Reduced Ca^{2+}-activated force explains increased skeletal muscle fatiguability in heart failure

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Congestive heart failure (CHF) is accompanied by a skeletal muscle myopathy causing increased exertional fatigability. The underlying cellular mechanisms are unclear but the myopathy seems to affect predominantly slow-twitch fibres. We have investigated whether the intrinsic Ca^{2+}-activating properties of the myofilaments are altered in CHF in resting and fatigued skeletal muscle *soleus* fibres.

CHF was induced by coronary artery ligation in male wistar rats while under general anaesthesia (ventilated with isofluoran 2.5% in 30% O_2 and 70% N_2O). Sham-operated rats were used as controls (SHAM). Postoperatively the animals were given 0.2 mg kg^{-1} buprenorphine and kept under daily surveillance. Six weeks after ligation the degree of heart failure was assessed measuring left ventricle end diastolic pressure (LEVDP, with Millar catheter) under general anaesthesia (again isofluoran) and lung weight (post mortem, at the end of the experiment). The right soleus muscle was prepared *in situ* with the distal tendon attached to a force transducer. Blood supply to the muscle was left intact. The muscle was kept at ˜38°C by dripping warmed saline on the muscle. Fatigue was induced by subtetanic stimulation resulting in repeated partially fused trains of isometric contractions with a peak force of about 30% of maximum tetanic force (1 ms pulses at 5 Hz in trains of 6 s on, 4 s off for 30 minutes). Within 10s after completion of the fatigue-protocol the fatigued muscle was dissected out, subsequently pinned at resting length in a petri dish under paraffin oil and cooled on ice. The non-stimulated contralateral muscle served as resting control. The rats were then killed. From the muscles single fibres were dissected out and mechanically skinned. The skinned fibres were mounted between forceps and a force transducer, and stretched to 120% of slack length. Fibre diameter was measured while the fibre was still under oil. Subsequently the fibre was transferred to a series of baths containing cytosol-mimicking solutions with strongly EGTA-buffered free [Ca^{2+}] or [Sr^{2+}] ranging from 0.05 to 20 μM or 200 μM, pH 7.1. Maximal Ca^{2+}-activated force was calculated and relative force-pCa and –pSr plots were constructed and hill curves fitted. At last the fibres were put in 10 μl SDS-buffer solution for later gel-electrophoresis analysis of myosin light chain phosphorylation (MLC) and myosin heavy chain (MHC) isoforms. The fatigue protocol resulted in a 44 ± 5 % (mean ± SE) decrease in peak force produced by the whole muscle during the 5 Hz trains in CHF rats, whereas peak force was decreased by only 24 ± 9% in SHAM rats (n = 5 each in CHF and SHAM). In the resting contralateral skinned single fibres, no differences were found in maximum Ca^{2+}-activated force, fibre cross-sectional area (CSA) or [Ca^{2+}] eliciting 50% force (pCa_{50}) between SHAM and CHF rats (each n = 10 fibres from 5 rats). Fatigued fibres from both SHAM and CHF rats, tended to have lower maximum Ca^{2+}-activated force per CSA than the contralateral resting fibres (each n = 10 fatigued and 10 contralateral fibres from 5 rats). However, in SHAM rats, fibre CSA was 28% higher in fatigued than in contralateral resting fibres ($p < 0.02$), whereas CSA was slightly lower in fatigued CHF fibres ($p < 0.05$). The lack of activity-induced cell-swelling in CHF is to be confirmed with microscopy analysis of whole muscle cross sections. When disregarding CSA, absolute maximum Ca^{2+}-activated force in fatigued fibres in CHF rats was 23 ±10 % (n = 10) lower than contralateral resting fibres ($p < 0.02$), whereas in fatigued fibres from SHAM rats absolute Ca^{2+}-activated force was not altered ($p > 0.1$). Ca^{2+}-sensitivity was not altered compared to contralateral resting fibres in either fatigued CHF or SHAM fibres. Sr^{2+}-activation curves indicated that two of in all 42 fibres dissected were either fast twitch or mixed fibre types (both from SHAM rat), and these fibres were not included. All other fibres had Sr^{2+}-activation curves typical for predominantly slow-twitch type MHC. The MHC isoforms in each included fibre are to be confirmed with gel electrophoresis.

In conclusion, increased fatigability in slow muscles in CHF seems to be due to intrinsic alterations in actin-myosin interaction of slow twitch fibres during activity leading to a decrease in absolute force produced at all [Ca^{2+}]. The lack of activity-induced cell swelling in CHF fibres may in some way play a role.