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Training effects on skeletal muscle calcium handling in human chronic heart failure

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Running title: Skeletal muscle calcium handling in CHF

Abstract

Purpose. Patients with chronic heart failure (CHF) typically complain about skeletal muscle fatigue. In rat experiments reduced intracellular calcium release seems to be related to fatigue development in normal skeletal muscle, but not in muscle from rats with CHF. We therefore hypothesize that training may not improve intracellular calcium cycling to the same extent in muscles from patients with CHF as compared to healthy controls (HC).

Methods. Thirteen HC and 11 CHF patients performed 6 weeks of unilateral knee extensor endurance training. CT examinations of the thigh and biopsies of vastus lateralis were obtained bilaterally before and after the training period.

Results. Peak power of the trained leg was 10 and 14% greater than in the untrained leg in HC and CHF respectively. For the HC training resulted in a higher Ca^{2+} release rate and lower leak in the trained leg associated with a tendency of increased ryanodine receptor (RyR) content with reduced phosphorylation level. In the trained leg of CHF patients RyR content was reduced without associated changes of either Ca^{2+} leak or release rate.

Conclusion. Training in HC has effect on Ca^{2+} leak and release of the sarcoplasmic reticulum (SR), but in CHF patients training is achieved without such changes. Thus, calcium handling seems not to be the site of decreased exercise tolerance in CHF.

Key words: Sarcoplasmic reticulum, Knee extensor, Male, Citrate synthase, Computed tomography, MHC isoform

Introduction

Paragraph Number 1 Limited exercise capacity is a hallmark symptom of chronic heart failure (CHF) patients originally thought to result from reduced skeletal muscle blood flow due to impaired cardiac pumping capacity (36). However, this is unlikely to be the only mechanism involved as improvement of cardiac performance is not paralleled by increase in exercise tolerance (44). Exercise capacity is also poorly correlated with ejection fraction (EF)(10), and reduced muscle performance is evident even when exercising smaller muscle groups in which bulk blood flow is not limited by reduced cardiac output (4).

Paragraph Number 2 Alternative explanations for the skeletal muscle dysfunction have been proposed during the last decades. Altered changes in metabolites, like accumulation of lactate and decreased oxidative capacity may both contribute to the reduced exercise capacity in heart failure. Recently Okada *et al.* suggested that alterations in myosin content could explain skeletal muscle strength deficit in CHF patients (27). Also the role of Ca^{2+} handling as a factor in the CHF associated skeletal muscle dysfunction has been investigated. It has been reported that CHF rats have increased levels of sarcoplasmic Ca^{2+} ATPase (SERCA) and ryanodine receptor (RyR) in the soleus muscle (19), although a reduction in SERCA is also reported (30). The results regarding the Ca^{2+} transient during fatigue is also inconclusive as both maintained (20) and reduced transients have been reported (29) in skeletal muscle from CHF rats. Even though experimental studies seem to be inconclusive, they point to significant alterations of intracellular Ca^{2+} handling in skeletal muscle in CHF. Some researchers even argue that reduced Ca^{2+} release is the major determinant of skeletal muscle fatigue (43). If altered Ca^{2+} handling also is part of the multifactorial skeletal muscle dysfunction in CHF has not been tested in humans.

Paragraph Number 3 Prior to the early 1980's, heart failure patients were advised to live sedentary lives. Today it seems clear that exercise reduces both hospitalization and mortality and increases quality of life for these patients (26). Even high intensity exercise appears to be well tolerated (45), and physical inactivity can accelerate the disease progression (17).

Training by running or cycling taxing cardiac output consistently leads to higher VO_{2max} and improves exercise tolerance in CHF patients (11), but also local muscle training has beneficial effects, like increasing peak work load, endurance and oxidative capacity, contributing to improved skeletal muscle function (12).

Paragraph Number 4 By performing endurance training only with the quadriceps femoris muscle (QF) of one leg (approximately 2 kg), healthy subjects as well as CHF patients can tolerate much higher workloads per kg muscle for rather long exercise times as compared to whole body exercise as bicycling (21). This ensures high demand on the exercising muscles including the contractile apparatus, the energy metabolism as well as the Ca^{2+} handling proteins, despite limited pumping capacity of the failing heart. Thus, effects of training can be evaluated independently of heart function. Such training has been investigated in a limited number of studies, none of which have included a healthy control group for other purposes than baseline characteristics. Training effects of CHF patients can thus not be distinguished from that of the healthy, age matched population. In the present study we include a training healthy control group and investigate two main hypotheses. First, at baseline we expect that skeletal muscle SR Ca^{2+} handling will be different in muscle from CHF patients compared to healthy controls. Secondly, we also expect that SR function may not be improved by training to the same extent in CHF as in HC by exercising the QF muscle of one leg.

