Role of Contraction Duration in Inducing Fast-to-Slow Contractile and Metabolic Protein and Functional Changes in Engineered Muscle

ALASTAIR KHODABUKUS, LESLIE M. BAEHR, SUE C. BODINE, AND KEITH BAAR*

Division of Neurobiology, Physiology and Behavior, University of California Davis, Davis, California

The role of factors such as frequency, contraction duration and active time in the adaptation to chronic low-frequency electrical stimulation (CLFS) is widely disputed. In this study we explore the ability of contraction duration (0.6, 6, 60, and 600 sec) to induce a fast-to-slow shift in engineered muscle while using a stimulation frequency of 10 Hz and keeping active time constant at 60%. We found that all contraction durations induced similar slowing of time-to-peak tension. Despite similar increases in total myosin heavy (MHC) levels with stimulation, increases in contraction duration resulted in progressive decreases in total fast myosin. With contraction durations of 60 and 600 sec, MHC IIx levels decreased and MHC Ila levels increased. All contraction durations resulted in fast-to-slow shifts in TnT and TnC but increased both fast and slow Tnl levels. Half-relaxation slowed to a greater extent with contraction durations of 60 and 600 sec despite similar changes in the calcium sequestering proteins calsequestrin and parvalbumin and the calcium uptake protein SERCA. All CLFS groups resulted in greater fatigue resistance than control. Similar increases in GLUT4, mitochondrial enzymes (SDH and ATPsynthase), the fatty acid transporter CPT-1, and the metabolic regulators PGC-1α and MEF2 were found with all contraction durations. However, the mitochondrial enzymes cytochrome C and citrate synthase were increased to greater levels with contraction durations of 60 and 600 sec. These results demonstrate that contraction duration plays a pivotal role in dictating the level of CLFS-induced contractile and metabolic adaptations in tissue-engineered skeletal muscle.

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Correspondence to: Keith Baar, Division of Neurobiology, Physiology and Behavior, University of California Davis, Davis, CA 95616.
E-mail: FMBlab@googlemail.com

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Skeletal muscle can be classified as fast or slow based on myosin ATPase activity (Barany, 1967; Close, 1972). Rodent muscle fibers can be classified into 4 distinct phenotypes via their metabolism and myosin heavy chain isoforms: 1) slow-twitch oxidative type I, 2) fast-twitch oxidative type Ila, 3) fast-twitch glycolytic type IIx/d, and 4) fast-twitch glycolytic type IIb (Pette and Staron, 1990; Schiaffino and Reggiani, 1996). Cellular origin (Stockdale, 1992), developmental state (Condon et al., 1990) and neural input (Hennig and Lomo, 1985) all regulate muscle phenotype. However, neural activity is the main regulator of muscle phenotype. The neural activity is the main regulator of muscle phenotype and neural input (Hennig and Lomo, 1985) all regulate muscle phenotype. However, neural activity is the main regulator of muscle phenotype.

During the development of the rat soleus muscle, neural input to the muscle not only increases over time (i.e., an increase in active time towards 60%) but the duration of single contractions also increases (Eken et al., 2008). Despite CLFS patterns attempting to mimic the natural neural input to slow fiber type muscles, some muscles, such as the rat extensor digitorum longus muscle, require a continuous 20 Hz electrical stimulation pattern for more than 60 days to induce myosin shifts (Windisch et al., 1998).

CLFS patterns are based off electromyography (EMG) recordings that have shown that slow muscles have a natural tonic firing frequency of 10–20 Hz and can fire 300,000 impulses per day (Hennig and Lomo, 1985; Eken et al., 2008). During the development of the rat soleus muscle, neural input to the muscle not only increases over time (i.e., an increase in active time towards 60%) but the duration of single contractions also increases (Eken et al., 2008). Despite CLFS patterns attempting to mimic the natural neural input to slow fiber type muscles, some muscles, such as the rat extensor digitorum longus muscle, require a continuous 20 Hz electrical stimulation pattern for more than 60 days to induce myosin shifts (Windisch et al., 1998).

We have previously found that engineered muscle can adapt to an adult-like 60% active time when using 0.6 sec long contractions and that frequency dictates the level of slowing...
(Khodabukus and Baar, 2014b). However, despite observing functional slow shifts we did not observe changes in MHC isoform. In this paper we sought to determine whether we could engineer a more complete slow phenotype in vitro by increasing the duration of each contraction and rest period while maintaining the 60% active time. We hypothesized that mimicking the neural patterns experienced by developing slow muscles at different stages of development would enable us to find an electrical stimulation protocol that produced fast-to-slow shifts at both the functional and protein level. By varying contraction durations from short, 0.6 sec long embryonic-like, contractions to adult-like, 600 sec long, contractions we found that contraction duration plays a key role in the development of a more complete slow phenotype muscle in vitro.

