{287}-CaMKII, respectively, with all isoforms analyzed conjointly. The values shown are means ± standard errors and expressed in arbitrary units (a.u.). *Significantly different from pre-training, $P < .05$; †Significantly different from VL20 at the same time point, $P < .05$

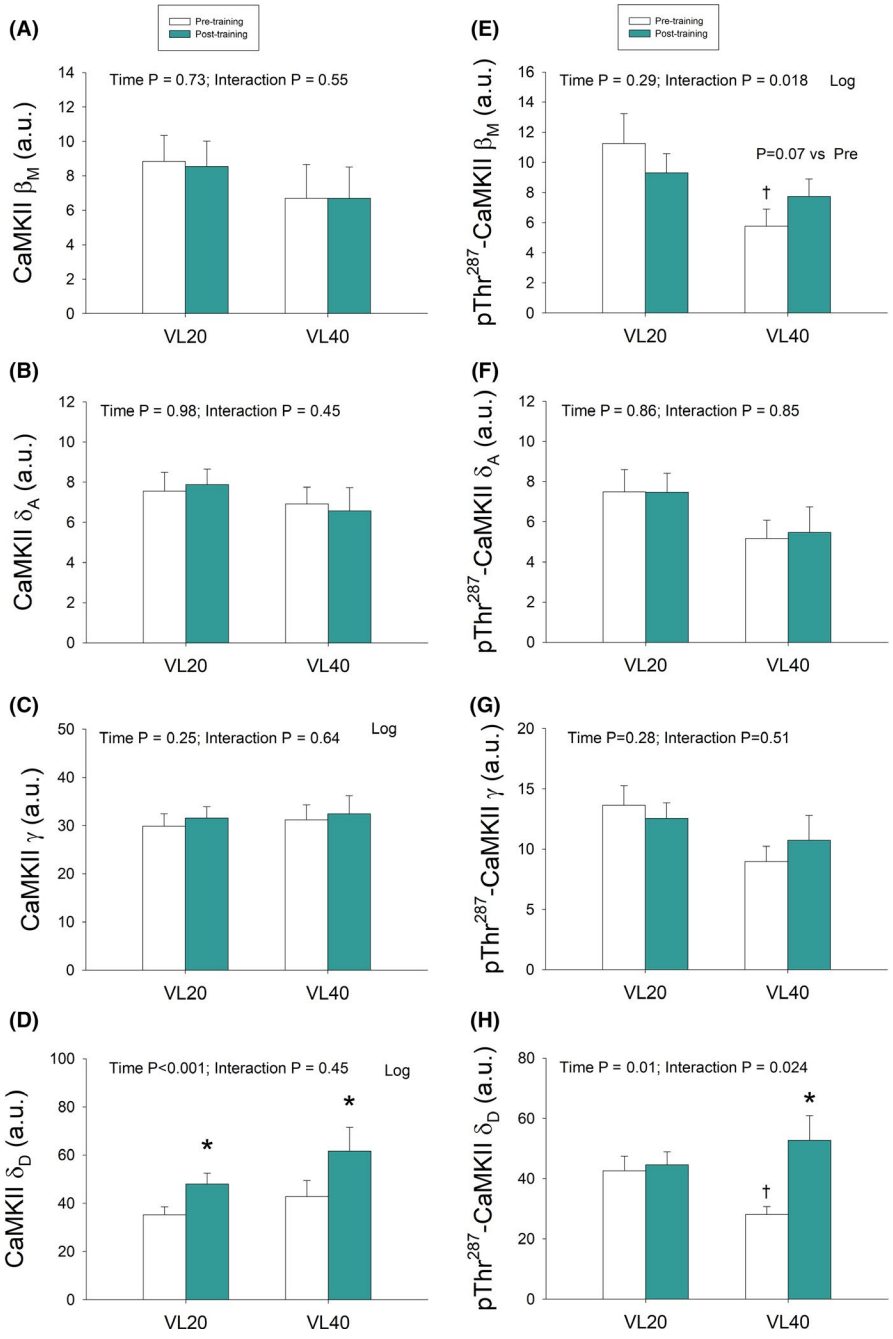

FIGURE 2 Skeletal muscle protein expression levels of total and phosphorylated CaMKII isoforms β_M, δ_A, γ, and δ_D analyzed separately before (pre-training) and after (post-training) an 8-week velocity-based resistance training program using the full squat exercise in two groups of subjects allowing an intra-set repetition velocity loss of 20% (VL20, n = 12) or 40% (VL40, n = 10). Left panel, total CaMKII expression: (A) CaMKII β_M, (B) CaMKII δ_A, (C) CaMKII γ, and (D) CaMKII δ_D. Right panel, phosphorylated form: (E) Thr^{287}-CaMKII β_M, (F) Thr^{287}-CaMKII δ_A, (G) Thr^{287}-CaMKII γ, (H) Thr^{287}-CaMKII δ_D. See the top of Figure 1 for representative immunoblots. The values shown are means ± standard errors and expressed in arbitrary units (a.u.). *Significantly different from pre-training, $P < .05$. † Significantly different from VL20 at the same time point, $P < .05$. Log: statistical analysis performed with logarithmically transformed data

were observed in the fractional phosphorylation of the other isoforms.

There was a positive association between the change in the expression of phospho-Thr^{287}-CaMKII δ_D and the total number of repetitions performed during training ($r = 0.59$, $P = .004$, n = 22) (Figure 4A), and the degree of *vastus lateralis* and *intermedius* hypertrophy ($r = 