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Original Article

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Electrically induced muscle cramps induce hypertrophy of calf muscles in healthy adults

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Abstract

Objectives: Skeletal muscles usually cramp at short lengths, where the tension that can be exerted by muscle fibers is low. Since high tension is an important anabolic stimulus, it is questionable if cramps can induce hypertrophy and strength gains. In the present study we investigated if electrically induced cramps (EIMCs) can elicit these adaptations. **Methods:** 15 healthy male adults were randomly assigned to an intervention (IG; n=10) and a control group (CG; n=5). The cramp protocol (CP) applied twice a week to one leg of the IG, consisted of 3x6 EIMCs, of 5 s each. Calf muscles of the opposite leg were stimulated equally, but were hindered from cramping by fixating the ankle at 0° plantar flexion (nCP). **Results:** After six weeks, the cross sectional area of the triceps surae was similarly increased in both the CP (+9.0±3.4%) and the nCP (+6.8±3.7%). By contrast, force of maximal voluntary contractions, measured at 0° and 30° plantar flexion, increased significantly only in nCP (0°: +8.5±8.8%; 30°: 11.7±13.7%). **Conclusion:** The present data indicate that muscle cramps can induce hypertrophy in calf muscles, though lacking high tension as an important anabolic stimulus.

Keywords: Muscle Cramps, Neurogenic Calf Enlargement, Hypertrophy, Maximal Voluntary Contraction, Pseudo-hypertrophy

Introduction

Enlarged calf muscles are occasionally seen in patients suffering from neuromuscular[1-3] or metabolic disorders[4-6]. In most cases the underlying mechanisms for this observation remain unclear. Generally a true hypertrophy of muscle tissue is distinguished from a *pseudo*-hypertrophy, which describes a calf enlargement due to an infiltration of fat or other tissues[7,8]. Though these infiltrations are often seen in biopsies of affected muscles, the *pseudo*-hypertrophy seems to account for only a minority of calf enlargements in neurological disorders. Reimers et al.[8], who found 80 out of 350 neurological patients to be affected by enlarged calf muscles, reported that only 21% of these revealed the aforementioned pseudo-hypertrophy, while in 79% true hypertrophies were detected.

We hypothesize that, apart from disease specific factors there may be a common anabolic stimulus that is present in many neurological diseases and accounts for the calf enlargements of some patients. A potential candidate for this common stimulus may be the forceful contractions of muscle cramps, which frequently occur in patients with neurogenic hypertrophy[9,10]. The concurrent presence of repetitive muscle cramps and enlarged calf muscles have been reported in patients suffering from malignant hyperthermia[2], congenital dermal sinus[3], idiopathic generalized myokymia[11], familial x-linked myalgia[12], Issaacs' syndrome[13], S1-root lesion[14], multifocal motor neuropathy[15], Charcot-Marie-Tooth (type 1A)[16], long-term sequela of myelitis or poliomyelitis[1], Hoffmann's syndrome[17], hypothyroid myopathy[6], and some undefined neuromuscular disorders[18].

However, muscles are known to almost exclusively cramp at short muscle lengths. That is, based on the sarcomere length-tension relationship[19], the mechanical stress placed on the muscle fibers is low. Since muscle tension is an important anabolic stimulus[20], it is unclear whether these contractions can actually contribute to the aforementioned calf enlargements. Therefore, the primary aim of the present study was to discover the anabolic potential of skeletal muscle cramps. For that purpose, we investigated the hypertrophic response of calf muscles to a 6

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Abbreviations

BIA	bioelectric impedance analysis
CTF	cramp threshold frequency
CP	cramp protocol
EIMC	exercise induced muscle cramps
mCSA	muscle cross-sectional area
MP	motor point
MRI	magnetic resonance imaging
MVC	maximal voluntary contraction
nCP	non cramp protocol
NMES	neuromuscular electrical stimulation
CTF	cramp threshold frequency
VAS	visual analog scale

week cramp protocol (CP), consisting of electrically induced muscle cramps (EIMCs). These data were then compared to a stimulation protocol, in which the muscles were hindered from cramping by fixing the ankle joint in a neutral position (nCP). Further, it was tested if hypertrophic responses were accompanied by functional improvements, in terms of strength gains of plantar flexion.

