Biotransformation of Skeletal Muscle Used for Long-term Dynamic Aortomyoplasty

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Abstract

Dynamic aortomyoplasty using autologous transpositioned latissimus dorsi (LD) muscle has been developed to produce extra-aortic counterpulsation as an alternative to ventricular assist devices in a goat animal model. This study was intended to analyze the chronic biochemical transformation of LD muscle as well as the metabolic potential, calcium regulating capacity and the contractile machinery of the long-term stimulated skeletal muscle-powered extracardiac counterpulsation device. The results showed that LD muscles continuously conditioned for 12 months presented with greater adaptive capacity for aerobic oxidation through an increase in the oxidative phosphorylation and β-oxidation of fatty acids, and decreased glycolytic metabolism and myosin and myofibrillar-ATPase activity than their control counterpart. These changes are similar to those seen after 4 to 6 weeks of continuous or intermittent electrostimulation showing that conversion of fast-twitch type II fibers to slow-twitch fatigue-resistant type I fibers is maintained when the muscle is conditioned long-term. This in turn suggests that long-term conditioned LD muscle presents with all necessary adaptive capabilities for use in the development of chronic muscle-powered counterpulsation biomechanical assist devices.

Key words: cardiac assist device, intra-aortic balloon pump, counterpulsation, skeletal muscle, aortomyoplasty

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Conventionally, chronic end-stage, low cardiac output states of various causes have been sustained with the intra-aortic balloon counterpulsation (IABC) [9-11, 13, 15, 17, 32, 36, 37]. Since the application of IABC is associated with aortic dissection, leg ischemia, mesenteric infarction, septicemia and neurologic sequelae [12, 14, 29], attempts have been made to develop extra-aortic counterpulsation devices using autologous progressive electrostimulated latissimus dorsi (LD) muscle. Although acute dynamic aortomyoplasty has been proven to represent an effective way to significantly augment cardiac function for short-term in an animal model [6], there is no information regarding functional and structural changes seen after long-term chronic electrostimulation of LD used for extracardiac counterpulsation. This preliminary study intended to analyze the chronic biochemical transformation of LD muscle used continuously for approximately 12 months as a biomechanical assist device. The metabolic potential, calcium regulating capacity and the contractile machinery of the long-term stimulated skeletal muscle-powered extracardiac counterpulsation device are examined.

Materials and Methods

Aortomyoplasty

Three adult goats (30 to 40 kg in weight) of random sex were used for this study. Anesthesia was induced with ketamine (25-30 mg/kg i.m.) atropine (1 mg i.m.) and thyamylal sodium (28 mg/kg i.v.). The animals were intubated and artificially ventilated with halothane and oxygen and operated upon under sterile conditions. The left LD muscle was divided from its insertion and prepared as a pedicle flap. A portion of the flap was transpositioned into the thoracic cavity through resection of the left second rib. Particular care was taken to secure hemostasis and the preservation of the neurovascular pedicle. Thoracotomy was performed through the left fourth intercostal space.

The descending aorta was carefully dissected for approximately 5 to 8 cm below the aortic isthmus and the LD muscle flap wrapped around the aorta was fixed under minimal tension with interrupted 3-0 prolene sutures. An unipolar electrode was used to stimulate the muscle. A modified pacing lead was placed in direct contact with the thoracodorsal nerve and the sensing electrode was attached via a screw lead into the left ventricle. The electrode wires were placed interscapularly through a subcutaneous tunnel. The chest was closed in layers. Particular care was taken to preserve the blood flow to the muscle flap. A chest tube was left in the thorax until skin closure. At the end of the procedure, the tube was removed upon lung inflation and the air was evacuated from the chest. All animals tolerated the procedure well, recovered uneventfully, and were fully ambulatory on postoperative day 1. Postoperatively, they received antibiotics for at least 7 days. At the end of the first postoperative week, all animals were moved to a farm and allowed to move freely.