0.48$, $P = .03$, n = 22) (Figure 4B) as well as with the degree of *quadriceps* muscle hypertrophy ($r = 0.43$, $P = .048$, n = 22); while the association was negative with the change in MHC-IIX ($r = -0.72$, $P < .001$, n = 22) (Figure 4C). These associations depended specially on the changes observed in the VL40 group ($r = 0.59$, $P = .07$; $r = 0.72$, $P = .02$, and $r = -0.86$, $P = .002$, n = 10, for repetitions performed during training, degree of *vastus lateralis* and *intermedius* hypertrophy, and change in MHC-IIX, respectively).

Sarcolipin expression was increased by 33% in VL20 ($P = .048$) while the 11% reduction observed in VL40 did not reach statistical significance ($P = .38$, group × time interaction $P = .04$) (Figure 5A,B). No association was observed between the changes in muscle volume and those of SLN expression when all subjects were analyzed together ($r = -0.07$, $P = .76$, n = 22) or each group separately ($r = 0.30$, $P = .35$, n = 12; and $r = -0.28$, $P = .44$, n = 10, for the VL20 and VL40, respectively). Similar results were obtained between the changes in muscle CSA from the

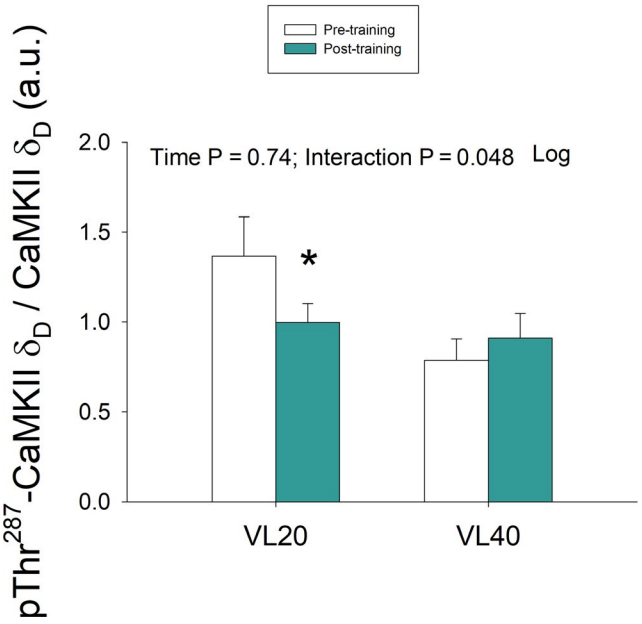

FIGURE 3 Phosphorylation fraction of the CaMKII δ_D isoform (phospho-Thr^{287}-CaMKII δ_D/CaMKII δ_D) before (pre-training) and after (post-training) an 8-week velocity-based resistance training program using the full squat exercise in two groups of subjects allowing an intra-set repetition velocity loss of 20% (VL20, n = 12) or 40% (VL40, n = 10). *Significantly different from pre-training, $P < .05$. Log: statistical analysis performed with logarithmically transformed data