Materials and methods

Subjects

Fifteen male sport students between the ages of 20 and 35 years volunteered to participate in this study. All of them were engaged in regular training for different sports (e.g. soccer and basketball) at least two hours per week over the last 6 months. Subject characteristics are summarized in Table 1. Prior to the study, participants had to complete a medical screening questionnaire to identify any study-relevant pathological conditions. That is, subjects were excluded from the study if they reported any injuries of the lower extremities within six months prior to the intervention period or any cardio-respiratory disease. Further exclusion criteria were as follows: chronic medication, surgeries within six months prior to the intervention period, and any major illness that would affect the ability to perform the protocol. All individuals were informed about the procedures and possible risks of the study protocol, and written informed consent was obtained prior to the onset of the intervention. Study procedures were approved by the ethics committee of the German Sport University Cologne and followed the principles outlined in the Helsinki Declaration.

Procedures

Subjects were randomly assigned to an intervention (IG; n=10) or control group (CG; n=5). Using neuromuscular electrical stimulation (NMES), the calf muscles of both legs were stimulated in the IG in an alternating fashion. A coin was tossed to determine which leg was assigned to the CP or the nCP. Consequently, four subjects underwent the CP in the dominant leg, while in six subjects the CP was applied to the non-dominant leg. The control group did not undergo any NMES or other intervention. Measurements of functional and morphological muscle properties were taken at baseline (pre-), at three weeks (mid-), and at six weeks (post-test). Subjects were advised to record their habitual and training activities throughout the whole intervention period. All subjects were asked to maintain their physical activity routine, including their regular training program. However, participants were not permitted to conduct any additional resistance training of the calf muscles during the 6-week intervention period and were advised to avoid any intense loading of calf muscles three days prior to all testing occasions (i.e. pre, mid, and post).

	IG (n=10)	CG (n=5)
Age (years)	25.9±3.3	23.8±2.4
Body height (cm)	179.6±6.1	180.4±5.2
Body weight (kg)	78.9±6.2	79.2±8.3
Body fat (%)	11.0±3.6	10.5±2.5

Abbreviations: IG= intervention group, CG= control group.

Table 1. Baseline subject characteristics.

Stimulation protocol

The applied stimulation protocol consisted of two stimulation sessions per week. During the calf muscle stimulation, subjects were seated on an elevated platform with hip and knee joints flexed at ~90° and both legs hanging down freely. Following a warm-up, consisting of three sets of five voluntary calf raises, calf muscles (Mm. gastrocnemii) of both legs were stimulated alternately using a portable battery-powered stimulator (Compex 3, Compex, Ecublens, Switzerland). The stimulation protocol comprised three sets of biphasic rectangular-wave pulsed currents at 30 Hz above the individual cramp threshold frequency (CTF; see measurements), an impulse width of 150 μs per leg. The 30 Hz was added to the CTF as it has been previously reported that cramps do not occur soon after a first cramp episode[21], which may be a function of cramp induced CTF increments[22]. However, the present study design was not able to determine the exact number of elicited number of muscle cramps during the protocol and it is conceivable that the number of cramps decreased over the six week period.

Each set consisted of 6 x 5 s contractions, intermitted by a 10 s break - resulting in a duty cycle of 0.33. Each set was followed by a 90 s pause. During this pause, the opposing leg was stimulated using the same stimulation protocol. Electrical stimulation was delivered to the medial (MG) and lateral (LG) head of the m. gastrocnemius by placing one self-adhesive gel electrode (5 x 5 cm) over the motor points (MP) of both muscle heads and an equally sized reference electrode over the proximal part of the muscle belly, just below the popliteal cavity. MPs, defined as the area over the muscle at which minimal current induces visible muscle contractions, were located using

a small size pen electrode (~0,1 cm^2 surface; Compex, Ecublens, Switzerland). To ensure invariant electrode placement, the detected motor points were marked with permanent ink and participants were advised to remark those locations throughout the intervention period. Intensity of electrical stimulation was set to 85% of the maximally tolerated current (range 0-120 mA). The latter was tested at the beginning of each session. In order to keep the total amount of muscle stimulation equal for both legs throughout the intervention, the opposite shank was stimulated with the same NMES parameters applied during the maximal tolerance test, but with a fixed ankle joint in a neutral position (0° plantar flexion - describing a ~90° angle between the foot and shin axis).