Muscle Conditioning

The LD muscle flap was left undisturbed for six weeks, then a Medtronic® Symbios Model 7005 pulse stimulator (Medtronic, Inc., Minneapolis, MN) was implanted subcutaneously under local anesthesia and attached to the previously implanted leads. This implantable pulse generator is a multimodal, programmable device with bidirectional telemetry. The pacemaker was programmed non-invasively using the Medtronic® Model 9710 Programmer in the A-V Universal (DDD) mode. This allows an adjustable A-V delay from 25 to 250 msec in increments of 25 msec. Throughout its pacing cycle, the pacemaker ignores all spontaneous atrial activity. Pacing was started synchronously with the intrinsic heart beat with an original A-V delay of 250 msec at 1:4 synchrony and this increased weekly at 1:2 up to 1:1 with the cardiac diastole. The final A-V delay remained 250 msec. Pulse width was 0.5 msec at a rate of 2 Hz (120 per minute). The amplitude was 2.5, 2.5 and 5.0 V. respectively. Gradual and progressive increase in stimulating power was necessary in order to avoid fiber deterioration. The LD muscle flap was conditioned for approximately 12 months. Chronic electrostimulation did not cause any discernable discomfort to the animals. After 12 months of continuous conditioning, all animals were anesthetized as described previously in this article and biopsies were taken from the conditioned LD and its unconditioned counterpart. Particular attention was paid to the location and depth of the biopsy in order to analyze strictly comparable samples of LD. Muscle samples were frozen as quickly as possible by immersion in liquid nitrogen and stored at -70°C until the time of analysis. All animals tolerated the conditioning process very well, however, 2 animals developed skin infections at the site of the subcutaneous pacemaker pocket necessitating drainage and relocation of the pacemaker. At 6 and 12 months postoperatively, all animals underwent echocardiographic assessment of the function of the aortomyoplasty. In all cases, it was determined that the aorta was synchronously squeezed with the diastolic phase of the cardiac cycle, however, no hemodynamic measurements were performed.

During the whole study period, all animals received humane care according to the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health (NIH Publication No. 80-231 revised 1985).

Biochemical Procedures

Stimulation lasted until 2 hours before starting to take biopsies. Fifty mg muscle samples were diluted 1:20 in a 33 mM phosphate buffer and homogenized for 3 bursts of 15 s at number 7 on a Brinkman polytron. Maximal activity of total 6-phosphofructokinase (PFK, EC 2.7.1.11) was determined spectrophotometrically by measuring the rate of oxidation of NADH at 340 nm, 30°C, pH 7.58 [31]. A minor modification was made by changing the buffers to TEA and sodium phosphate. Citrate synthase (CS, EC 4.1.3.7) was selected as a marker enzyme for aerobic capacity. Maximal activity of the enzyme was determined spectrophotometrically at 30°C, pH 8.1, by measuring the formation of thionitrobenzoic acid [34]. Myocardial capacity for β oxidation of fatty acids was determined by measuring the maximal activity of 3-hydroxyacyl-CoA dehydrogenase (HADH, EC 1.1.1.35). The activity was measured spectrophotometrically at 30°C, pH 7.4 [4].

SR isolation and Ca^{2+} ATPase activity

From approximately 200 mg of tissue, microsomal fraction enriched with SR vesicles was isolated according to the method described by O'Brien et al. [23] with minor modifications. Isolated fractions were prepared by an initial sedimentation at 10,000 x *g* and then at 125,000 x *g* with 25% sucrose (w/v). Protein concentration of the fraction was determined using a procedure originally described by Lowry et al, [18] and bovine serum albumin was used as the standard.

The Ca^{2+} stimulated Mg^{2+} dependent ATPase activity reported in this study is the difference between the total Ca^{2+} and Mg^{2+} and basal Mg^{2+} activities. These activities were estimated by measuring the amount of inorganic phosphate liberated by enzymatic hydrolysis at 37°C, pH 7.0, as reported previously [23]. The reaction was initiated by the addition of 5 mM Tris ATP and terminated after 5 min by the addition of 1 ml of 5% sodium dodecyl sulphate (SDS), pH 7.0. The Ca^{2+}-ATPase activity was assayed in the presence of 50 mM sodium azide, an inhibitor of mitochondrial Ca^{2+} transport.

Myosin and myofibrillar-ATPase activities

Ca^{2+}-stimulated myosin-ATPase (M-ATPase) and myofibrillar-ATPase (MF-ATPase) activities were determined using myofibrils prepared by the method of Baldwin et al. [3], from a 100 mg tissue sample which is a modification of the original procedure described by Solaro et al. [33]. When prepared in this manner, the isolated fraction

*Table I. PFK, phosphofructokinase; CS, citrate synthase; HADH, 3-hydrohoxyacyl CoA dehydrogenase; SR yield, the amount of protein in the microsomal fraction; SR-ATPase/mg, SR-ATPase/mg protein in the microsomal fraction; SR-ATPase/g net wt., SR-ATPase activity/g net weight of muscle at 37 ℃: M-ATPase, myosin-ATPase activity in μmoles/mg myosin protein/min at 30 ℃: MF-ATPase, myofibrillar ATPase activity in μmoles/mg myofibrillar protein/min at 30 ℃. NS, not significant. * results are expressed in μmoles/g wet wt/min. All values are mean ± standard deviation.*

