Functional and Structural Characteristics of Sheep LD Conditioned to Fatigue Resistance by a Half-Day Cardiac-Like Electrostimulation Protocol: Implications for Dynamic Cardiomyoplasty

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Abstract

The sustainable power output and the working capacity of fatigue-resistance-converted muscle is one of the constraints of dynamic cardiomyoplasty and biomechanical circulatory supports. Fatigue-resistance at relatively high sustainable power output is achieved by different electrostimulation patterns which are implementable in clinical settings. We are testing the effects on functional and structural characteristics of the sheep LD of a half-day protocol which strictly mimics for any other parameter the clinical protocol actually tested in FDA trials. Results are encouraging, since the muscle maintains its mass and an intermediate state of fast to slow transformation. With the actual protocol we find only minor changes of power output in comparison to the standard electrostimulation protocol. Major result is the demonstration that the life-span of implantable devices could be more than doubled. Furthermore resting the muscle each day for periods of several hours will decrease risk of muscle damage. Though accidental experiences of a few surgeons demonstrate that periods of several hours or days of LD resting are not detrimental to the patient's cardiac function, the question remains if the new pattern of cardiac assistance, which open a full new set of options (muscle hypertrophy oriented, included) could be acceptable on regular basis.

Key words: sheep, Latissimus Dorsi, muscle conditioning to fatigue resistance, dynamic cardiomyoplasty, electrostimulation, sustainable power output, skeletal muscle cardiac assistance.

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Fatigue resistance at moderate power output is achieved in two-three weeks and is dependent from increased oxidative metabolism, calcium handling, but not changes in contractile machinery [22, 26]. The level of sustainable power output of fatigue-resistance-converted muscle is one of the major constraint of skeletal muscle-cardiac assistance [1, 27, 28]. Fatigue resistance at relatively high sustainable power output could be achieved by different patterns of muscle stimulation, and long-term fatigue resistance is maintained in spite of short-term (hour or day) changes in overall muscle activity. Evidence are: 1) for long-term changes the conditioning-deconditioning experiments in rabbit [25] and sheep [10]; 2) for short-term changes: i) anecdotes of clinical experience of electrostimulation disconnection without detrimental effects or major worsening of hemodynamic results during or after one-day of cessation of electrostimulation [2, 9, 15, 20, 21]; ii) animal experiments with 8 or 12 hrs per day electrostimulation (for review see [22]); iii) for very short-term changes: the data here reported and those of others during sessions of power and fatigue testing [5, 12, 19].

We are studying the effects on power output and on anatomical and molecular characteristics of the sheep latissimus dorsi (LD) of a half-day electrostimulation protocol which strictly mimics for any other parameter the clinical protocol [2, 6] which is under FDA testing [11]. Preliminary data show that an intermediate state of muscle transformation can be maintained if muscles are activated height-ten hours per day. The results are interesting, since: i) the muscle maintains its mass and the half-day electrostimulation prolongs the life-span of the devices; ii) interrupted electrostimulation (one hour on / one hour off) is known to be less damaging than continuous electrostimulation [16, 17], being the slow fibres more susceptible to exercise-induced muscle damage [23]; and iii) partially transformed muscles being faster are expected to be more powerful [13, 14]. Preliminary results of our pilot study were previously presented in abstract form [6].

Materials and Methods

Surgical procedures and measures of dynamic characteristics of sheep LD were performed at the Experimental Surgery Unit, Cardiovascular Institute, University of Bologna, S. Orsola Hospital, Bologna; histochemical and molecular analyses on muscle specimens were accomplished at the Department of Biomedical Sciences, University of Padova, Italy. A pilot study was conducted in three sheep. To minimize dissection and vascular disturbances intramuscular electrodes were implanted according to the Medtronic Protocol of dynamic cardiomyoplasty in the LD maintained *in situ* as described in [1]. Stimulation protocols: Four days after surgery, to allow wound healing, the ITREL stimulator was switched on 10 hrs per day (usually from 8 a.m. until 6 p.m.), single pulse mode (impulse frequency: 2 Hz; impulse duration: 210 µsec, 3-5 Volts; ON stimulation period: 0.1 sec; OFF stimulation period: 2 sec), to mimic the one to two ECG mode, that is at a mean frequency of 40 stimuli (single pulse) per min. After two weeks of stimulation the LD was activated for the successive two weeks with a couple of impulses (in-burst frequency: 15 Hz) every 1.5 seconds, then for the successive two weeks with a three-impulse-burst. Finally from week-9 a 0.13 sec burst at 30 Hz was discharged every 1.5 seconds. Power output and fatigue tests were assessed at four and six months after surgery according to [1, 5]. Histochemistry, isomyosin profile, myoglobin content, and quantitation of molecular markers of muscle damage/regeneration were performed on small biopsies at four and six months to limit damage of the muscle during the experiment. Methods are detailed in: [6, 24, 29-31].