Electrical stimulation of calf muscles is accompanied by a plantar flexion of the foot due to a shortening of muscle fibers. According to the literature and pre-studies from our work group (unpublished), cramps are induced almost solely in shortened muscles[23]. Therefore, a custom-built ankle brace that fixates the foot in a neutral position (0° plantarflexion) was used to avoid the development of muscle cramps within the nCP. By contrast, plantar flexion was unhindered in the CP to elicit EIMCs.

Measurements

Baseline measurements of muscle cross-sectional area (mCSA), cramp threshold frequency (CTF), maximal voluntary contraction (MVC), and a bio-impedance analysis (BIA) were performed ~72 h prior to the six week training intervention. MVC and mCSA measurements were repeated ~72 h after the last training session. Further, perceived discomfort of contractions were rated following each set. Since daytime changes in neuromuscular efficiency of triceps surae (in voluntary and induced contractions) have been previously reported [24], measurement time points were held constant between pre- and post-tests.

Muscle cross sectional area (mCSA)

To assess if the applied NMES protocol induced hypertrophic structural adaptations, a magnetic resonance imaging (MRI) scanner (1.5 Tesla, Magnetom Espree, Siemens, Germany) was used. T-1 weighted, spin-echo, axial plane sequences were performed with 860 ms repetition time and 13 ms echo time. Subjects were instructed to lie supine on the provided examination platform and to avoid any movements throughout the test to reduce the risk of movement artifacts. Scout-views in the frontal plane were preceded each scan to localize the femorotibial cleft in which the proximal ending of the tibia was marked as reference line. Aligned to this reference, transverse scans of 7 mm slice thickness were obtained for calf muscles of both legs. Subsequently, the MRI data were analyzed by an experienced investigator using the Jive-X Dicom Viewer Light (Version 4.3.0.2, Visus Technology Transfer GmbH, Bochum, Germany). To describe the slice with the greatest circumference at baseline, measuring snakes (provided in Jive-X software) were aligned to the outer border of the muscles on each image. Thereafter, the mCSA of the m. triceps surae was measured on this slice. To ensure the same measurement position at post-test, the slice with the same distance from the tibia plateau was analyzed. The test-retest reliability of this procedure was high, with an ICC of 0.921.

Maximal voluntary contraction (MVC)

MVC of plantar flexion was measured on a seated leg press (Edition-Line; Gym80 International GmbH, Gelsenkirchen, Germany) that was connected to a force sensor (KM1506, megaTron; Munich, Germany), with a measuring range from 0 to 5000 N. Since previous studies reported a link between the joint angle during stimulation and strength gains[25], MVC of calf muscles was measured at two different ankle positions (0° and 30° plantar flexion). Subjects were seated on the machine with their backs pressed firmly against the back pad. They were advised to keep their knees locked out in a fully extended position throughout the MVC measurement. If this was not possible, the knee angle was fixed at 0° using a knee brace (Cool IROM, short; DonJoy, DJO Global, CA, USA). Subjects were asked to perform three MVCs (four seconds duration) at both ankle positions, with alternating legs. The force values were determined as the highest sliding average over a 7 ms time window. The highest value of the three trials was recorded. ICC values for MVC tests at 0° and 30° plantar flexion were 0.984 and 0.978.

Cramp threshold frequency (CTF)

Subjects were lying comfortably in a prone position with straight legs and the ankles plantar flexed at ~30°, to facilitate cramp generation. Self-adhesive stimulation electrodes (~3 cm2 surface, Axion GmbH, Germany) were placed over the previously detected motor point (cathode) of the medial head of the m. gastrocnemius medialis and the proximal part of the muscle (anode). Electrical stimulation was provided by a portable battery-controlled device (Stim-Pro X9, Axion GmbH, Germany) using rectangular stimuli with an impulse width of 150 μs and a current intensity of 40 mA. Electrical bursts were applied for 5 s, starting with an initial frequency of 4 Hz, followed by a 55s rest period. Using this protocol, stimulation frequency was incrementally increased by 2 Hz until a cramp was induced. Subjects were instructed to relax the calf muscles during stimulation to prevent any unwanted activation. Cramp occurrence was determined by an involuntary, painful contraction of the M. gastrocnemius medialis reported by the subject, observation and palpation of the cramped muscle by a clinical investigator, and the absence of muscle contraction in the M. gastrocnemius lateralis. The minimum electrical stimulation frequency capable of eliciting muscle cramps was defined as the threshold frequency[23,26]. The reliability of this procedure to induce muscle cramps electrically has been reported to be high with ICC values between 0.844 and 0.984[27].