VARIABLES	CONTROL (n = 9)	LONG-TERM CONDITIONED (n = 9)	SIGNIFICANCE
PFK*	49.2 ± 15.4	7.2 ± 1.8	$p < 0.05$
CS*	10.8 ± 6.2	17.4 ± 5.4	$p = 0.08$
HADH*	2.3 ± 1.1	3.3 ± 0.8	NS
SR yield	0.8 ± 0.2	0.4 ± 0.3	NS
SR-ATPase/mg	2.0 ± 0.5	0.6 ± 0.5	$p < 0.05$
SR-ATPase/g net wet weight	1.6 ± 0.8	0.3 ± 0.2	$p = 0.06$
M-ATPase	0.7 ± 0.2	0.5 ± 0.1	NS
MF-ATPase	0.4 ± 0.1	0.2 ± 0.0	$p < 0.05$

has been demonstrated to be free of mitochondrial, sarcoplasmic reticulum, and Na^+/K^+-ATPase activity [33]. Protein concentration of the myofibrillar fractions was measured according to Lowry et al [18] and then adjusted to 6 mg/ml. Ca^{2+}-activated MF-ATPase activity was determined at 30°C, pH 7.0. The amount of inorganic phosphate liberated by enzymatic hydrolysis was measured by the method described previously [28]. M-ATPase activity was determined in a similar manner [21] and then specific activity was calculated assuming that myosin constitutes 42% of the myofibrillar protein [21]. From each muscle sample 3 biochemical and enzymatical determinations were performed.

All values are expressed as mean ± standard deviation. Statistical analysis was performed with SPSS PC Plus (version 3.0). Paired *t*-tests were employed for analysis of differences between groups. A p value less than 0.05 was considered statistically significant.

Results

All animals survived the full extent of the experimental procedure. None of the animals developed postoperative signs of paraplegia. The biochemical and enzymatic markers of contractile machinery of the chronic electrostimulated muscle vs. control are presented in Table I. The autotransplanted chronic conditioned LD muscle presented with statistical significant changes in the glycolytic metabolism as expressed by a decrease in the activity of PFK (49.2 ± 15.4 vs. 7.2 ± 1.8 μmoles/g net wt/min), and a decrease in the calcium regulating capacity of the sarcoplasmic reticulum (SR) as represented by the amount of protein yielded by the microsomal fraction (0.8 ± 0.2 vs 0.4 ± 0.3, p = NS). SR-ATPase activity per mg protein in the microsomal fraction (2.0 ± 0.5 vs. 0.6 ± 0.5 μmoles inorganic phosphate released from ATP/mg protein/min, $p < 0.05$) and SR-ATPase activity per g net wet weight (1.6 ± 0.8 vs 0.3 ± 0.2, p = 0.06). The energy produced by fat catabolism (β-oxidation of fatty acids) was increased in the stimulated muscle as the activity of 3-hydroxyacyl CoA dehydrogenase (HADH) increased by 43% in a nonstatistical significant manner (2.3 ± 1.1 vs. 3.3 ± 0.8, μmoles/g wt/min $p > 0.05$). The long-term conditioned muscle showed an increase in the aerobic oxidative capacity of the Krebs cycle as the citrate syntase (CS) raised from 10.8 ± 6.2 to 17.4 ± 5.4 μmoles/g net wt/min ($p = 0.08$). The analysis of myosin and myofibrillar-ATPase activities revealed a decrease in M-ATPase (0.7 ± 0.2 vs 0.5 ± 0.1 μmoles/ mg myosin protein/min, p = NS) and a statistically significant reduction of the MF-ATPase (0.4 ± 0.1 vs 0.2 ± 0.0, μmoles/mg/min $p < 0.05$) in the electrostimulated muscle.

Discussion

Transposition of autologous skeletal contractile tissue and the development of new implantable pacemaker technology capable to deliver low-frequency stimulation allowing the fast-twitch muscles to change their physiological and histochemical properties, has opened new avenues in the development of biomechanical cardiac assist devices. Of those, skeletal muscle-powered extra-aortic counterpulsation devices have proven to be the most effective with regard to their capabilities to improve hemodynamics when used for short-term cardiac augmentation in the acute setting [6, 22]. The conversion of type II fast-twitch fibers of the skeletal muscle to type I fiber similar to the cardiac muscle has been obtained through relatively short-term chronic electrostimulation. However, some authors have found that long-term electrostimulation

of the skeletal muscle may result in the development of progressive fatigue which might diminish the efficacy to augment the cardiac function [20, 38]. This has been attributed in part to a decrease in fiber diameter (area) and maximum force-generating capacity [7, 19, 30]. Most of the studies addressing this issue present data related to changes in functional status of chronic electrostimulated skeletal muscle of different animal species or report the transformation of LD muscle *in situ* [1, 26].

