Electrical Stimulation-Induced Changes in Double-Wrapped Muscles for Dynamic Graciloplasty

Dalila Bouamrirène, MD; Jean-Paul Micallef, PhD, DSc; Philippe Rouanet, MD, PhD; Francis Bacou, PhD, DSc

Hypothesis: Treatment of fecal incontinence has been greatly improved by electrical stimulation of gracilis muscle transposed around the anal canal. Various configurations of the muscle have been used: single α, γ, ε muscle loops, split sling, or double wrap. We report herein experimental data on muscle transformation and damage induced by the latter surgical approach.

Design, Interventions, and Main Outcome Measures: This study was conducted on 4 groups of New Zealand white rabbits. Group 1 had unstimulated transposed gracilis muscles. Group 2 had left transposed gracilis muscles stimulated only. Group 3 had both right and left transposed gracilis muscles stimulated. Group 4 were the controls (not operated on). Muscle properties were studied by electrophysiological, immunohistochemical, and biochemical techniques.

Results: Transformation from fast-contractile glycolytic muscle fibers into fast-intermediate to slow-contractile oxidative muscle fiber types induced a fatigue resistance of the transposed muscle that has undergone long-term stimulation and muscle alterations characterized by fiber atrophy and fibrosis.

Conclusions: Whatever technique of dynamic graciloplasty is used, muscle degeneration associated with mobilization might result primarily from the surgical dissection, whereby collateral blood supply to the gracilis is interrupted and exacerbated by long-term stimulation.

Arch Surg. 2000;135:1161-1167

SKELETAL MUSCLES differ in contraction rate, metabolism, fiber type, composition, and expression of different isoforms of muscle proteins that are modulated, within certain limits, by innervation and/or contractile activity.1,2 Thus, electrical stimulation at low or high levels of activity that resemble normal slow- or fast-twitch muscle motor unit activities specifies the role of motor innervation via motor activity in leading to muscle transformation.3 These changes essentially concern their metabolic properties4,5 and contractile characters.6,8 The latter are associated with transformations of the myosin isoform spectrum and the regulatory proteins of myofilaments.9 In the last decade, plasticity of skeletal muscle properties has been widely used in several surgical approaches, such as cardiomyoplasty,10 urinary graciloplasty,11 and particularly sphincteroplasty for treatment of fecal incontinence12 and abdominoperineal reconstruction.13,14 Since the pioneer work of Pickrell et al,15 who first used the gracilis muscle in the human for sphincteroplasty, treatment of fecal incontinence of congenital, cancer, or trauma origin has been largely improved. A new graciloplasty approach in which gracilis muscle is electrically stimulated via either an intramuscular electrode or direct stimulation of the obturator nerve was independently developed by Baeten et al16 and Williams et al,17 respectively. Various configurations of the muscle have been used: single α, γ, ε muscle loops, split sling, or double wrap. One of the recent improvements of the latter approach consists of suturing the distal ends of the 2 gracilis muscles to each other. This procedure is supposed to decrease muscle fibrosis that occurs when each muscle is fixed to the abdominal wall.18 However, no studies have been done to assess the effect of this construction on muscle properties. We report herein experimental data on muscle transformation and damage induced by this surgical approach. Taken together,
MATERIALS AND METHODS

This study was conducted on female New Zealand white rabbits (3 months old, 2.7 kg) from our own breeding (INRA, Montpellier, France). All surgical experiments were performed under aseptic conditions. The animals were kept in individual cages and were provided with pelleted rabbit food and water ad libitum. Rabbits were divided into 4 groups: group 1: unstimulated transposed gracilis muscles (n = 3); group 2: left transposed gracilis muscles were stimulated only (n = 3); group 3: both right and left transposed gracilis muscles were stimulated (n = 4); and group 4: controls (not operated on) (n = 4).

SURGICAL PROCEDURES

Rabbits were anesthetized with pentobarbital sodium (Nembutal, 30 mg/kg intravenously) plus ketamine hydrochloride (Ketalar 50, 100 µL/kg intravenously). Rabbits were perfused with 5% glucose (Plasmalyte) during the operation. Supplementary doses of ketamine were administered intravenously as required.