Perceived discomfort

Immediately after each set, participants were asked to record the perceived discomfort during the electrical stimula-

Muscle	Group	Pre [cm²]	Post [cm²]	Change [%]	Hedges g
$mCSA_{TS}$	CP	49.0±6.8	53.4±7.6[a]	+ 9.0±3.4	0.63 (-0.5-1.7)
	nCP	47.8±7.0	51.1±8.4[a]	+ 6.8±3.7	0.45 (-0.6-1.5)
	CG	50.5±4.9	50.7±5.0	+ 0.4±1.1	

Abbreviations: CP, cramp protocol; CG, control group; nCP, non-cramp protocol; [a]p<0.05, significantly different from pre.

Table 2. Cross-sectional area (mCSA) of the triceps surae muscles before and after training. Pre and post-test values are presented as mean ± SD, while effect sizes (Hedges g) are presented as mean (95% CI).

tion and the development of the cramp on a 100 mm unmarked visual analog scale (VAS; 0 mm represented ''no pain'' and 100 mm represented ''intolerable pain'')[28,29]. That is, after each session discomfort was rated three times for each leg.

Bioelectric impedance analysis (BIA)

BIA measurements were taken using a segmental body composition analyzer (Tanita, BC 418MA, Japan). According to the manual, the subjects were asked to step barefooted on the scale so that the heels were placed on the posterior electrodes and the forefeet were in contact with the anterior electrodes. Subjects were then instructed to grasp one handle with each hand. From the recorded impedance, the scale estimates different parameters such as muscle mass, fat mass, and total body water. Reliability of this method has been reported to be good with excellent limits of agreement (<1%)[30].

Statistics

Statistical analyses were performed using Statistica software package (Statistica Version 7.1, Statsoft Inc., Tulsa, OK, USA). Since the Shapiro-Wilk test showed that all variables followed a normal distribution, parametric tests were applied. To investigate if the perceived discomfort, averaged over all sets performed, differed between CP and nCP, a paired t-test was performed. A two-way (group x time) analysis of variance (ANOVA) with repeated measures was used to assess if mCSA and MVC changed differently among the analyzed groups. If ANOVA revealed significant results, a Bonferroni post-hoc analysis was used to examine which of the within- and between-treatment differences were significant. The critical level of significance in the present study was set to $p<0.05$.

In order to compare cramp induced effects on mass gains with training induced adaptations of calf muscles, a literature search was performed by using Medline (1966 - June 2014). The search strategy combined the following terms: training, resistance, stimulation, calf, gastrocnemius, triceps surae, leg, hypertrophy, and growth. Thereafter, standardized mean effect sizes (ESs) were calculated from relevant studies as follows: the difference between the standardized mean change for the treatment and control groups were divided by the pooled pretest standard deviation multiplied by the correction factor suggested by Hedges[31]. The latter counteracts the phenomenon that ESs of small samples tend to be overestimated. Even though formulas have been provided to calculate ESs from trials that lack a control group (i.e. from one-group pre-post designs), we refrained from that possibility as it was suggested by others that the internal validity of those trials is threatened by the non-experimental design and that estimated ESs of those trials are positively biased[32].

Results

Participation compliance for the IG, defined as the number of sessions attended, was 100% at the completion. None of the subjects experienced any injury throughout the intervention period or dropped out due to any other reason. Thus, the 6-week data were able to be collected from all 15 subjects (30 calf muscles) that were initially enrolled. The development of muscle cramps was successfully avoided in the nCP legs by using the custom-built ankle brace. The paired t-test revealed that the perceived discomfort was significantly higher during the CP protocol (48.63±17.38 mm) than during nCP (26.38±14.29 mm) at $p<0.05$.

Muscle cross sectional area (mCSA)

Prior to the intervention, the muscle cross sectional area of analyzed calf muscles did not differ between groups. A significant interaction ($p<0.05$) between group x time could be found for mCSA measurements of the m. triceps surae (Table 2), at an observed power of 0.14. Post-hoc Bonferroni tests revealed that mCSA increased in both legs of the IG (CP and nCP), while mCSA values remained unchanged in CG. An overview of mCSA values and respective ESs can be found in Table 2. All analyzed MRI slices revealed isointense calf muscles without abnormal deposition of fat, when compared to pretest scans.