Methods

Paragraph Number 5 Eleven sedentary patients with stable chronic heart failure (CHF), NYHA class II-III, caused by ischemia were recruited from Oslo University Hospital, Ullevål. Subjects with other heart failure etiologies were not included in the study. Ejection fraction (EF) was below 35%, measured not longer than 3 months prior to inclusion. Thirteen healthy controls (HC) were included as controls. To further minimize confounding factors we choose to include only male subjects as previous studies have reported differences in skeletal muscle properties in men and women with CHF (9). Refer Supplemental Digital Content (SDC) for additional inclusion and exclusion criteria (Table 1). At inclusion, all subjects underwent a series of tests: Knee extensor peak torque, spirometry, ergometer cycle test, biopsies from vastus lateralis bilaterally and CT examination of both thighs. After the training period, the CT examination and the biopsy procedure were repeated. Also peak torque was retested along with evaluation of peak power. All CHF patients used β -blockers and either angiotensin conversion enzyme inhibitors (ACEi) or angiotensin II (ATII) receptor blockers (Table 2). Medical treatment in the CHF patients was continued throughout the study period, as cessation would have been considered ethically unacceptable. Written informed consent was obtained from all the subjects included. The investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the regional ethics committee.

Spirometry, $VO_{2\text{peak}}$ test and work-ECG

Paragraph Number 6 With patients on an ergometer bicycle (Schiller ERG911, Baar, Switzerland), VO_2 was measured every 20 s (Vmax 229, SensorMedics, Yorba Linda, CA, USA) during stepwise increments of intensity (10 W/min) until exhaustion to determine $VO_{2\text{peak}}$. The CHF patients started at 50 W while the HC started at 100 W. For the test to be

valid a Borg score ≥ 18 and respiratory exchange rate (RER) > 1.0 at exhaustion was required. Electrocardiograms were obtained from all the test persons during cycling.

CT examination

Paragraph Number 7 CT scans were performed on a HiSpeed or a LightSpeed scanner (General Electric, Paris, France). The examination was a helical scan from spina iliaca anterior inferior through patella on both thighs. 5 mm slices were reconstructed every 5 mm. Quadriceps volume was calculated using an Advantage Workstation (General Electric, Paris, France) and cross sectional area (CSA) was measured in the middle of the thigh by personnel blinded for patient group and training status. The inter-observer correlation between the two technicians measuring CSA and outlining the muscle for volume analysis was very good ($r^2 > 0.99$).

Skeletal muscle biopsy

Paragraph Number 8 Percutaneous needle biopsy of the vastus lateralis was performed under sterile conditions with local anesthesia (Xylocain® adrenaline, 10 mg/ml+5 μ g/ml, AstraZeneca, Oslo, Norway), using a 6 mm Pelomi needle (Albertslund, Denmark) with manual suction. Samples for preparation of SR vesicles for measurements of Ca^{2+} uptake and release were homogenized (Polytron 1200 homogeniser, Kimemtica AG, Luzern, Switzerland) on ice in a Tris/ sucrose buffer (pH 7.9) supplied with a phosphatase inhibitor (P2850, Sigma-Aldrich, Oslo, Norway). The rest of the biopsies were frozen in isopentane on dry ice and stored at -80°C for other analyses.

Ca^{2+} uptake, release and leak

Paragraph Number 9 SR function was analysed in a LS50B fluorometer (Perkin Elmer, Oslo, Norway) at 37 °C by the use of indo-1. $K_{d \text{ indo-1}}$ at 37°C is 0.13 μM (38). Calcium pumping in the SR vesicles was initiated by addition of 1.1mM MgATP and blocked by 1.5 μM thapsigargin (SDC, Figure 1). SR leak was evaluated during the next 60 s and reported as the linear rate of rise in arbitrary units ($\text{au}\cdot\text{s}^{-1}$). Calcium release was initiated by addition of 11mM 4-Chloro-m-Cresol. The fluorescence ratio was converted to $[\text{Ca}^{2+}]$ by the following equation: $[\text{Ca}^{2+}] = (\text{R} - \text{R}_{\text{min}}) / (\text{R}_{\text{max}} - \text{R}) * K_{d \text{ indo-1}} * (\text{S}_{f2} / \text{S}_{b2})$, where $\text{S}_{f2} / \text{S}_{b2} = 2.7$. R_{min} and R_{max} were determined by reading the indo-1 ratio after applying 3.3 mM EGTA and 4.8 mM CaCl_2 , respectively.

Citrate Synthase (CS)

Paragraph Number 10 Enzyme activity was determined in homogenized vastus lateralis biopsies according to an established spectrophotometric assay (34). DTNB (5,5'-ditiobis-(2-nitrobenzoic acid)) was added and the free mercaptide ion that was measured at 412 nm.

Protein immunoblot

Paragraph Number 11 Western blot was performed on total homogenate. SDS-PAGE (4-15% for ryanodine receptor, 7.5% for Serca1 and 15% for phospholamban) was performed before blotting and probing with primary antibodies (Serca1: MA3-912, Serca2: MA3-919 all Affinity Bioreagents, Golden, CO, USA. Total PLB: A010-14, Ser16 P-PLB: A010-12, Thr17 P-PLB: A010-13, all Badrilla, Leeds, UK). Immunoreactivity was detected by enhanced chemiluminescence method (Amersham, Piscataway, NJ, USA) with a Fujifilm camera (LAS-1000 or 4000, Fujifilm, Stockholm, Sweden). Myofibrillar proteins were separated by glycerol/SDS polyacrylamide gel electrophoresis. Identical amounts of protein were applied

in each well. Gels were stained sequentially by Pro Q Diamond and SYPRO Ruby (both M33305, InVitrogen, Oslo, Norway) for phosphorylated and total proteins, respectively. The fluorescence was detected by Typhoon laser scanner (9410, GE Healthcare, Oslo, Norway), and quantified by Imagequant (GE Healthcare, Oslo, Norway). Protein amounts after the training period were normalized to the protein content in biopsies from that individual muscle before training.