Materials and Methods

2D Cell culture

The C2C12 myoblast cell line (ATCC) was grown in growth media consisting of high glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 100 Units/ml penicillin until 70% confluent. C2C12 cells were used between passages 6 and 10.

3D Cell culture

Muscles were engineered using fibrin casting as reported previously (Khodabukus and Baar, 2009). Briefly, the muscle constructs were engineered between two 6 mm long silk sutures set 12 mm apart on Sylgard (PDMS)-coated dishes. 500 μl of growth media containing 10 U/ml thrombin, 0.2 μg/ml genipin, and 0.5 μg/ml aprotinin was added to the plate and agitated until it covered the entire surface. Two hundred microliters of 20 mg/ml fibrinogen was added dropwise and the gels were left to polymerize for 1 h before addition of 100,000 cells. Two days after plating cells, the constructs were switched to differentiation media consisting of high-glucose DMEM supplemented with 10% horse serum and penicillin (100 U/ml) for 2 days. Following the second day in differentiation media, the constructs were moved to high-glucose DMEM with 7% FBS and penicillin (100 U/ml) for the remainder of the experiment. We have previously shown that the shift back to 7% FBS maximizes force production compared to 10% horse serum (Khodabukus and Baar, 2009).

Electrical stimulation

Electrical stimulation was performed using a custom made electrical stimulator previously described in detail before (Khodabukus and Baar, 2012). Constructs were differentiated for 7 days and then were initially electrically stimulated for 24 h with an electrical stimulation protocol consisting of a continuous 0.4 sec 10 Hz train followed by a 3.6 sec rest. The constructs were then electrically stimulated for 14 days with the appropriate electrical stimulation protocol (Fig. 1). Once electrical stimulation was started, both the non-stimulated and electrically stimulated constructs were fed every 24 h.

Contractile testing

Functional testing of the C2C12 constructs was performed 14 days after the onset of electrical stimulation as described previously (Dennis et al., 2001). To determine both passive tension and contractile properties of the engineered tissue, one of the anchors was freed from the Sylgard substrate and attached to a custom-made force transducer via one of the minuten pins. After 2 min equilibration, the length of the engineered tissue (L) was set to a baseline length (L0) that generated...

![Fig. 1. Force profile of different contraction durations. Representative force traces of non-stimulated engineered muscles stimulated to contract at 10 Hz for A. 0.6; B. 6; C. 60; and D. 600 sec. Note the increased fatigue with longer contraction duration.](image-url)
zero passive tension. Rheobase (R50) and chronaxie (C50) were then determined as described previously (Dennis et al., 2001). Rheobase was calculated as the electric field strength (V/mm) eliciting 50% peak twitch force (Pt) with a 4 ms pulse width. Chronaxie was calculated as the pulse width required to elicit 50% peak force at twice rheobase. Once excitability had been determined all impulses were delivered with a 4 ms pulse width at 4/C2R50 as described previously (Khodabukus and Baar, 2012). Time-to-peak tension (TPT) and half-relaxation time (1/2RT) were measured in each individual construct a minimum of three times following a single impulse (twitch). Force-frequency (1, 5, 10, 20, and 40 Hz) was then determined with a 1-second train duration and the maximum force recorded designated as peak force. Fatigue was determined by stimulating for 0.75 sec with 0.75 sec rest at 50 Hz for 3 min at four times rheobase with a 4 ms pulse width. A 50 Hz stimulation was used to induce maximal force in both unstimulated and stimulated muscles. If a 10 Hz stimulus were used, the stimulated muscles would be contracting at 93–98% peak force and non-stimulated muscles at 80% peak force. At 50 Hz all fibers are recruited in both groups.