muscle biopsies (previously reported[1]) and SLN expression when all subjects were analyzed together ($r = -0.20$, $P = .37$, n = 22) or each group separately ($r = 0.02$, $P = .95$, n = 12; and $r = -0.55$, $P = .07$, n = 10, for the VL20 and VL40, respectively).

There was a negative association between the changes in SLN and the changes in phospho-Thr^{287}-CaMKII δ_D ($r = -0.50$, $P = .018$, n = 22) and a positive association between the improvements in MPV during the fatigue test and the increase in SLN with strength training ($r = 0.42$, $P = .05$, n = 22). No associations were observed between the change in SLN expression on one side and the improvement in CMJ height ($r = 0.29$, $P = .18$, n = 22) and 1RM ($r = 0.35$, $P = .11$, n = 22). Before training, no association was observed between the relative cross-sectional area of type I fibers and SLN expression ($r = -0.37$, $P = .09$, n = 22) nor between the MHC-I percentage and SLN expression ($r = -0.28$, $P = .21$, n = 22).

In general, no significant changes were observed in OXPHOS proteins, except NDUFB8 which was reduced after training by 22% (time effect $P = .03$, group × time interaction $P = .26$) (Figure 6A-F). No association was observed between the changes in OXPHOS proteins and phospho-Thr^{287}-CaMKII isoforms.