Maximal voluntary contractions (MVC)

A significant group x time interaction was also found for strength gains measured at 0° and 30° plantar flexion. Post-hoc analysis revealed for both ankle positions that force significantly ($p<0.05$) increased only in nCP legs. However, a similar trend ($p=0.10$) could be observed for the MVCs of the CP legs measured at 30°. Force values in the CG remained virtually unchanged. The observed power of analyses were 0.60

Test	Group	Pre [N]	Post [N]	Change [%]	Hedges g
MVC 0°	CP	1647.3±252.4	1733.1±213.1	6.2±11.6	0.27 (-0.8-1.4)
	nCP	1587.7±294.8	1710.6±268.1[a]	8.5±8.8	0.36 (-0.7-1.4)
	CG	1809.3±400.5	1806.7±407.9	- 0.2±1.1	
MVC 30°	CP	1318.0±283.3	1431.1±253.6	10.7±19.1	0.37 (-0.7-1.5)
	nCP	1271.7±291.0	1397.0±254.9[a]	11.7±13.7	0.41 (-0.7-0.5)
	CG	1361.1±331.1	1356.2±341.5	-0.2±2.2	

Abbreviations: CP, cramp protocol; MVC, maximal voluntary contraction; nCP, non-cramp protocol; CG, control group; [a]p<0.05, significantly different from pre.

Table 3. Force values measured during MVC tests at 0° and 30° plantar flexion before and after training. Pre and post-test values are presented as mean ± SD, while effect sizes (Hedges g) are presented as mean (95% CI).

for MVC measures at 0° and 0.60 for MVC measures at 30° plantar flexion. A summary of force values and respective ESs is given in Table 3.

Discussion

To the knowledge of the authors the present study investigated for the first time, if the painful contractions of muscle cramps are able to elicit hypertrophy in calf muscles and may therefore explain or at least contribute to the calf enlargement seen in some neuromuscular and endocrinological diseases.

Muscle cross-sectional area (mCSA)

The main finding of the present study was that the applied six week intervention de facto induced significant mCSA changes in both the CP and the nCP legs, while mCSA of CG legs remained virtually constant. The performed ANOVA did not find a significant difference between both stimulation programs and the calculated ESs for mCSAT even point towards a slightly higher anabolic effect in the cramping protocol. Thus the present data indicate that muscle cramps itself may act as an anabolic stimulus though the maximal filament overlap at short muscle lengths prevents high tension during these contractions. From these data it is generally conceivable that muscle cramps contribute to the true hypertrophy seen in some neuromuscular disorders. However, due to the healthy subjects in the current study, application of the results to pathological conditions is limited, as those subjects may have other factors influencing contraction-induced hypertrophy.

Some of the previously published reports already attributed true hypertrophy of muscles in patients with neurological disorders to an increased motor unit activity (continuous or intermittent) that induces myocloni, fasciculations, and cramps[1,7,15,33-35]. While the present data support this "increased activity theory" in the case of muscle cramps, we doubt that continuous motor activity in terms of chronic fasciculations would be able to elicit meaningful calf enlargements. This claim is based on the fact that continuous stimulation of muscles has been reported to decrease and not to increase the cross sectional area of muscles[36,37]. Therefore, it is more likely that muscle cramps and not fasciculations contribute to the hypertrophy of calf muscles in some neuromuscular disorders and endocrine myopathies.

Since neurogenic calf enlargements can reach tremendous dimensions in some patients[14], we compared the magnitude of cramp induced muscle growth with the hypertrophic response to different training regimen, reported in the literature. In order to enable this comparison and to rank effects of the applied stimulation protocols (CT and nCT) on structural and functional adaptations among previously presented calf-training regimens, we calculated standardized mean effect sizes (ESs). By using this meta-analytical approach, the estimated overall weighted mean ES of all relevant trials (i.e. that investigated the effect of resistance training or NMES on muscle growth of the lower limb), including the present study, was 0.30 (95% CI:0.01-0.59; p=0.05; random effects model). That is, CP and nCP induced effects on muscle growth were almost twice as high as those of various calf training regimen. This underlines the anabolic potential of muscle cramps despite the mechanically unfavorable muscle fiber length during the contractions. An overview of all included trials can be found in the supplemental material in form of a forest plot (Figure S1) and a table, which lists the respective study characteristics (Table S1).