Cross-sectional area was calculated from the measured width of each construct (at its narrowest point), assuming a rectangular cross section and a depth of 500 μm. Specific force was calculated

Fig. 2. Force production, force-frequency and MHC and MRF protein levels following 2 week electrical stimulation. For 2 weeks engineered muscles were continuously electrical stimulated at 10 Hz with contraction durations of 0.6, 6, 60, and 600 sec with rest periods of 0.4, 4, 40, and 400 sec, respectively. A. Specific Force; B. Force-frequency; C. Western blots showing protein levels of terminal markers of differentiation total and neonatal (Neo) MHC and the MRF proteins (Myf-5, MyoD and Myogenin) and β-Tubulin as a loading control. D. The quantification of the protein by densitometry relative to β-Tubulin. * indicates significantly different from CTL (P < 0.05).
as kilonewtons per square meter: the force generated by the construct (kN) divided by its cross-sectional area (m²).

Western blot

Tissues were washed in ice-cold PBS, and then blot-dried before freezing in liquid nitrogen and storing at −80°C. At the time of processing, samples were powdered in a 1.5 ml microcentrifuge tube on dry ice, suspended in 200 μl ice-cold sucrose lysis buffer (50 mM Tris pH 7.5, 250 mM sucrose, 1 mM EGTA, 1 mM EDTA, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 5 mM Na₂(PO₄)₂, and 0.1% DTT) and shaken at 1,400 rpm for 1 h at 4°C in an Eppendorf thermomixer (Eppendorf, Hauppauge, NY). The samples were then centrifuged at 4°C for 1 min at 10,000 g to remove insoluble material. The supernatant was transferred to a new tube, and protein concentration was determined using the DC protein assay (Bio-Rad, Berkeley, CA). Equal aliquots of protein in 1x Laemmli sample buffer were boiled for 5 min before separation on a 10% acrylamide gel by SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to a nitrocellulose membrane (Protran, Whatman, Piscataway, NJ) at 100 V for 1 h. The membrane was blocked for 1 h in 3% milk in Tris-buffered saline + 0.1% Tween (TBST). Membranes were incubated overnight at 4°C with the appropriate primary antibody in TBST at 1:1,000. The membrane was then washed three times in TBST before incubation for 1 h at room temperature with the

Fig. 3. The effect of contraction duration on time-to-peak-tension and myosin isoform shifts. A. Time-to-peak tension. B. Western blots showing protein levels of the MHC isoforms (slow, total fast (F59), Ila, and IIx) and β-Tubulin as a loading control. C. The quantification of the protein by densitometry relative to total MHC* indicates significantly different from CTL (P < 0.05).

Fig. 4. The effect of contraction duration on troponin isoform protein levels. A. Western blots showing the slow and fast isoform of the 3 troponins (TnC, TnI, and TnT) and β-Tubulin as a loading control. B. The quantification of TnC, TnT and C. TnI isoform by densitometry relative to β-Tubulin. Data are mean ± SEM. * indicates significantly different than CTL (P < 0.05).
appropriate peroxidase-coupled secondary antibody in TBST at 1:10,000 (Pierce, Rockford, IL). Antibody binding was detected using an enhanced chemiluminescence horseradish peroxidase substrate detection kit (Millipore, Billerica, MA). Imaging and band quantification were carried out using a Chemi Genius Bioimaging Gel Doc System (Syngene, Cambridge, UK). Each of the target protein band was then normalized to the level of tubulin in the same gel. We have previously shown that tubulin levels are not changed following stimulation (Khodabukus and Baar, 2014b). The primary antibodies used in this study were MF20 (detects all MHC isoforms), F59 (detects all fast MHC isoforms), CaF2-5D2 (Fast SERCA), E7 (ß-Tubulin), MANDYS13B7 (Dystrophin), N3.36 (Neonatal MHC) (Hybridoma Bank, Iowa), total eEF2, SERCA 2a, (Cell Signaling, MA), SDH, ATPsynthase, (Millipore, MA), Parvalbumin (GeneTex, CA), PFK, GLUT4, Cytochrome C, CPT-1, PGC1-a, MEF2, MyoD, Myogenin, Myf5, Troponin C-SS, Troponin C-FS, Troponin I-SS, Troponin I-FS, Troponin T-SS, and Troponin T-FS (Santa Cruz Biotechnology, Santa Cruz, CA).

**Statistical analysis**

Data is presented as means ± S.E.M. The experiments were repeated three times, with an n = 6 for each experiment.
Results

To study the role of contraction duration on the response of engineered muscle to chronic low-frequency electrical stimulation (CLFS) we electrically stimulated constructs with a frequency of 10 Hz and an active time of 60% for 2 weeks. We utilized contraction durations of 0.6, 6, 60, and 600 sec which were followed by rest periods of 0.4, 4, 40, and 400 sec respectively, to maintain an active time of 60%. As would be expected, at the onset of stimulation longer contractions resulted in greater levels of fatigue at the end of each contraction (Fig. 1).