Results and Discussion

Three sheep were implanted and final stimulation protocol according to [3] was achieved in two sheep. After four and six months of ten hours per day electrostimulation at 3 V, 130 µsec train at 30 Hz every 1.5 sec (40 tetanic contractions per min) major results are: i) Muscle gross mass does not decrease, as is the obligatory results of all-day electrostimulation patterns. Myoglobin, a molecular marker of oxidative myofibers, only slightly increases (it remains near to 4% of total muscle proteins, that is near to 0.6% of tissue weight). MHC1 (the peculiar marker of slow-type, fatigue-resistant myofiber [4]) increase from 20% of normal LD to 60-65%, but does not achieve the 100% transformation which usually occurs in continuous electrostimulation (Table 1). These results are in keeping with the rabbit 12 hr per day experiments discussed in Pette and Vrbova [22] vs the 24 hrs per day electrostimulation experiments performed by Salmons and collaborators [26]. The contractile characteristics are also incompletely converted: in the normal LD tetanic fusion was obtained at stimulation frequencies higher than 40 Hz, while after one month of 24 hrs per day conditioning the tetanic fusion was complete at 20 hr [1]. Figure 1 shows that 20 Hz stimulation produces a clone in normal LD (trace a), a fused tetanus in a full-day conditioned LD (trace c), and a non-fused tetanus in a 10 hours per day electrostimulated LD. After half-day electrostimulation sustainable power output is similar to that extractable from a 24 hrs per day electrostimulated LD (about 0.5 watts per LD, i.e., more than 2.5 watts per kg [1]). When the power output was doubled either by increasing duration of the tetanic contractions or the in-burst frequency, a fatigue index of about 80 (that is there was a loss of 20% power) was present after 3 min. If the power output was maintained four times higher than that continuously sustainable, the muscle fatigued in less than one minute. Sustainable level of power was obtained after short-time rest (a few minutes), that is, a moderate power output (30-50% of left ventricle mean power output) is maintained in spite of short-term (20-30 sec) increase of work demand up to four times the sustainable power output. During several hours of measurements the sustainable power output was maintained, even after manifested fatigue due to increased demands. Interestingly, the sustainable power was achieved with electrostimulation parameters very close to those of the final setting of the conditioning protocols. Questions worth to be tested are: could the muscle be conditioned with more demanding electrostimulation protocols? Could such an increased activity be imposed without long-term muscle damage?

In conclusion our results confirm that an intermediate state of muscle transformation can be maintained by half-day electrostimulation even in species, like the sheep, in which full conversion to slow type contractile properties and to slow myosin is achieved with tetanic cardiac-like electrostimulation or even with 2 Hz continuous electrostimulation, but applied all the day around. Resting the muscle each day for periods of several hours would decrease the risk of muscle damage. Experimental studies in rabbit show that substantially less muscle damage than in continuous electrostimulation had been observed in one hour on / one hour off electrostimulation [16]. Chekanow confirms that after dynamic cardiomyoplasty there is no need to stimulate the LD every heart contraction, and suggests that hour-periods of muscle rest could be intro-

Table 1. Structural and molecular characteristics of sheep LD: 12 vs 24 hrs per day cardiac-like electrostimulation; n.d., not determined.

	Weight gr	MHCl (%)	Myoglobin (%)
12 hrs per day electrostimulation			
Sheep Bo94-1			
Control LD	180		
axillary region biopsy		8	0.88
central biopsy		20	n.d.
Four-month stimulated LD	185		
axillary region biopsy		68	0.85
central biopsy		60	n.d.
Sheep Bo94-2			
Six-month electrostimulated LD			
axillary region biopsy		65	0.27
24 hrs per day electrostimulation			
Control unstimulated LD (12 normal LD)		14	0.72-0.59
2-month stimulated LD (3 LD)		95	0.35-0.98-0.80

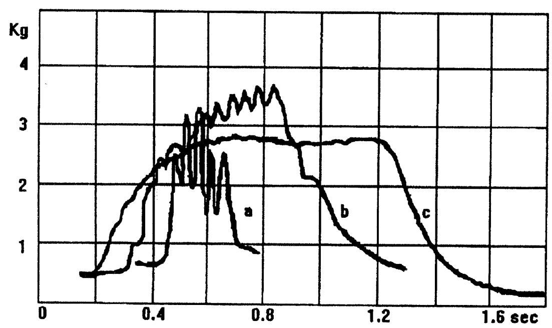

Figure 1. Contractile characteristics of conditioned LD. Tetanic fusion: The Figure shows that stimulation at 20 Hz produces a clone in normal LD (trace a, 0.25 sec), a non-fused tetanus in a half-day electrostimulated LD (trace b, 0.5 sec), and a fused tetanus in a full-day conditioned LD (trace c, 1 sec). After half-day electrostimulation sustainable power output is similar to that extractable from a 24 hrs per day electrostimulated LD (about 0.5 watts per LD, i.e., more than 2.5 watts per kg [1]).

duced in the stimulation regimen. We here demonstrate that the Magovern enthusiasm for such a proposal could stand on experimental evidence. The question remains if the patients could accept such a pattern of cardiac assistance. Occasional experiences of a few surgeons demonstrate that such an approach (which open a full set of new options) is not detrimental [2, 9, 20, 21]. The higher freedom in activating the patient's LD could add to pre-operative physical training [18] the long-standing advantages of muscle hypertrophy-oriented daily electrostimulation sessions. In any case, a major result of our preliminary observations is that the life-span of implantable devices could be more than doubled.

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