The mechanisms underlying the observed adaptation remain unclear. In consideration of the short muscle lengths during CP, out of the four putative, contraction induced anabolic signals, (i) high tension, (ii) stretch, (iii) swelling, and (iiii) muscle damage[38], only the latter two come into question. That is, it may be speculated that muscle damage and cell swelling induced muscle growth at short muscle lengths. Although some authors actually reported that eccentric contractions at short muscle lengths result in greater muscle damage when compared to exercises at long lengths[39], the majority of literature supports the opposite[40,41]. In line with this, data from isometric contractions indicate that muscle soreness and force deficit are significantly greater for muscles that were exercised at longer lengths[42]. To conclude, it is unlikely that the CP resulted in greater muscle damage, when compared to the nCP.

Several lines of evidence suggest that cell swelling is an anabolic stimulus that increases the protein synthesis while de-

creasing the proteolysis[20]. The increased pressure against the sarcolemma is thought to initiate the anabolic signaling and the membrane stretch is supposed to affect the transmembrane amino acid transport beneficially. Since the fluid pressure is highest at lengths where the muscle fibers have the greatest curvature[43], it is conceivable that the intense contractions at maximal plantar-flexion triggered similar mechanisms and led to the anabolic response in the CP legs.

Maximal voluntary contraction (MVC)

From mCSA data we would have expected that force increased similarly in both legs of the IG, with CP having a slight edge over the nCP legs, due to the larger ES for mCSA gains in CP. However, no such advantage on force values could be found for CP. Rather, the nCP legs showed significant force increments from baseline for both ankle positions tested, while CP legs only presented a trend for strength gains at 30° plantar flexion but not at 0°. The latter likely reflects the well-known length specificity during isometric training[44], which has been reported to be more pronounced at short muscle lengths[45]. That is, the shorter the muscle at which the isometric training is conducted, the more the achieved strength gains are limited to this specific angle.

The reason for the observed mismatch between functional and structural adaptations in CP legs remains unclear. However, it must be noted that the CP training induced an unusual stimulus compared to many other strength training regimens. That is, due to the maximal overlap of myofilaments, the actual tension exerted by plantar-flexors during the CP was conceivably low. Consequently, some of the signaling responsible for increasing muscle strength was absent[20]. Hypertrophy without concomitant improvements in isometric strength performance, as observed in CP legs, is known from early stages of strength training and has been suggested to be a result of an increased co-contraction of antagonists[46]. Noteworthy in this respect is that patients suffering from writer's cramps have been reported to exhibit an increased co-contraction of antagonist muscles of the forearm during voluntary contractions[47]. Thus the EIMCs of the CP may have induced other spinal and/or supraspinal adaptations than nCP, which resulted in a decreased reciprocal inhibition comparable to that seen in subjects suffering from writer's cramps[48,49].

In general, NMES is known to modulate spinal and supraspinal circuitries that affect the force output of voluntary contractions1. However, CP and nCP protocols did not differ in stimulation settings, thus excluding the stimulation itself as an explanatory variable of the present findings. Nevertheless, the sensory input to the central nervous system substantially differed between both protocols, as the perceived pain was significantly greater during the CP. It has been demonstrated that experimentally-induced pain through saline injections in calve muscles increases the activity of the tibialis anterior during the gait cycle[51]. Thus, the heightened nociceptive input elicited by EIMCs may have triggered adaptations of spinal and/or supraspinal circuitries, which led to an increased activation of antagonists during the MVCs. Since we did not measure electrical activity produced by agonists and antagonists or used electrical stimulation superimposed onto the MVCs, the present study design did not allow for determining the underlying mechanisms.

Apart from increased antagonist activities, it may be speculated that plantar flexors other than the triceps surae accounted for this outcome, by increasing in size only in nCP legs. However, as argued by Fouré et al[52], the contribution of synergists to plantar flexion torque is limited and even significant changes in physiological mCSA of these muscles should be without any major impact. Another possible explanation for the greater mCSA gains in CP legs without benefits for muscle force may be that the well-known exercise associated transmembrane shift of water, with concomitant cell swelling, was more pronounced following the cramp protocol. However, this cell swelling has been reported to recover immediately after cessation of exercise and to even out after 10-20 min[53]. Since MRI scans were performed 72 h after the last intervention, the phenomenon of cell swelling unlikely affected the present results. Finally, a pseudo-hypertrophy in form of a fatty infiltration or an increase of the intramuscular connective tissue would theoretically be able to explain the mismatch between structural and functional adaptations. Since gray scale intensity is inversely related to the T1 relaxation time, fatty or connective tissue replacements would have resulted in brightened images. However, posttest MRI scans of calf muscles were isointense to pretest images, precluding a pseudo-hypertrophy as the explanatory mechanism.