Role of contraction duration on active force production and MHC content following 2wk electrical stimulation

All contraction durations induced a similar increase in force production compared to non-stimulated constructs (CTL = 0.0008 ± 0.00005 kN/m²; 0.6 sec = 0.0031 ± 0.00015 kN/m²; 6 sec = 0.0030 ± 0.00022 kN/m²; 60 sec = 0.0031 ± 0.00017 kN/m²; 60 sec = 0.0028 ± 0.00023 kN/m²) (Fig. 2A). Force-frequency curves for all electrically stimulated muscles were significantly shifted up and to the left compared to control muscles (Fig. 2B). Peak force was achieved at 20 Hz and 40 Hz for electrically stimulated and non-stimulated controls, respectively. Muscles electrically stimulated at 60 and 600 sec produced significantly higher force at 5 and 10 Hz than other electrically stimulated groups (P < 0.05).

CLFS led to a significant increase in both total and neonatal myosin heavy chain (MHC) protein and the myogenic regulatory factor (MRF) proteins Myf-5 and MyoD but not myogenin compared to non-stimulated muscles (Fig. 2C and D).

Role of contraction duration on time-to-peak tension following 2wk electrical stimulation

We next looked at the change in time-to-peak tension (TPT) and half-relaxation time (1/2RT), classical markers used to help determine muscle phenotype. Electrical stimulation significantly slowed TPT (Fig. 3A) regardless of contraction duration (CTL = 55.8 ± 1.4 ms; 0.6 sec = 81.3 ± 2.7 ms; 6 sec = 81.0 ± 4.6 ms; 60 sec = 82.8 ± 4.5 ms; 600 sec = 90.6 ± 5.4 ms). In adult muscle, myosin isoform is the main determinant of TPT so we analyzed the relative levels of slow (sMHC) and fast (fMHC) MHC. Relative to total MHC protein content we found a significant decrease in fMHC (Fig. 3B) with contraction durations of 6 sec and longer (CTL = 1.00 ± 0.04 AU; 0.6 sec = 0.96 ± 0.06 AU; 6 sec = 0.66 ± 0.02 AU; 60 sec = 0.024 ± 0.02 AU; 600 sec = 0.025 ± 0.007 AU). Additionally, relative to total MHC proteins we found a significant increase in Ila MHC and a decrease in IIX MHC protein with contraction lengths of 60 and 600 sec (P < 0.05).

Role of contraction duration on troponin isoform following 2wk electrical stimulation

We next analyzed changes in the slow and fast isoforms of the three troponin proteins, TnC, TnI, and TnT (Fig. 4). Electrical stimulation resulted in an increase in slow and decrease in fast isoforms on TnC and TnT (Fig. 4A and B). Slow TnT increased to significantly greater levels with contraction durations of 6, 60, and 600 sec compared to 0.6 sec (Fig. 4B). Both the slow and fast isoforms of TnI increased following electrical stimulation, with the fast TnI isoform increasing to significantly greater levels with contraction durations of 6, 60, and 600 sec compared to 0.6 sec (Fig. 4A and C).

Role of contraction duration on half-relaxation time following 2wk electrical stimulation

To determine the cause of the shift in 1/2RT we looked at the protein content of the fast specific calcium sequestering protein parvalbumin (Parv) and both the slow and fast isoforms of the calcium sequestering proteins SERCA and calsequestrin (CSQ) (Fig. 5B). Following CLFS the fast CSQ and SERCA isoforms significantly decreased and slow CSQ and SERCA isoforms significantly increased compared to non-stimulated controls. Parvalbumin protein was not detected following electrical stimulation at any contraction duration.

Role of contraction duration on fatigue resistance and metabolic proteins following 2wk electrical stimulation