Because there are demonstrated interspecies variations with regard to muscle plasticity after long-term electrostimulation [26, 39], we undertook this study to determine the adaptive responses of goat LD muscle to 12 months of continuous conditioning (working transformation). The goat was chosen as the animal model for aortomyoplasty because its intrathoracic anatomy closely resembles to human counterpart. Since LD muscle is a noncritical, broad-base muscle which allows bidirectional stretch, it can be easily prepared surgically as a muscle flap and transferred into the thoracic cavity for preparation of an extra-aortic counterpulsation device.

Experiments using chronic electrical stimulated LD muscle for 6 weeks have demonstrated no signs of muscle fiber destruction or regeneration [8, 25, 34]. However, some authors [6, 25] and their critics have expressed concern over the adaptive responses to longer continuous use. Our study shows that LD muscle conditioned continuously for 12 months, presents with the same adaptive biochemical and histochemical changes as those seen after 6 weeks of electrostimulation. At 12 months there are persistent changes in the activity levels of enzymes of energy metabolism. Our experimental muscle preparation showed a significant reduction of cytosolic anaerobic capacities and an increase toward a more oxidative, aerobic metabolism as represented by a decrease in the activity of PFK (inducible enzyme whose activity is considered to play a major role to the regulation of glycolysis) and an increase in the CS activity, respectively. CS takes part in the major aerobic pathway (Krebs cycle) inducing the generation of ATP via oxidative phosphorylation. Although not statistically significant, the later changes represent an increase of 61% in the oxidative myofibrillar power which is generally attributed to an increase in the mitochondrial volume [24, 25]. It is clear that the 12 months conditioned LD muscle derives more energy from β-oxidation of fatty acids as expressed by an increase of over 43% in the activity of HADH. These results are consistent with other investigators' findings [1]. Moreover a statistical significant decline in the sarcoplasmic reticulum content of the long-term conditioned muscle might be responsible for the changes in the Ca^{2+} regulating capacity as determined by Ca^{2+}- ATPase activity. Our study demonstrated a reduction in Ca^{2+}-stimulated Mg^{2+} dependent-ATPase activity. Similar changes have been observed by others as early as 6 weeks after continued or intermittent electrostimulation of rabbit tibialis anterior and extensor digitorum longus muscles [27, 35]. Changes in the protein yield of fragmented sarcoplasmic reticulum seen after long-term stimulation correlate with a reduction in the extent of the sarcotubular system which further represents a decline in the calcium-transporting capabilities characterized by the slow-twitch muscle fiber [30].

As a result of 12 months of chronic, continuous conditioning, the goat LD muscle presented with a typical reduction of M-ATPase and MF-ATPase activity which reflects the conversion of type II fast-twitch fiber to type I slow-twitch cardiac-like muscle. This in turn induces a reduction of the intrinsic isometric contractile speed which reduces the maximum speed of muscle shortening [2]. Although this study does not address the issue of myosin composition and no histochemistry was performed, it is known that at one year there is still an incomplete transition with regard to the fast light chains subunits [24] or there may be a remaining component of fast light chains as the normal evolution of slow-twitch muscle [5]. Our biochemical analysis has shown that LD muscle could be continuously conditioned long-term and transformed to a slow-twitch type muscle which presents all the biochemical characters of the myocardial fiber. We did not observe any macroscopic degenerative changes after 12 months of stimulation and we assume that the transition of type II to type I muscle has achieved a plateau level with regard to the adaptive biochemical transformation.

Because in our animal model the descending aorta was wrapped for a maximum of 5 to 8 cm, no intercostal arteries had to be ligated. As a result, we encountered no signs of spinal cord ischemia (paresis or paraplegia). However, it should be stressed that the extrapolation of these findings to humans must be carefully considered. For an effective counterpulsation device, approximately 20 cm of descending aorta need to be wrapped and some intercostal arteries might have to be ligated.

No hemodynamics were measured at 12 months. Although this article does not intend to address the issue of hemodynamic changes observed by the use of aortomyoplasty, we envision that the use of a burst stimulator would provide better contraction of the muscle wrap than the single pulse DDD pacemaker used in this study.

In conclusion, the adaptive consequences of long-term biotransformation are suggestive for the development of a fatigue-resistant type I muscle fiber which is very suitable for the ultimate objective, i.e., being modeled as a chronic muscle-powered, cardiac assist device.

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