The skin was incised longitudinally in the medial part of each thigh. The right and left gracilis muscles were separated from connective tissue and carefully dissected free from surrounding muscles. The 2 peripheral arteries of gracilis muscles were localized and ligated. Gracilis was then tenotomized distally and mobilized down to their insertion into the tibial tuberosity, leaving the neurovascular bundle intact. A medial laparotomy was performed, and a silicone drain (4 cm long) was fixed to the rectus abdominis muscles with nonabsorbable surgical suture (Prolene 5-0). Gracilis muscles were wrapped around the tube and sutured to each other with nonabsorbable surgical suture (Vicryl 3-0).

IMPLANTATION OF THE STIMULATION EQUIPMENT

An intramuscular electrode (S5591; Medtronic, Minneapolis, Minn) was implanted into the left gracilis (group 2) or bilaterally (group 3) close to the nerve trunk perpendicular to muscle fibers. The electrode was tunneled to the abdomen and was connected by an extension wire to an electrostimulator (Intrel Medtronic, model 7421) implanted subcutaneously. The thighs and the abdomen were closed in 2 layers with polyglyactin 910 (Vicryl 3-0).

ELECTROSTIMULATION

Muscle electrostimulation began 28 days after implantation of the devices, and the electrical parameters were programmed by a telestimulator according to the following protocol: continuous electrostimulation with an impulse width of 210 microseconds and a voltage of 1 to 3 V, according to the postsurgical stage. Frequency was increased from 2 Hz for the first 15 days to 5 Hz for the second 15 days to 10 Hz from 30 days of stimulation onward. Muscle contractions were perceptible until the end of the experiments.

Our results show that transformation from fast-contractile glycolytic muscle fibers into fast-intermediate to slow-contractile oxidative muscle fiber types induces a fatigue resistance of the transposed muscle that undergoes long-term stimulation. However, this surgical procedure, as those previously used, induces important
muscle alterations characterized by fiber atrophy and fibrosis, particularly dramatic in the distal ends of the sutured muscles.

BIOCHEMICAL CHARACTERIZATION OF MYOSIN HEAVY CHAIN ISOFORMS

Myosin was prepared according to d’Albis et al. Briefly, small pieces of frozen muscles previously thawed on ice were washed in 5 volumes of 20-mmol/L sodium chloride, 3-mmol/L sodium phosphate, and 1-mmol/L ethylene glycoltetraacetic acid (EGTA) (pH 6.5). Myosin was extracted in 3 volumes of 100-mmol/L sodium pyrophosphate, 5-mmol/L EGTA, and 1-mmol/L dithiothreitol (pH 8) and centrifuged at 10,000g. The supernatant containing myosin was diluted with 1 volume glycerol and stored at −20°C until use.

Myosin heavy chains (MyHC) were separated in 8% polyacrylamide slab gels in the presence of 0.4% sodium dodecyl sulfate and 30% glycerol. Electrophoresis was carried out at 70 V for 28 hours in a cold room. Myosin heavy chain isoforms were stained with a silver staining kit (Bio-Rad Silver Stain Plus kit) according to the manufacturer’s instructions.

Gels were scanned with Adobe Photoshop/VistaScan system, and the relative amounts of the different MyHC isoforms were quantified with a National Institutes of Health program using an Apple Macintosh computer.