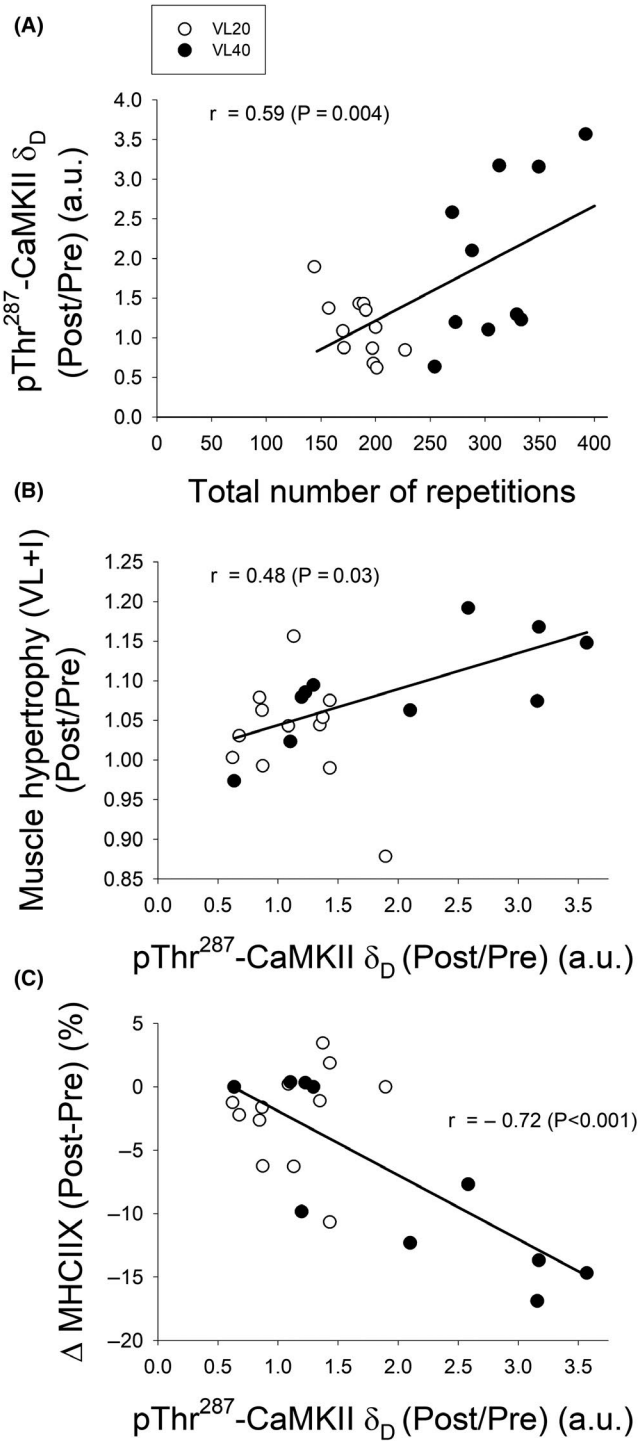

FIGURE 4 Relationship between the change in phospho-Thr^{287}-CaMKII δ_D and (A) the number of repetitions carried out during the training program, (B) the degree of muscle hypertrophy of m. *vastus lateralis* and *intermedius* (sum of both extremities) and (C) the delta change in myosin heavy chain IIX percentage (MHC-IIX) after 8 weeks of strength training allowing a 20% loss in mean velocity of the propulsive phase in the set (group VL20, n = 12, plain circles) or 40% (group VL40, n = 10, black circles). Note that in panel (B) there is an outlier, after removing this outlier the correlation improved to $r = 0.65$ ($P < 001$). Log: statistical analysis performed with logarithmically transformed data

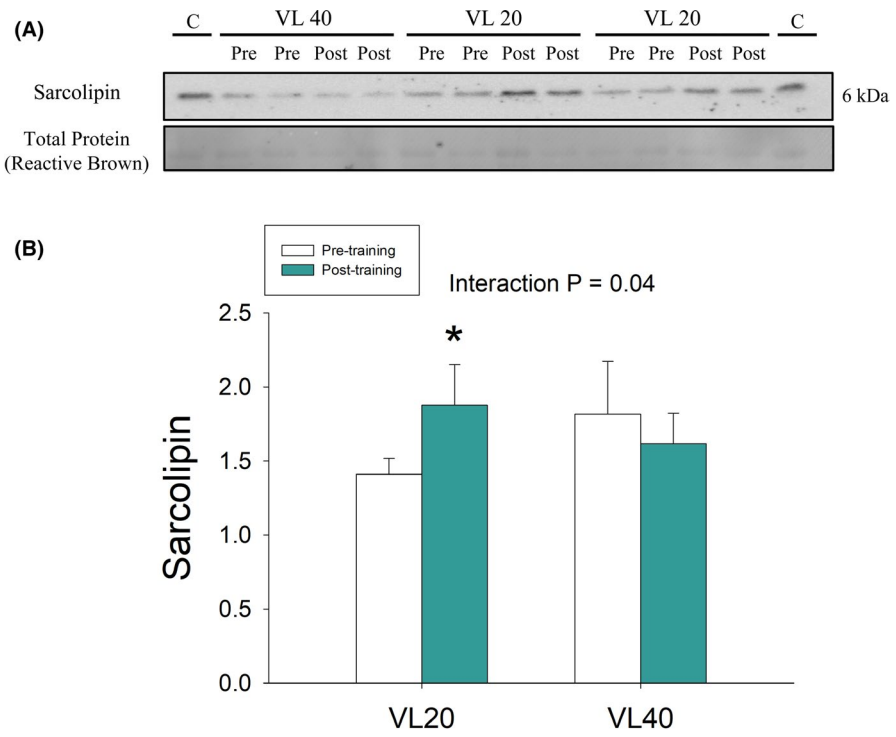

FIGURE 5 Skeletal muscle protein expression levels of sarcolipin before (pre-training) and after (post-training) an 8-week velocity-based resistance training program using the full squat exercise in two groups of subjects allowing an intra-set repetition velocity loss of 20% (VL20, n = 12) or 40% (VL40, n = 10). (A) Protein expression levels of sarcolipin, (B) Representative immunoblot image and total amount of protein loaded (Reactive Brown staining) in three subjects pre- and post-training. Duplicate assays pre- and post-training were included for all samples. A control human sample was run on duplicate on each gel as a loading control. Estimated molecular weights are indicated on the right side of the blot. The values shown are means ± standard errors and expressed in arbitrary units (a.u.). *Significantly different from pre-training, $P < .05$