Training protocol, peak torque and peak power

Paragraph Number 12 One legged dynamic knee extensor training was performed on a knee extension ergometer, modified by Hallén *et al.* (14). This setup restricts exercise to the quadriceps muscle unilaterally. The other leg was hanging loosely from the chair and did not move during exercise. This leg served as control. The limited muscle mass engaged will not challenge cardiac output (CO), ensuring sufficient oxygen supply to the working muscle despite the reduced CO of CHF patients (21). The patients were randomly assigned to exercise the dominant or the non-dominant leg. Two low intensity (LI), one moderate (MI) and one high intensity (HI) exercise session were performed weekly for 6 weeks with the same relative workload for the two groups. HI was defined as the workload that exhausted the test subject after 20 min of exercise. LI (~70% of HI) consisted of 60 min exercise while MI (~80% of HI) consisted of 20 min warm up at LI followed by 40 min exercise at MI. HI exercise consisted of 20 min exercise at HI with 10 min warm-up and 10 min cool down, both at LI. All exercise was performed at 60 rounds per min. During a separate exercise session at HI, a catheter was placed in the femoral vein on the trained leg under local anesthesia (10 mg/ml Xylocain, AstraZeneca AS, Oslo, Norway). Blood was sampled and analyzed for lactate (YSI 1500 Sport, USA).

Paragraph Number 13 Maximum voluntary isokinetic strength (peak torque) was tested on an isokinetic dynamometer (REV9000, Technogym®, Italy). The range of motion was set to a knee angle from 20° to 90°, and the angle speed to 60°·s⁻¹. Warm up was done on an ordinary cycle ergometer (50 W, 10 min).

Paragraph Number 14 Peak power was tested on the knee extension ergometer using a stepwise incremental (2 W min⁻¹) protocol. The tests were performed on one leg at the time on two consecutive days after the training protocol. Start load was individually adjusted to cause exhaustion between 4 to 10 min after the start of testing. A physician was always present during testing of CHF patients.

Statistics

Paragraph Number 15 Data are presented as average ± SEM. SEM is also indicated on the figures. Any p value less than 0.05 was considered statistically significant. Protein measurements and Ca²⁺ handling properties after the training period were related (either as ratios or as delta values) directly to the corresponding pre-training value. The post/pre ratios were log-transformed to approximate the data to normal distribution. Differences between groups were tested by Student's t test. All statistics was performed on either Statistica (StatSoft, Inc. (2007). Statistica, Tulsa, OK, USA) or Microsoft Excel 2007.

Results

Paragraph Number 16 CHF patients had 39% lower VO_{2peak} compared to HC and a low EF confirmed the heart failure diagnosis (Table 1). There were no incidences of angina suspect chest pain or ischemia suspect ECG alterations during the test period. At a one-leg HI

work load the femoral venous lactate level was not different between the groups (6.8 ± 0.4 mmol/l for CHF vs. 6.2 ± 0.5 mmol/l for HC). Furthermore, the duration of each training session was the same for the two groups.

Baseline characteristics

Paragraph Number 17 Baseline characteristics are summarized in Figure 1. There were no differences between left and right leg for any of the parameters. Thus, averaged values are presented. At inclusion CHF patients were 17% weaker than HC as measured by peak torque (Figure 1A), but if normalized to either quadriceps volume or CSA (Figure 1B), the statistical differences between the groups disappeared. Peak power was 29% lower in untrained leg of CHF patients compared to HC ($p < 0.001$). This difference persisted even when correcting for CSA and is indicative a reduced skeletal muscle function in CHF. CS activity was not significantly different (Figure 1C). Also, there was no significant difference in MHC distribution. Each of the three fiber types constituted about 1/3 of the total number of fibers in both groups (Figure 1D). The level of SERCA2 was 51% higher in CHF compared to HC, and the PLB monomer/pentamer ratio was 41% lower in the CHF group compared to HC (Table 3). There were no significant differences between CHF and HC as regards SR Ca^{2+} uptake and release rate (Figure 1E and F). However, SR Ca^{2+} leak was 24% lower in CHF patients compared to HC (Figure 1G).

Training effects

Paragraph Number 18 Six weeks of endurance training did not increase peak torque in trained leg (Figure 2A) in spite of an increased quadriceps CSA (by 9% and 7% for HC and CHF) and volume (by 6% and 9% for HC and CHF, Figure 2B). For the HC group, training

also increased both volume and CSA in the untrained leg significantly, by 7 and 8% respectively. However, after the training period peak power was higher in the trained leg compared to the untrained leg by 10% and 14% for HC and CHF respectively (Figure 2C). Compared to the baseline levels CS activity in the trained leg increased by $34\pm 9\%$ in HC and $27\pm 9\%$ in CHF patients (Figure 2D). There was no increase in CS activity in the untrained leg of either group. MHC distribution was not significantly changed in either group (SDC Table 2).