Following 2wk CLFS, fatigue resistance increased greatly in stimulated muscles compared to non-stimulated constructs (CTL = −58.4 ± 3.6 ms; 0.6 sec = −27.8 ± 1.7 ms; 6 sec = −28.7 ± 1.4 ms; 60 sec = −32.1 ± 1.6 ms; 600 sec = −31.9 ± 1.0 ms) (Fig. 6A) with no difference between different contraction durations.
To determine whether the change in fatigue resistance was due to changes in metabolic protein content we looked at the levels of glycolytic, mitochondrial, and fatty acid transport and oxidation proteins (Fig. 6B and C). Phosphofructokinase (PFK) protein was not detected in electrically stimulated constructs but was found in CTL constructs. GLUT4 protein was found to increase with CLFS in a contraction duration independent manner. Citrate synthase (CS), part of the Krebs Cycle, was significantly increased compared to CTL only with contraction durations of 60 and 600 sec. Cytochrome C (Cyt C), part of complex III and IV of the electron transport chain, was significantly increased with all stimulation groups but increased to significantly greater levels with contraction durations of 60 and 600 sec compared to 0.6 sec. Succinate dehydrogenase (SDH), part of the Krebs Cycle and complex II of the electron transport chain and the complex V enzyme ATP synthase (ATPsyn) were found to be significantly increased following electrical stimulation, independent of contraction duration.

The fatty acid transport protein carnitine palmitoyltransferase-I (CPT-I) and the β-oxidation enzyme β-hydroxyacyl-CoA dehydrogenase (β-HAD) increased following CLFS independent of contraction duration. The expression of the medium (MCAD) and very-long-chain Acyl-CoA dehydrogenase (VLCAD) were unchanged following stimulation but the long chain (LCAD) isoform decreased with electrical stimulation.

We also looked at the levels of the metabolic regulators peroxisome proliferator-activated receptor-γ coactivator (PGC-1α), myocyte enhancing factor 2 (MEF2) and sirtuin 1 (SIRT1) proteins levels (Fig. 7A and B). PGC-1α and MEF2 were increased by electrical stimulation regardless of contraction duration whereas SIRT1 levels were unchanged.

Discussion

In this article, we looked at the effect that contraction duration has on the functional and phenotype shifts induced by CLFS in engineered muscle tissue. Functionally, electrical stimulation increased force production and induced a slow shift in contractile dynamics and greater fatigue resistance compared to control. Increasing contraction duration resulted in a greater slowing of half-relaxation time but not TPT. Myosin shifts required contraction durations of at least 6 sec and were greater with increasing contraction durations.

Increased force generation and fatigue resistance with electrical stimulation demonstrated that the engineered C2C12 constructs can adapt to an adult-like slow phenotype neural pattern. We utilized a 24 h pre-conditioning stimulus of a continuous 10 Hz train of 0.4 sec followed by a 3.6 sec rest that we have used previously (Donnelly et al., 2010; Khodabukus and Baar, 2012), but found that this is not required for the cells to adapt to the adult-like stimulus (data not shown). Myoblasts of different origins have different ranges of plasticity in response to electrical stimulation (Wehrle et al., 2019). C2C12s originate from mouse thigh muscle which is a mixed phenotype muscle (Blau et al., 1985). The functional changes combined with the changes in myofibrillar, calcium-sequestering, and metabolic proteins suggest that they are a good cell model to study transitions to a slow muscle phenotype.

Contraction durations of at least 6 seconds were required to induce myosin shifts and longer contraction durations resulted in greater myosin shifts. Using the F59 antibody that recognizes all isoforms of fast MHC (Miller et al., 1989) we found a progressive decrease in fast MHC with contractions lengths of 6, 60, and 600 sec. This suggests that longer contractions are needed to shut down the production of fast myosin heavy chain proteins. The loss of F59 signal may also reflect changes in embryonic fast myosin, which would be expected to be high in engineered muscle. However, the progressive decrease in Ilx and increase in Ila MHC with increasing contraction duration (Fig. 3B and C), indicates that adult fast MHC isoforms were shifted to a slower phenotype.

Fast-slow myosin shifts do not occur directly but through progressive shifts in fast MHC isoforms from I/Ilb to I/Ilx to I/lla to slow (Gundersen et al., 1988; Hamalainen and Pette, 1997). Smaller mammals such as mice and rats (Windisch et al., 1998) are less adaptive in fast-to-slow shifts than larger animals such as rabbits (Salmons and Sreter, 1976; Ashley et al., 2008), particularly when it comes to shifts in myosin isoform. Therefore the lack of a complete MHC shift is not that surprising seeing that it can take up to 60 days of chronic 20 Hz stimulation (i.e., 24 h a day) to see a shift in rat muscle (Windisch et al., 1998; Peuker et al., 1999). It is possible that the stimulation protocol utilized, cell source and/or the 2 week time period is not sufficient to drive a full myosin shift and this will require further study.