4 | DISCUSSION

This study shows that strength training induces specific adaptations in basal levels of CaMKII and SLN protein depending on the level of fatigue allowed in the sets. Both types of training resulted in muscle hypertrophy and increased expression of CaMKII, particularly of the isoform δ_D. However, basal phospho-Thr^{287}-CaMKII phosphorylation levels responded in an isoform- and training-specific way, such that Thr^{287}-CaMKII δ_D was increased only when training with more fatigue, and a similar trend was observed for Thr^{287}-CaMKII β_M isoform. The fact that there was a positive association between the total number of repetitions carried out and the change in basal Thr^{287}-CaMKII δ_D phosphorylation is compatible with a cause-and-effect relationship. The latter is also supported by the observed association between the increase in Thr^{287}-CaMKII δ_D phosphorylation and the reduction in MHC-IIX expression, and the increase in muscle mass elicited by the training program. In contrast with our hypothesis, the changes in SLN expression were not associated with the changes in muscle mass, MHC-IIX percentage, or strength, while a negative association was observed between SLN expression and the mitochondrial proteins ATP5A and SDHB, and with the changes in phospho-Thr^{287}-CaMKII δ_D.

4.1 | Muscle phenotype changes with strength training and CaMKII

Although little is known regarding the specific role played by each isoform in human skeletal muscle, it has been reported that the protein expression of the CaMKII δ/γ isoforms is increased after sprint training[20] and endurance training[39,40] in humans, as observed in the present study. The increase in basal CaMKII δ/γ expression has been strongly associated with the increase in maximal CaMKII enzymatic activity and basal Thr^{287}-CaMKII protein expression.[40]

Also in agreement with our results, a significant increase in the total amount of CaMKII has been reported a few days after a blood flow-restricted strength training program,[41] although the phosphorylated form of CaMKII was not assessed in that study.[41] Likewise, rodent experiments have shown that a higher CaMKII activity promotes muscle hypertrophy[11-13] and a fast-to-slow MHC shift.[2] Collectively, the present investigation and these studies show that the basal protein expression levels of CaMKII are increased in response to diverse training stimuli. However, in these studies, the δ and γ isoform were analyzed conjointly[20,39,40] precluding any conclusion regarding the specific CaMKII isoform accounting for the reported effects.