Paragraph Number 19 For the HC training increased the Ca^{2+} release rate (from 0.029 ± 0.003 to $0.043\pm 0.002 \mu\text{M}\cdot\text{s}^{-1}$) and reduced leak (from 15.5 ± 1.3 to $10.9\pm 2.4 \text{ au}\cdot\text{s}^{-1}$) of Ca^{2+} from SR in the trained leg (Figure 3B and C). Also in the untrained leg the release rate was higher after the training period (increased from 0.029 ± 0.003 to $0.049\pm 0.003 \mu\text{M}\cdot\text{s}^{-1}$; Figure 3B). Related to this the RyR amount tended to be elevated ($p=0.053$) and RyR phosphorylation level was nominally reduced in the trained leg (Figure 4A) of the HC group. For the CHF patients there was no significant effect of training on SR Ca^{2+} release rate or leak and RyR amount decreased by 18% in the trained leg. For this group, however, the Ca^{2+} uptake rate in untrained leg was significantly higher after the training period (from 0.008 ± 0.001 to $0.014\pm 0.003 \mu\text{M}\cdot\text{s}^{-1}$; Figure 3A). There was no change in SERCA amount or isoform in any of the groups, nor were there alterations in PLB that could explain the effects of training. However, the training induced alteration in PLB was different for the two groups (Figure 4A and B). Ser16 phosphorylation was upregulated in trained leg in HC (Figure 4A), but lower in the untrained leg of the CHF group (Figure 4B). Both for the total RyR amount and the phosphorylated form, the two groups showed different effects of training. Total RyR was

reduced but phosphorylation was increased in CHF compared to HC. See SDC for representative Western blots (Figure 2-4).

Discussion

Paragraph Number 20 The main finding of this study is that only by a lower Ca^{2+} leak from SR, is Ca^{2+} handling in skeletal muscle from CHF patients different from that of HC. Peak power was lower in patients compared to HC, even when correcting for CSA. Training of QF resulted in increased CS activity and improved peak power in trained leg in both groups without alterations in MHC distribution. Ca^{2+} release rate was higher and Ca^{2+} leak was reduced in trained leg for HC. As hypothesized, in the CHF group training had no significant effects on calcium handling.

Baseline characteristics

Paragraph Number 21 There were no significant differences in peak torque between HC and CHF patients when correcting for CSA. This is consistent with several previous studies (24), although some also report that reduced skeletal muscle strength cannot solely be accounted for by a reduction in muscle mass (39). The lack of agreement in the literature could arise from differences in severity and treatment of heart failure, or be due to different techniques utilized to measure muscle mass or strength. Concurrent with previous reports, we found a functional deficit in skeletal muscle of heart failure patients, with a lower peak power in untrained leg of CHF patients compared to HC.

Paragraph Number 22 Several investigators have reported that skeletal muscle of CHF patients is characterized by an isoform switch towards a less fatigue resistant fiber type (35).

This switch could be unrelated to deconditioning (8). However, this finding is not confirmed in the present study. One explanation for this could be that the patients enrolled might have milder degrees of heart failure, and consequently only minor alteration of the muscle phenotype. However, the patients in the present study have about the same EF and NYHA class as patients in other studies that evaluate skeletal muscle alterations in CHF (40). An alternative explanation for the lack of isoform switch could be medication of the heart failure patient. Over the last decades heart failure is treated more intensely and with drugs such as ACEi. Several investigators have reported that this drug seems to have effects also on the skeletal muscle in heart failure. ACEi could induce a switch from type II to type I fibers (40) and protect mitochondrial function (46). Another drug group, the ATII receptor blockers, have similar effects (6). However, there are also contradictory reports that fail to demonstrate the ACEi effects in skeletal muscle of CHF patients (32). All CHF patients in the present study used either ACEi or ATII receptor blockers. Thus, we cannot exclude that usage of these drugs could explain why CHF patients had the same MHC isoform distribution as HC.

Paragraph Number 23 Other drugs used by the CHF patients in the present study (Table 2) probably do not affect MHC isoform. High doses of furosemide has been reported to decrease the level of potassium in rat skeletal muscle (3) and statins could have negative effects on skeletal muscle mitochondrial ATP-producing capacity (18). The usage of statins is, however, associated with skeletal muscle complaints, and although the underlying mechanisms remain unclear it is hypothesized that increased Ca^{2+} leak could contribute to the muscle ache (33). In the present study there is no relation between the usage of statins and increased rate of Ca^{2+} leak.

Training effects in healthy controls

Paragraph Number 24 Effects of training were readily identified as increased peak power and CS activity in the trained leg. Hypertrophy was evident in both trained and untrained leg after training, but no change in muscle strength or MHC distribution were demonstrated.

Paragraph Number 25 After the training period the release rate of Ca^{2+} increased bilaterally, and in trained leg Ca^{2+} leak was reduced (Figure 3). This contrasts with “leaky” and dyssynchronous RyRs that are identified after an acute bout of exercise (2). Increased release rate and reduced leak corresponded to the nominal rise in RyR amount and reduced RyR phosphorylation. It has been shown that dephosphorylation of RyR leads to binding of calstabin 1 (23), reducing leak and an increasing release rate of Ca^{2+} from SR. The reduced leak and increased release rate could be related to higher levels of Ca^{2+} in the SR after training compared to before training. This could, in turn, result in an improvement of muscle function.