IMMUNOCYTOCHEMICAL STUDIES

Unfixed muscle fragments were frozen in carbon dioxide–cooled isopentane. Cross sections 8 µm thick were cut in a cryostat. The expression of MyHC isoforms was performed with monoclonal antibodies raised against embryonic (11H7), perinatal (also called fetal or neonatal; 4C10), adult fast (14G2), and adult slow (8H8) myosins (kindly provided by F. Pons, U300 INSERM, Montpellier, France). Monoclonal antibody to collagen IV was purchased from Boehringer. Sections were incubated first with the different antibodies for 30 minutes at 37°C, followed by fluorescein-conjugated goat anti–mouse IgG (Cappel Research Products, ICN Pharmaceuticals Inc, Costa Mesa, Calif) for 30 minutes at 37°C. Incubation of the antibody was in phosphate-buffered saline and bovine serum albumin (10 mg/mL), and all washes were in phosphate-buffered saline. Stained sections were mounted in glycerol containing 1-mg/mL para-phenylenediamine and viewed with fluorescein optics with a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

STATISTICS

To test for statistically significant differences in the case of metabolic enzyme activities MyHC proportions, non-parametric test of Mann-Whitney was applied (StatView; Abacus Concepts Inc, Berkeley, Calif). Statistical significance was assumed at P < .05.

RESULTS

MECHANICAL ASPECTS OF MUSCLE CONTRACTION

Both control or transposed unstimulated gracilis muscles presented a typical pattern of fast skeletal muscle contractile properties characterized by a short time to peak twitch and to half relaxation (Figure 1). Fusion was reached from 40-Hz frequency onward. Figure 2, A, showed contraction curves recorded from group 2 gracilis muscles. The right unstimulated gracilis presented contractile properties similar to controls. They contrasted with
those of the left stimulated muscle, which showed a longer course of contraction than control, and almost fused at 20 Hz. These properties characterized a slow-twitch muscle. Fatigue resistance was in agreement with these data (Figure 2, B). Amplitude of contraction was most important in the right muscle but decreased sharply with stimulation, whereas the left stimulated muscle was resistant to fatigue.

METABOLIC PROPERTIES

Figure 3, A, showed the aldolase-ICDH ratio as marker of predominant type of oxidative or glycolytic metabolism. The semimembranosus proprius, constituted by 100% of type I fibers,21 was used as model of highly oxidative muscle. It was characterized by its low aldolase and its high ICDH activities, with an aldolase-ICDH ratio of 2. This contrasted with control or transposed unstimulated gracilis, which showed high aldolase and low ICDH activities, with an aldolase-ICDH ratio close to 60. Gracilis muscles from the bilaterally stimulated group presented an oxidative type of metabolism, particularly in muscles from the left side (aldolase-ICDH ratio, 7). In contrast, right gracilis muscle samples from the unilaterally stimulated group were highly glycolytic (aldolase-ICDH ratio, 50), whereas left stimulated gracilis showed highly oxidative properties (aldolase-ICDH ratio, 1.5), similar to those observed on the semimembranosus proprius (Table).
Figure 3, B, represents the percentages of MyHC isoforms in different control or operated-on muscles as deduced from the different gels (not shown). The semimembranosus proprius was an example of a slow-twitch muscle constituted in the rabbit by 100% of type I MyHC.8 In contrast, the rabbit gracilis muscle was a fast-twitch muscle containing less than 2% of type I MyHC. The 3 adult fast IIb, IId, and IIa MyHC isoforms were present at 12.5%, 79.5%, and 6.5%, respectively. When MyHC isoforms were pooled according to the metabolic properties of their corresponding fibers, 8.4% of types I and IIa MyHC (slow-twitch and fast-twitch oxidative fiber types, respectively) and 91.7% of types IIb and IId MyHC isoforms (both glycolytic fiber types) were expressed in control gracilis. Similar percentages were detected on transposed unstimulated gracilis. In the group of transposed gracilis stimulated bilaterally, MyHC I and IIa increased to 34% and 70% in the right and left stimulated gracilis, respectively. In the unilaterally stimulated group, the right unstimulated gracilis expressed predominantly the MyHC IIb and IId isoforms (91%), whereas the I and IIa isoforms were largely predominant (83%) in the left stimulated muscle.

Figure 4. Immunohistochemical staining with antibodies to fast (A, E, I, M), slow (B, F, J, N), and perinatal (C, G, K, O) myosin and antibody to collagen IV (D, H, L, P) of the unstimulated right loop (A-D), the stimulated left loop (E-H), the right unstimulated gracilis middle part (I-L), and the left stimulated gracilis middle part (M-P). Scale bar in P=200 mm.