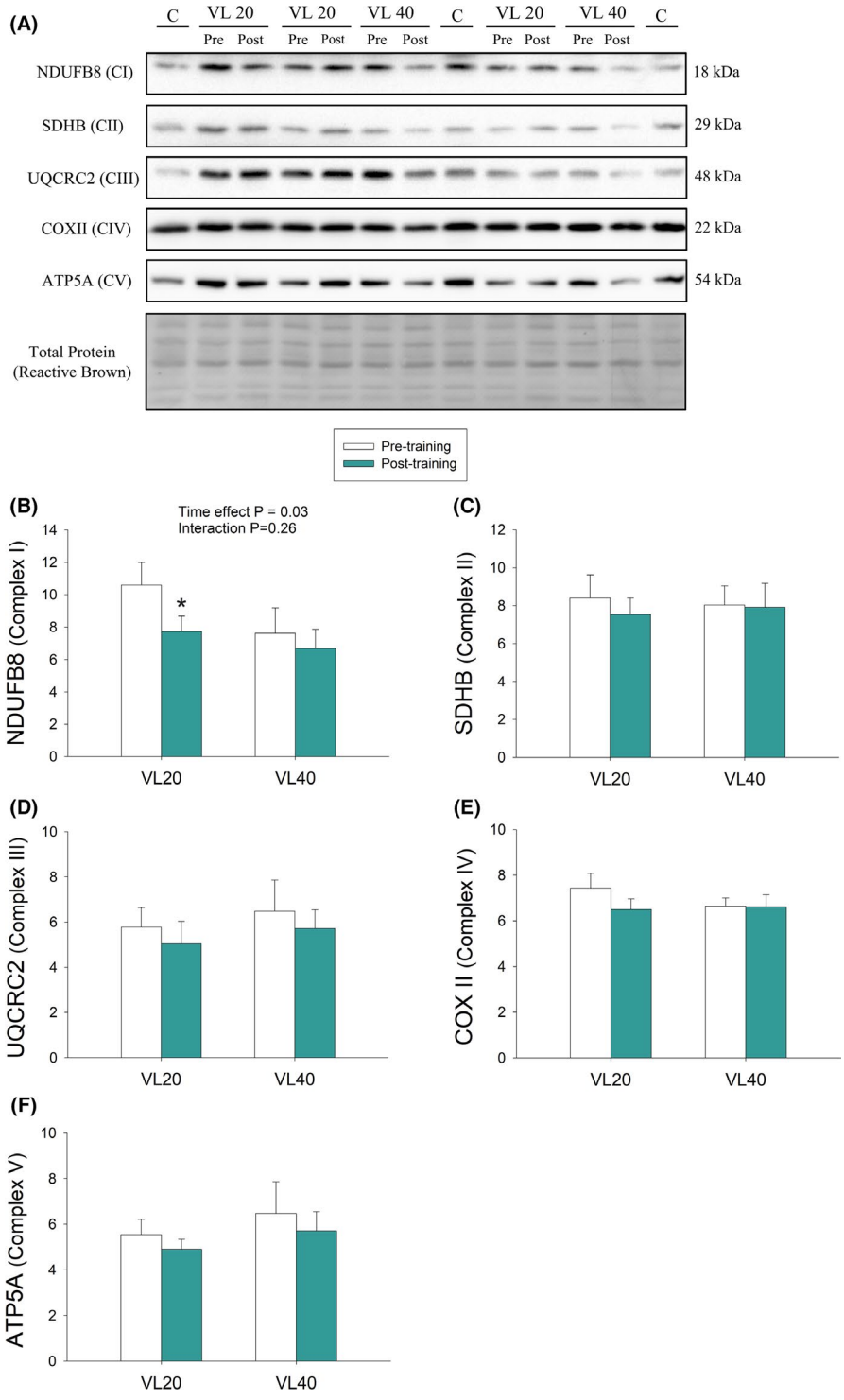

FIGURE 6 Skeletal muscle protein expression of different subunits of OXPHOS complexes: (A) Representative immunoblot images and total amount of protein loaded (Reactive Brown staining) from five participants in the study belonging to both training groups at pre- and post-training. A control human sample was run on triplicate on each gel as a loading control. Estimated molecular weights are indicated on the right side of the blot, (B) NDUFB8 (Complex I), (C) SDHB (Complex II), (D) UQCRC2 (Complex III), (E) COX-II (Complex IV), and (F) ATP5A (Complex V) before (pre-training) and after (post-training) an 8-week velocity-based resistance training program using the full squat exercise in two groups of subjects allowing an intra-set repetition velocity loss of 20% (VL20, n = 12) or 40% (VL40, n = 10). The values shown are means ± standard errors and expressed in arbitrary units (a.u.). *Significantly different from pre-training, $P < .05$

Thr^{287}-CaMKII phosphorylation is more pronounced right after sprint exercise,[42] prolonged-moderate intensity exercise,[43] and workouts eliciting more metabolite accumulation,[44] than with less severe exercise.[43,44] Strength training to failure is accompanied by higher activation of the anaerobic metabolism[45] and possibly greater Ca^{2+} transients than training with considerably less fatigue in each set.[2] The latter, combined with the higher oxidative stress associated with fatiguing contractions, should have facilitated the activation of CaMKII.[46,47] These facts could explain the observed associations between the change in basal Thr^{287}-CaMKII δ_D phosphorylation and the total number of repetitions completed during the training intervention, the reduction in MHC-IIX expression, and the increase in muscle volume elicited by the training program. Likewise, since CaMKII promotes recovery of muscle pH via activation of the Na^+/H^+ exchanger,[48] increased phospho-Thr^{287}-CaMKII could indirectly promote adaptive changes that could accelerate the recovery between sets.