Paragraph Number 26 A similar training regimen as in the present study using young healthy subjects resulted in a switch toward a slow fiber type isoform, with depressed SERCA1 and reduced SR uptake rate of Ca^{2+} (13). Our data do not support these findings which could be due to an age effect. Judging from the relatively large increase in ser16 PLB in the trained leg, the unaltered Ca^{2+} uptake kinetics was unexpected because phosphorylation of PLB on either ser16 or thr17 is thought to relieve the PLB induced inhibition of SERCA (41). In the present study also relative SERCA abundance was unaltered. However, the fact that skeletal muscle training responses seem to be age dependent is not surprising. Even though there is good evidence that training increases muscle mass and strength also in the elderly (25), muscle plasticity may decrease with age (7), and training induced activation of the mTOR pathway is attenuated (28) with a lower differentiation of satellite cells (5). The satellite cell pool is also reduced with ageing (16). Further, six weeks of endurance training is

possibly not enough time to allow for the MHC isoform switch to occur. However, endurance training programs lasting as long as 6 months have also failed to show any significant changes in MHC isoform in the elderly (15).

Paragraph Number 27 In summary, in HC, local exercise gives rise to alterations in calcium handling mostly affecting RyR and Ca^{2+} leak and release.

Training effects in CHF patients

Paragraph Number 28 One-leg exercise could be maintained at HI for 20 min with about the same lactate concentration in the femoral venous blood as in HC. Thus, anaerobic metabolism in the exercising muscle was not more prominent in CHF, indicating that the CHF condition did not compromise muscle perfusion when only one quadriceps femoris muscle was active. Training effects were apparent as increased peak power, elevated CS activity and increased volume and CSA in trained leg (Figure 2B-D). There were no alterations in MHC fiber type distribution.

Paragraph Number 29 The heart failure condition is characterized by a hyperadrenergic state, and in heart failure animal models RyR1 is hyperphosphorylated, depleted of calstabin 1 and leaky (31,37). This is associated with increased frequency and decreased amplitude of Ca^{2+} sparks (42). However, little is known about the phosphorylation status of RyR in skeletal muscle from CHF patients. Because all CHF patients in the present study use β -adrenoceptor blockers as part of their standard medication (Table 2), it is possible that RyR phosphorylation status is normal or even reduced in these patients. Further, the β -adrenergic receptors are probably downregulated or less sensitive in heart failure patients (22). Accordingly, the altered adrenergic drive due to training could be blunted. We failed to find any regulation in

the degree of RyR phosphorylation in the CHF group. Instead we found a down regulation of the channel itself, but with no changes of Ca^{2+} release or leak. Since CHF patients had a lower SR Ca^{2+} leak at baseline, and that exercise did not alter the leak in this group while it did in the HC group, it is tempting to speculate that whatever positive effect training had on leak, this effect seemed to be already attained in the CHF group, maybe due to medication. It seems at least that the observed training effect can be attained without any effect on intracellular Ca^{2+} cycling. Further studies including training of patients with atherosclerosis that use fewer drugs than heart failure patients are needed to elucidate this issue.

Systemic effects of local muscle training?

Paragraph Number 30 It is unexpected that local muscle training decreased SR Ca^{2+} leak and increased both quadriceps CSA and volume in the untrained leg for HC. It is also surprising that these effects are absent in the CHF group. The most obvious explanation for the alterations in untrained leg in HC is that participation in the study led to a higher awareness regarding the beneficial effects of training resulting in an increase in physical activity. However, the participants were specifically instructed to maintain physically inactive throughout the study, and when specifically asked none in either group reported to have increased their daily activity level. Also, an increase in physical activity should affect the HC and CHF group equally. Another possible explanation for the changes in untrained leg is that local training could trigger the production of humoral factors in the skeletal muscle that in turn might have beneficial effects also in the resting control muscle. Local training influences the level of cytokines in the muscle and in plasma (1), but whether these cytokines have effects also in resting muscle tissue throughout the body is a captivating idea that should be pursued further. Lastly, the effects in resting leg would be relatively smaller if the training

effects were more pronounced. Therefore, it could be argued that the training intensity in the present study is set to low, and that the intensity should be higher for future studies.

Paragraph Number 31 The present study is the first to investigate skeletal muscle Ca^{2+} handling in muscle from human heart failure patients. In addition, it is the first study on local skeletal muscle function and trainability that includes a healthy control group that undergoes the same training protocol as the heart failure patients. In conclusion, CHF patients had similar effects of local muscle training as HC, but for CHF patients this effect was unrelated to altered calcium handling. For HC training effects were associated with changes in RyR function and some of the changes could be systemic in nature. Local skeletal muscle function in CHF patients compared to HC was unexpectedly normal as compared to what has previously been reported in experimental studies. We speculate that medication, such as β -blockers and maybe also ACEi/ ATII receptor blockers, to some extent reverse the dysfunction and explain why findings from experimental studies are not reproduced here.