Perceived discomfort

The perceived discomfort was distinctly higher in the CP when compared to the nCP legs, despite the fact that both legs were stimulated with the same frequency, current amplitude, and impulse width. To date, the underlying mechanisms for the pain sensation associated with muscle cramps are still unknown. From light and electron microscopic data it is known that free nerve endings (i.e. free from encapsulation but ensheathed by a single layer of Schwann cells) of type III (A delta) and type IV (C) fibers are located within the perimysium surrounding bundles of muscle fibers and the adventitia of arterioles, venules, and lymphatic vessels[54]. Though these free nerve endings, referred to as nociceptors, are sensitive to a broad range of endogenous substances and strong mechanical forces[55], the high mechanical stimulation threshold impedes activation by low pressure or ordinary muscle movement[54]. In the case of skeletal muscle cramps at short lengths, muscle stretch and high muscular tension are absent as potential stimulators of mechanical nociceptors. As mentioned earlier, it is well known that the fluid pressure is highest at lengths where the muscle fibers have the greatest curvature[43] and it has been speculated that fluid pressure is capable to activate the intramuscular nociceptors by compression[56]. Further, muscle cramps have been proposed to evoke nociceptor activation by mechanically deforming terminal branches of the sensory nerves[57].

Conclusion

For the first time, the present study investigated if muscle cramps represent a potent anabolic stimulus in calve muscles. The perceived discomfort was rated distinctly higher during the cramping protocol (CP), though stimulation intensity did not differ between CP and nCP. The mCSA significantly increased following both protocols. Following the six week intervention, the mCSA significantly increased in both, the CP and nCP legs. Therefore, the present data indicate that muscle cramps are able to induce hypertrophy in calve muscles, despite the maximal shortening of muscles, where high tension is lacking as an important anabolic signal. The muscle growth in CP legs was not accompanied by any strength gains. The reason for this observation remains unclear and needs further investigation.

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Supplemental material

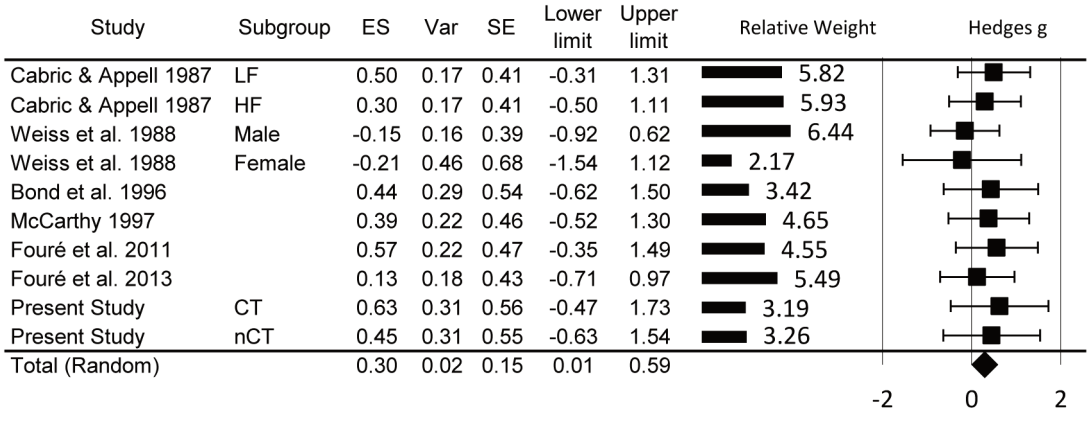

Figure S1. Forest plot of interventions that investigated the effect of resistance training or NMES on muscle growth in calf muscles.