An isoform and muscle type-specific Thr^{287}-CaMKII phosphorylation pattern have been reported in rodent skeletal muscle depending on stimulation frequency with special responsiveness of the δ_A isoform,[38] which has been implicated in the regulation of gene expression[49] via histone deacetylase 4 (HDAC4) phosphorylation to promote a fast-to-slow transition.[3] However, no association was observed here between the changes in the basal level of CaMKII phosphorylation and the expression of OXPHOS proteins, indicating that just a moderate rise in basal Thr^{287}-CaMKII phosphorylation may not suffice to induce an increase of mitochondrial protein.

4.2 | Sarcolipin protein expression is increased by strength training with less fatigue and is not associated with muscle hypertrophy

Animal studies have suggested that SLN may also determine the muscle phenotype by modulating the resting sarcoplasmic $[Ca^{2+}]$.[24,29] In the present research, no significant changes in SLN protein expression were observed in the group experiencing more muscle hypertrophy, and no relationship was found between muscle hypertrophy and SLN expression. This is at odds with some previous studies in rodents.[24,29] Riedl et al.[29] overloaded the *plantaris* muscle of mice by synergist ablation of the *soleus* and *gastrocnemius*. Consequently, the *plantaris* muscle doubled its mass in 14 days, likely because this model of overloading may cause muscle hyperplasia in mice[24] and also due to potential inaccuracies in the dissection.[24] This level of hypertrophy was associated with a ~20- to 40-fold increase in *Sln* mRNA, in part explained by the fact that murine adult *plantaris* muscles do not typically express SLN protein.[24] Using the same experimental model, Fajardo et al.[24] determined the changes in SLN protein expression in the overloaded *plantaris* and the unloaded/tenotomized *soleus* muscle of *Sln-null* ($Sln^{-/-}$) and WT mice. In Fajardo et al.[24] experiments, the compensatory hypertrophy of the *plantaris* was more modest and similar in WT and $Sln^{-/-}$ mice (~20%-25%). SLN protein expression increased 14-fold in the unloaded *soleus* of the WT and was accompanied by a slow-to-fast fiber type shift and atrophy, being these effects more pronounced in the $Sln^{-/-}$ mice. Thus, the two studies published in mice indicate that SLN protein expression occurs with overloading and unloading. This apparent counterintuitive adaptation could be due to the fact that immobilization and disuse cause inflammation, increase oxidative stress and elevate resting sarcoplasmic $[Ca^{2+}]$.[50-53]

In the present investigation, the highest level of *vastus lateralis* muscle hypertrophy was 19%, but SLN protein expression was reduced by 16% in the same subject. The apparent discrepancy between our findings and those obtained in mice may be due to the extreme nature of the intervention used in animal studies (gene manipulation and compensatory hypertrophy after surgery), as well as differences between species. In fact, nucleotide identity between human and mice SLN cDNAs is no more than 44%; thus, some functional between-species differences are expectable.[54]

The changes in SLN expression were not associated with the improvements in either maximal strength (1RM) nor vertical jump performance. Nonetheless, the improvement in jumping performance was greater in the group that experienced an increase in SLN protein expression. A weak positive association was observed between the changes in SLN and the improvement in the fatigue test. Rodent experiments have shown that SLN overexpression stimulates mitochondrial biogenesis and oxidative metabolism,[28] resulting in improved endurance capacity.[27] Even though SLN expression was increased in VL20, this was not accompanied by an increase in OXPHOS proteins. This indicates that the small changes elicited by strength training in SLN proteins levels in human skeletal muscle do not seem to play a direct or indirect role in the regulation of mitochondrial biogenesis. However, we cannot rule out a transient effect that may have escaped detection given the fact that we only did single-point measurements under basal conditions. Likely, a higher level or more sustained activation of CaMKII is required to induce mitochondrial biogenesis with strength training.