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Paragraph Number 34 The results of the present study do not constitute endorsement by ACSM.

Conflict of Interest

Paragraph Number 35 None declared

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Figure Captions

Figure 1. Baseline characteristics

Muscle characteristics before the training period. Black bars are HC, dark grey are CHF. Peak torque (Panel A), quadriceps volume and CSA (Panel B; vertical lines are volume, horizontal are CSA), CS activity (Panel C), MHC distribution (Panel D; lines pointing up are MHCI, pointing down are MHCIIa and horizontal lines are MHCIIx) and Ca^{2+} uptake rate, release rate and leak (E, F and G) are shown. Data are average of left and right leg \pm SEM (except panel D were SEM is written). * $p < 0.05$ vs HC.

Figure 2. Effects of training

Muscle characteristics after the training period. White bars are untrained leg, grey bars are trained leg. Data (except for Panel C) are presented as change relative to baseline characteristics (Figure 2). Peak torque (Panel A), quadriceps volume and CSA (Panel B; empty bars are volume, hatched are CSA), peak power (Panel C), and CS activity (Panel D) are shown. * $p < 0.05$ vs baseline value, # $p < 0.05$ vs changes in untrained leg, □ $p < 0.05$ vs untrained leg. ** $p < 0.05$ vs HC.

Figure 3. Calcium handling after training

SR Ca^{2+} uptake rate (Panel A) release rate (Panel B) and leak (Panel C) presented as percent change relative to baseline level. White bars are untrained leg, grey bars are trained leg. A positive value in Panel A and B means increased rate, while a positive value in Panel C means a higher leak. * $p < 0.05$ vs baseline value.

Figure 4. Calcium handling proteins and MLC after training

Regulation of proteins after the training for HC (Panel A) and CHF (Panel B) presented as percent change relative to baseline level. White bars are untrained leg, grey bars are trained leg. Positive values are increased concentration. * $p < 0.05$ vs baseline value. # $p < 0.05$ vs trained leg (HC).

Supplemental Digital Content (SDC)

Tables

Table 1. Inclusion and exclusion criteria for CHF patients and healthy controls

Table 2. MHC fiber type distribution after training period relative to distribution before training

Figure legends

Figure 1

Indo-1 emission ratio in black and gray from a typical experiment. Ratio was sampled at 25 Hz and smoothed (TableCurve 2D, version 5.01, Systat Software Inc, San Jose, CA, USA) using Savitzky-Golay (red) and spline estimation (green) before converted to $[Ca^{2+}]$.

Figure 2

4%-15% gradient SDS-PAGE gel. (A) Total protein, stained with SYPRO Ruby, (B) Same gel as in panel A stained with Pro Q Diamond for phosphorylated proteins.

Figure 3

7.5% SDS-PAGE gels blotted and probed with primary antibodies against SERCA1 and 2.

Figure 4

15% SDS-PAGE gel blotted and probed with (A) primary antibodies against PLB, (B) pSer16-PLB and (C) pThr17-PLB.

Table 1. Clinical characteristics of the subjects participating in the study

	HC	CHF
n	13	11
Age (years)	67.7±1.8	68.4±1.5
BMI (kg·m ⁻²)	27.7±0.7	27.7±1.2
VO _{2max} (ml·min ⁻¹ ·kg ⁻¹)	29.3±1.5*	17.8±1.7
EF (%)	67.5±2.1*	29.1±2.1
NYHA class	-	2.4±0.2

All values are average ± SEM.* p<0.05 vs CHF

Table 2. Medication of the subjects participating in the study.

	HC (n=13)	CHF (n=11)
β -blockers	1	11
Statins	3	10
ACEi	-	7
ATII blocker	2	4
Furosemide	-	7
Warfarin	1	6
ASA	5	7

Table 3. Protein concentration relative to HC

	HC (n=12)	CHF (n=10)
Actin	100±4	109±11
SERCA1	100±5	100±10
SERCA2	100±8	151±21*
Total PLB		
Mono	100±7	103±5
Penta	100±10	166±17*
RATIO	100±13	59±6*
PLBser16		
Mono	100±9	100±12
Penta	100±13	142±34
RATIO	100±15	100±32
PLBthr17		
Mono	100±10	87±13
Penta	100±17	111±37
RATIO	100±14	126±29
RyR	100±10	123±20
RyR-p	100±8	134±25
pRyR/RyR	100±8	102±11