BIOCHEMICAL CHARACTERIZATION OF MyHC ISOFORMS

IMMUNOHISTOCHEMICAL STUDIES

Figure 4 illustrated the immunohistochemical expression of fast, slow, and perinatal MyHC and collagen IV of tissue samples from right and left gracilis of the transposed unilaterally left-stimulated group. Unstimulated right and stimulated left loops were illustrated on the first and second upper lanes of Figure 4, respectively. Fast MyHC were the main isoforms expressed in the right loop, and the architecture of muscle fiber basal lamina as shown by anti-collagen IV staining is regular. No perinatal MyHC were expressed (Figure 4, A-D). These staining patterns are similar to those obtained on unstimulated right gracilis middle part (Figure 4, I-L) and on control gracilis (not shown). In contrast, the left stimulated loop (Figure 4, E-H) showed a different pattern of MyHC expression. Type I MyHC isoform was predominant, and coexpression of fast and perinatal MyHC isoforms was observed. Embryonic MyHC was never detected (not shown). Moreover, collagen IV staining showed alterations of fiber basement membrane architecture, and muscle fibers were considerably atrophied. Similar observations were done in left stimulated gracilis middle part (Figure 4, M-P). Almost all muscle fibers were transformed into slow contractile fibers. However, most of them coexpressed fast and perinatal MyHC isoforms. Although muscle architecture was much more regular than in the loop, it is note-
worthy that muscle fibers were atrophied in comparison to right unstimulated gracilis samples.

COMMENT

It is well known that muscle fiber type transformation is due to the aggregate number of pulses (average number of pulses per second) delivered to the muscle, that is, fiber type transformation can be achieved with a constant low frequency or with intermittent low-frequency bursts as long as the latter is equivalent to a constant low frequency. The primary concern in electrical stimulation of a newly transposed muscle is to avoid stimulation-induced muscle damage by using an initial low frequency (2 Hz under our experimental conditions). This is also better at inducing the growth of more capillary fibers and mitochondria within the first 2 weeks of stimulation, the frequency can then be increased progressively with a reduced risk of stimulation-induced ischemic damages.

However, our studies show that, even under these conditions, electrostimulation of the transposed gracilis induces damage particularly important at the muscle distal end. Similar observations have been previously reported for cardiomyoplasty and graciloplasty. Apart from long-term stimulation, several other factors might induce muscle alterations in relation with gracilis mobilization that require both tenotomy and collateral blood vessel ligation.

Previous studies showed that tenotomy induces dramatic alterations of muscle structure, associated with fatty degeneration in the rabbit and a 30% decrease of muscle mass and maximal muscle strength. In our studies, however, the proximal and distal muscle parts both present a similar structure characterized by the regular shape and size of muscle fibers that express the same pattern of MyHC isoforms as controls. Thus, tenotomy is not implicated in the observed damage to the muscle distal end, certainly because of the permanent tension of the tenotomized gracilis that are tightly sutured to themselves.

Another factor that might be involved in muscle alteration is blood vessel ligation. According to Mannion et al., blood vessel ligation associated with electrical stimulation induces a vessel ischemia that alters the muscle distal end only. Damage is worse when electrical stimulation is associated with muscle mobilization. We obtained similar results on transposed gracilis stimulated unilaterally (group 2). In its distal end, the stimulated muscle presents a striking degeneration, in contrast to the nonstimulated distal end, which shows no significant damage. Taken together, these results led us to conclude that muscle degeneration associated with mobilization results primarily from the surgical dissection, whereby collateral blood supply to the gracilis is interrupted, which is exacerbated by long-term stimulation but is not caused by stimulation alone.