Although overexpression of SLN induces a fast-to-slow shift in muscle fiber type in rodents,[24,28] no such effect was observed in the present investigation. The reduction in MHC-IIX was statistically significant and larger in the VL40 group, which trained with more fatigue and did not experience significant changes in SLN expression. In agreement with Odermatt et al.,[54] SLN expression was enhanced after training in the VL20 group, which maintained a faster muscle fiber phenotype. Since SLN is more abundantly expressed in type II fibers, a muscle fiber type shift from type II to I could, in theory, explain a reduction in SLN in VL40 (if we assume a type II error in VL40 SLN results). However, no significant changes were observed in MHC-I % with training in either group, that is, such a type II to I shift did not occur.[1] Moreover, in VL40 there was significant hypertrophy of type II fibers,[1] which should have been associated with increased SLN rather than the observed reduction. This indicates that the changes in SLN expression observed herein cannot be explained by a training-induced shift in muscle fiber types. We also examined whether SLN changes were associated with the changes in muscle volume (MRI) and CSA (histology) in the VL20 group, to avoid the potential confounding effects of the changes in MHC composition observed in the VL40 group. This analysis also yielded negative results. Thus, SLN may change in response to certain types of exercise, but its physiological role in humans remains uncertain.

In summary, the level of fatigue permitted during the sets is a crucial variable determining the adaptations to strength training, as recently confirmed[55,56] when all repetitions are performed with the maximal intended velocity during the concentric phase. CaMKII δ_D increases in response to strength training and executing repetitions within each set close to failure is associated with elevated resting Thr^{287}-CaMKII δ_D phosphorylation levels, which seem dependent on the total number of repetitions performed during the training program. The increase in Thr^{287}-CaMKII δ_D phosphorylation is positively associated with muscle hypertrophy but also with the reduction in MHC-IIX expression. The latter could limit the enhancement of muscle power with training. Sarcolipin protein expression increases in response to strength training, but this effect is only observed when the level of fatigue allowed in the set is low. An inverse association has been revealed between the changes in basal Thr^{287}-CaMKII δ_D phosphorylation and those of sarcolipin, which is compatible with the existence of a negative loop limiting an excessive expression of SLN after fatiguing muscle contractions. Lastly, changes in basal SLN protein expression do not seem to play a role in muscle hypertrophy in human skeletal muscle, at least under physiological conditions.

5 | PERSPECTIVE

The amount of fatigue allowed during strength training emerges as a critical variable influencing skeletal muscle adaptations and performance improvements through specific signaling events. Future studies should examine whether similar effects are observed in other populations of different sex, age, and fitness. It will also be interesting to obtain additional data during the training program and the detraining phase. Since this is the first time that the effects of two different strength training programs on CaMKII isoforms and SLN protein expression in human skeletal muscle have been tested, our findings will need independent confirmation by other researchers. We induced only modest changes in SLN, which were similar to those observed after sprint training[57]; therefore, it remains unknown whether larger changes in SLN expression could produce adaptations similar to those described in mice overexpressing SLN. In the present investigation, there was a negative association between the changes phospho-Thr^{287}-CaMKII δ_D and those of SLN suggesting a potential negative feedback loop of phospho-Thr^{287}-CaMKII δ_D on SLN expression that requires testing in animal and cellular models.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

JJGB conceived and designed the training study with assistance by FPB, DRR, JSM, CD, and JAC. JJGB, FPB, and JAC collected data. JJGB, FPB, DRR, MGR, MMC, MMR, AGS, DMA, JSM, and CD analyzed and interpreted data. MMC, MGR, MMR, and JAC drafted the manuscript. MMR prepared all figures. All authors read and approved the final version of the manuscript.

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