We found that TPT slowed to the same degree regardless of contraction duration. Contraction rate closely correlates to MHC isoform in vivo (Close, 1972) but we found no change in MHC isoform when utilizing a 0.6-second train duration, suggesting that MHC was not responsible for the shift in TPT. The troponin complex can also regulate contraction rate and we found clear shifts in TnC and TnT isoform from fast to slow. In contrast, both the slow and fast isoforms of Tnl increased with electrical stimulation suggesting that Tnl responds to electrical activity in a different manner to TnC and TnT. In vivo, it has been shown that Tnl (Härtner and Pette, 1990; Leeuw and Pette, 1993) and TNT (Härtner and Pette, 1990) isoform changes occur more slowly than that of TnC and TnT in response to CLFS. Functionally, TNT is typically the dominant troponin isoform in dictating contractile dynamics (Yu et al., 2007) and our data suggest that TnT may underlie the slowing in TPT observed in this study. Although the lack of complete MHC shift is not that surprising, the slower contraction rates may not be due to shifts in myosin isoform but rather other factors such as changes in the amount of fast thin filaments (see below). The shifts in SERCA isoform showed a trend to be greater at 60 and 600 sec but this is unlikely to cause the much greater slowing in half-relaxation time in those groups. Other modifications such as phosphorylation of Tnl (Zhang et al., 1995; Roman et al., 2004; Layland et al., 2005) or other regulatory proteins may also play a role in the greater slowing of 1/2RT at 60 and 600 sec and warrants further investigation.

Electrical stimulation induced improvements in fatigue resistance and changes in metabolic proteins (Fig. 4). PFK, the rate-limiting step of glycolysis (Mor et al., 2011), was decreased following electrical stimulation. In vivo, GLUT4 expression increases relative to the time that muscle is active (Megenedy et al., 1993) and we found GLUT4 increased to similar levels with all contraction durations. We found contraction duration-dependent increases in the mitochondrial proteins citrate synthase (CS) and cytochrome C (Cyt C) but not SDH and ATP synthase, which increased independent of contraction duration (Fig. 7). Fatigue resistance (Westgaard and Lomo, 1988) and mitochondrial enzyme activity and protein levels (Gundersen et al., 1988) have been shown to increase with...
increasing active time following electrical stimulation. As active time was consistent it is likely that the greater fatigue induced by longer contractions (Fig. 1) provided the signal for increased CS and Cyt C. However, the mRNA levels of nuclear respiratory factor 1 (NRF-1), a key regulator of mitochondrial biogenesis (Virbasius and Scarpulla, 1994), only increase during rest periods between stimulation bouts (Nghou and Hood, 2011) and hint that not only the contraction duration but the rest period regulate metabolic adaptations to electrical stimulation.

Slow fibers have higher oxidative capacity than fast fibers and oxidative capacity increases following CLFS. We found no change in MCAD or VLCAD but surprisingly a decrease in LCAD following electrical stimulation. In contrast, δ-HAD which catalyses the third step of β-oxidation was increased with electrical stimulation, suggesting that different mechanisms regulate the levels of different enzymes of the β-oxidation pathway. We have previously found that compared to high glucose (25 mM), culturing in low glucose (5.55 mM) results in higher levels of LCAD and VLCAD (Khodabukus and Baar, 2015). This suggests that either ACAD content does not increase in response to electrical stimulation or that the high glucose used in this study prevented changes in ACAD content.

Exercise (Bru et al., 2002) and in vitro electrical stimulation-induced mitochondrial biogenesis (Atheron et al., 2005; Burch et al., 2010) is thought to be primarily regulated by PGC-1α. PGC-1α is deemed a master regulator of mitochondrial biogenesis due to its effect on peroxisome proliferator-activated receptors (PPARs) (Aubert et al., 2013), estrogen-receptor-related receptors (ERRs) (Willy et al., 2004), NRF-1 and 2 and other proteins involved in mitochondrial biogenesis and itself is thought to play a role in promoting the slow-fiber type program (Rasbach et al., 2010). Following electrical stimulation we found no change in its deacetylation SIRT1 but an approximate twofold increase in PGC-1α and MEF2, an upstream regulator of PGC-1α which is implicated in promoting slow-fiber type (Wu et al., 2000) and mitochondrial biogenesis (Naya et al., 2002) and likely induced some of the metabolic adaptations seen.

Using the C2C12 cell line we have been able to induce a functional contractile and metabolic shift to a slow phenotype using electrical stimulation. While keeping active time and the number of impulses received each day consistent, we have demonstrated that the duration of each contraction and/or rest period play a key role in inducing myofibrillar and metabolic protein shifts.
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