These observations are of the utmost importance in human graciloplasty, in which the distal end of the gracilis enmeshes the anal canal or the mobilized colon after abdominoperineal resection. Decrease in the number of muscle fibers or their disappearance alters the dynamic and elastic characteristics of muscle properties in the region where they need to be the most functional. Whatever technique of dynamic graciloplasty is used (single wrap, double wrap, or split sling), the occurrence of ischemia induces damage in the muscle distal end. To maintain blood flow and neoangiogenesis, whose importance have been experimentally shown for preserving muscle structures, 2 improvements for human graciloplasty can be suggested. One concerns low-frequency electrical stimulation before grafting, which has been shown to improve the distal blood flow. In this case, undesirable movements of the leg could be limited by stimulating the not-yet-transposed gracilis at low frequency (2 Hz) and at the threshold of minimal contraction. Another improvement is related to the use of neoangiogenesis-stimulating molecules. In agreement with the latter, it has been shown that treatments with basic fibroblast growth factor, platelet-derived growth factor, or heparin increase the number of muscle flap capillaries and decrease muscle fiber atrophy and fibrosis. These growth factors that improve skeletal muscle function and its survival might then be a useful adjunct for dynamic graciloplasty.

This work was supported by the Ligue Régionale Contre le Cancer, Comités de l’Hérault et de l’Aveyron, France.

Stimulators and electrodes were kindly provided by Medtronic Interstim, Maastricht, the Netherlands.

Reprints: Francis Bacou, MD, PhD, DSc, Laboratoire de Differenciation Cellulaire et Croissance, INRA, 2 Place Pierre Viala, 34060 Montpellier Cedex 1, France (e-mail: bacou@ensam.inra.fr)

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Why Don’t Patients and Physicians Talk About End-of-Life Care? Barriers to Communication for Patients With Acquired Immunodeficiency Syndrome and Their Primary Care Clinicians

J. Randall Curtis, MD, MPH; Donald L. Patrick, PhD, MSPH; Ellen S. Caldwell, MS; Ann C. Collier, MD

Background: Patients with chronic and terminal disease frequently do not talk to their physicians about end-of-life care. Interventions to improve this communication have generally been unsuccessful, suggesting that important barriers to this communication must exist.

Objectives: To determine the barriers to and facilitators of patient-physician communication about end-of-life care and to identify barriers and facilitators that are more common among those patients who are least likely to discuss end-of-life care: minorities and injection drug users.

Methods: We conducted a prospective study of 57 patients with advanced acquired immunodeficiency syndrome and their primary care physicians who were recruited from university and private clinics. Barriers to and facilitators of end-of-life communication were identified from a prior qualitative study and assessed for frequency and importance and for an association with the occurrence and quality of end-of-life communication.

Results: Clinicians identified more barriers than patients. Barriers identified by patients and clinicians fell into 3 categories: education about end-of-life care, counseling to help address end-of-life concerns, and health care system changes to facilitate patient-physician communication. Although none of the patient-identified barriers was associated with the occurrence of communication, 2 clinician-identified barriers were associated with less communication: “the patient has not been very sick yet” and “the patient isn’t ready to talk about end-of-life care.” Nonwhite patients were more likely to identify the following 2 barriers than white patients: “I feel that if I talk about death, it could bring death closer” and “the patient isn’t ready to talk about end-of-life care.” Nonwhite patients were more likely to identify the following 2 barriers than white patients: “I feel that if I talk about death, it could bring death closer” and “the patient isn’t ready to talk about end-of-life care.”

Conclusions: The diversity of barriers and facilitators relevant to patients with acquired immunodeficiency syndrome and their clinicians suggests that interventions to improve communication about end-of-life care must be focused on individual needs and must involve counseling interventions and health system changes in addition to education. Clinician barriers are more common and more strongly associated with the occurrence of end-of-life communication than patient barriers, suggesting that clinicians are an important target group for improving this communication. (2000;160:1690-1696)

Corresponding author: J. Randall Curtis, MD, MPH, Division of Pulmonary and Critical Care Medicine, Harborview Medical Center, Box 359762, 325 Ninth Ave, Seattle, WA 98104-2499 (e-mail: jrc@u.washington.edu).