Numbers are average ± SEM. * p < 0.05 vs HC

Figure 1

Figure 1

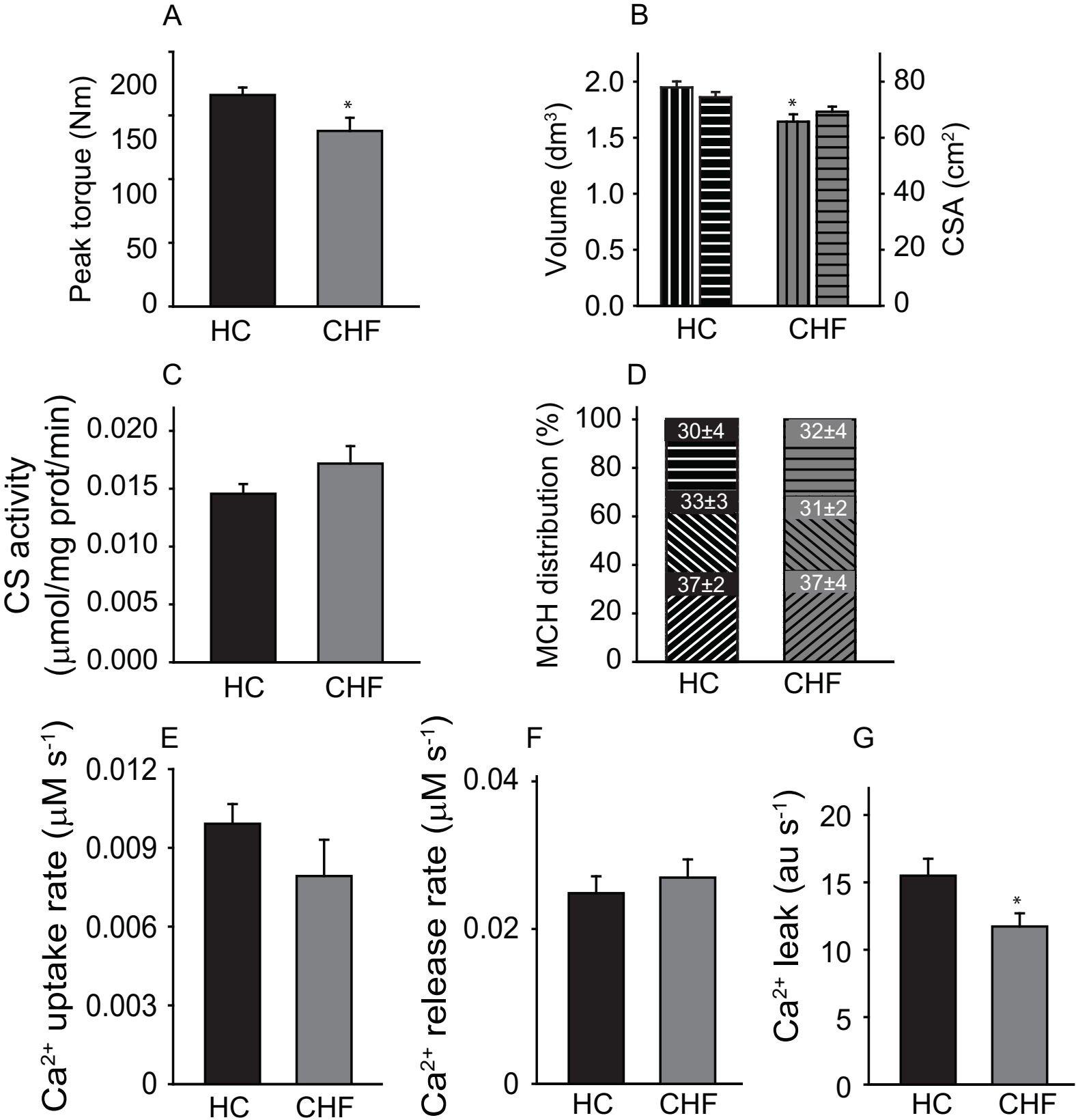


Figure 2