Authors	N	Intervention	D [w]	F [s/w]	Method	Pre	Post
Cabric & Appell[58]	12 IG / 12 CG (LF)	NMES 15-25 contractions (40-45 mA, 50 Hz, 200 µs)	3	7	Girth	EG: 37.9±2.2 cm CG: 38.2±1.6 cm	EG: 39.0±2.2 cm CG: 38.3±1.5 cm
	12 IG / 12 CG (HF)	NMES 15-25 contractions (40-45 mA, 2000 Hz, 200 µs)	3	7	Girth	EG: 38.4±1.0 cm CG: 38.3±1.5 cm	EG: 39.0±1.0 cm CG: 38.5±1.5 cm
Weiss et al. 1988[59]	(male) 12 IG / 14 CG	4 x 9-13 RM seated heel raises	8	3	Net girth (US & girth)	EG: 34.53±2.06 cm CG: 34.28±2.90 cm	EG: 34.12±2.09 cm CG: 34.26±2.25 cm
	(female) 14 IG / 14 CG	4 x 9-13 RM seated heel raises	8	3	Net girth (US & girth)	EG: 29.96±4.28 cm CG: 29.59±1.39 cm	EG: 29.24 ± 4.06 cm CG: 29.77±1.94 cm
Ishida et al. 1990[60 a]	5 EG	15 heel rasises at 70% 1RM	8		Girth	37.5±3.1 cm	38.1±3.0 (4 w)
Martin et al. 1993[25 a]	6 IG / 6 CG	NMES 30 contractions (5 s on, 5 s off, 70Hz, 200 µs IW, 0.25 DC)	4	3	CT	EL: 50.8±5.2 cm^2	EL: 50.8±4.8 cm^2
Bond et al. 1996[61]	7 IL / 7 CL	6 x 15 max. plantar flexions at 15°/ sec	4	4	MRI	EL: 540±42 cm^3 CL: 558±35 cm^3	EL: 552±43 cm^3 CL: 552±36 cm^3
McCarthy 1997[62]	10 IG / 9 CG	3 x 4-12 RM inclined and seated heel raises	12	3	MRI (mVOL)	EG: 799±72 cm^3 CG: 672±31 cm^3	EG: 829±72 cm^3 CG: 679±31 cm^3
Kubo et al. 2007[63 a]	IG 10 (Plyo)	hopping and drop jump using 40% of 1RM	12	4	MRI (mVOL)	EG: 576.5±49.7 cm^3	EG: 604.8±51.8 cm^3
	IG 10 (Wght)	5x10 plantar flexions at 80% 1RM	12	4	MRI (mVOL)	EG: 579.4±48.8 cm^3	EG: 610.8 ± 51.4 cm^3
Fouré et al. 2010[52]	9 IG / 10 CG	Progressive Plyometric jump training	14	2-3	US (mCSA)	EG: 3432±538 mm^2 CG: 3900±612 mm^2	EG: 3659±487 mm^2 CG: 3783±646 mm^2
Fouré et al. 2013[64]	11 IG / 11 CG	200-600 eccentric contractions	14	2-3	US (mCSA)	EG: 3952±750 mm^2 CG: 3623±679 mm^2	EG: 3960±735 mm^2 CG: 3535±698 mm^2
Present study	10 IG / 5 CG (CT)	NMES 3x 6 tetani (5 sec, 30 Hz above CTF, 150 µs IW, 85% of mTSC, 0.33 DC)	6	2	MRI	EG: 49.0±6.8 cm^2 CG: 50.5±4.9 cm^2	EG: 53.4±7.6 cm^2 CG: 50.7±5.0 cm^2
	10 IG / 5 CG (nCT)	NMES 3x 6 tetani (5 sec, 30 Hz above CTF, 150 µs IW, 85% of mTSC, 0.33 DC)	6	2	MRI	EG: 47.8±7.0 cm^2 CG: 50.5±4.9 cm^2	EG: 51.1±8.4 cm^2 CG: 50.7±5.0 cm^2

Abbreviations: CG: control group, CL: control leg; D: duration; ES: effect size; F: frequency; HF: high frequency; IG: intervention group; IL: intervention leg; LF: low frequency; mVOL: muscle volume; MRI: magnetic resonance imaging; mTSC: maximal tolerated stimulation current; N: sample size; Plyo: plyometric training; Wght: weight training.

[a] Study was excluded from analyses due to the fact that it lacked a CG or data from CG were not presented.

Table S1. Overview of training programs that assessed exercise induced muscle growth of plantar flexors. Pre and post-test values are presented as mean ± SD.