Figure 2

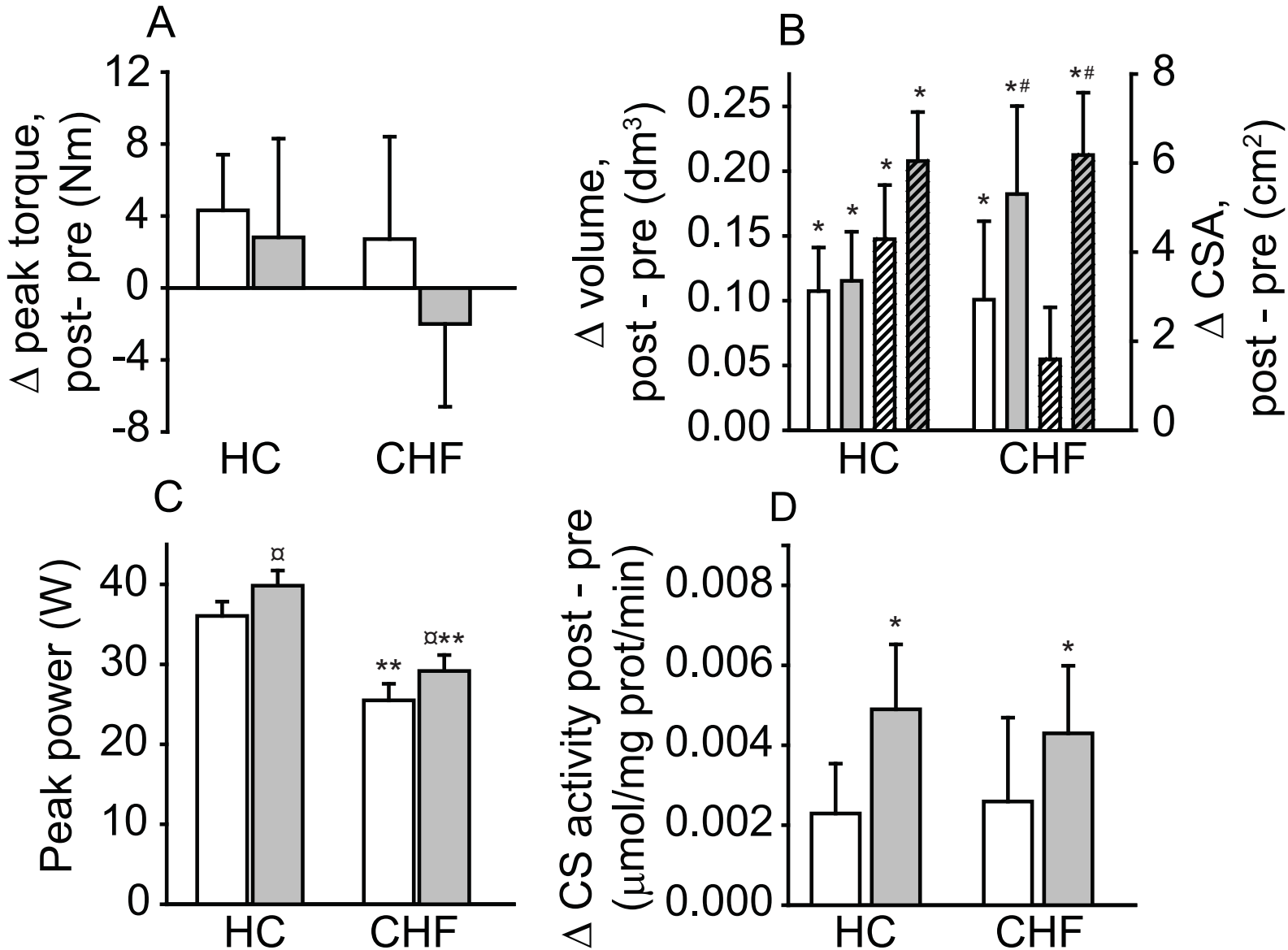


Figure 3

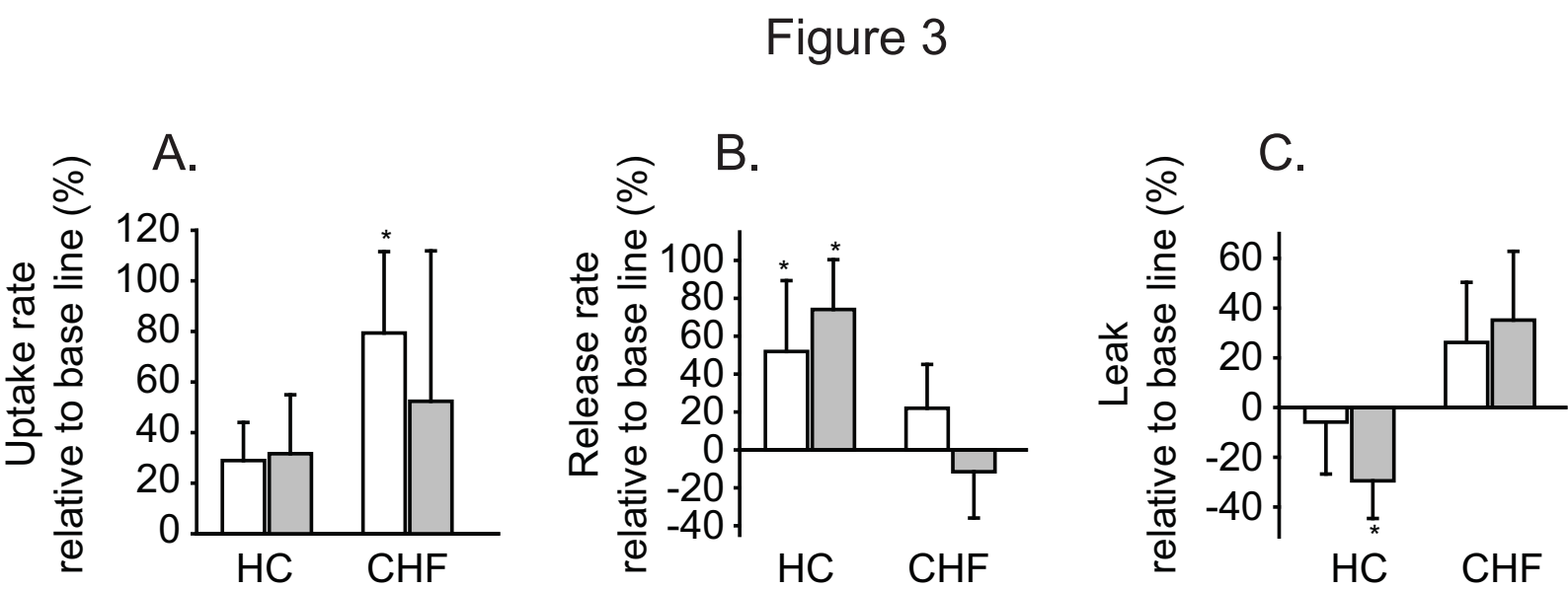


Figure 4

Figure 4

