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, double, triple, and quadruple 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.
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 pellet 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 insertions with nonabsorbable surgical suture ( mortal 1). Gracilis muscles were wrapped around the tube and sutured to each other with nonabsorbable surgical suture (Prolene 5-0).

IMPLANTATION OF THE STIMULATION EQUIPMENT

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 polyglactin 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 to 40 Hz, and those for biochemical analyses were stored at −75°C until assayed.

TISSUE SAMPLING

Muscle fragments were taken from the right middle muscle, the left middle muscle, the right loop, and the left loop after evaluation of muscle contractile properties. Samples for immunohistochemistry were cut immediately, and those for biochemical analyses were stored at −75°C until assayed.

ENZYMATIC CHARACTERIZATION OF MUSCLE METABOLIC PROPERTIES

To detect metabolic transformation of the different types of muscles, the activities of 2 enzymes were assayed because of their importance in the principal metabolic pathways: nicotinamide adenine dinucleotide phosphate isocitrate dehydrogenase (EC 1-1-1-42; ICDH) for the citric acid cycle and fructose 1-6 diphosphate aldolase (EC 4-1-2-13; aldolase) for the glycolytic pathway.

Frozen muscles previously thawed on ice were homogenized in 50-mmol/L ice-cold Tris buffer (pH 7.4, 1:10 dilution). The homogenates were centrifuged at 4°C for 30 minutes at 20000 g, and enzymatic assays were performed on supernatants at 37°C on a recording spectrophotometer (Uvikon 930; Kontron Instruments, Zurich, Switzerland). In addition, ICDH was assayed in 0.1-mol/L triethanolamine buffer (pH 7.5), 52-mmol/L sodium chloride, 4.6-mmol/L d, l-isocitric acid, 0.12-mol/L manganese sulfate, and 9.1-mmol/L nicotinamide adenine dinucleotide phosphate. The kit ALD MPR3 UV-Test (Boehringer, Mannheim, Germany) was used for aldolase determination. Protein concentrations were carried out with a part of the whole homogenates with a protein assay (Bio-Rad DC protein assay; Bio-Rad Laboratories, Hercules, Calif).

EVALUATION OF MUSCLE CONTRACTILE CHARACTERISTICS

After 2 months of stimulation (3 months after operation), rabbits were anesthetized as previously described. The muscles were exposed and dissected free of surrounding tissues. The muscular loop was excised, and its extremity was attached by a wire to a strain gauge transducer that measures the forces exerted by muscle contractions. The force transducer and its electronic amplifier (Captels SA, Saint Mathieu de Treviers, France) were connected to an analog to digital converter (Minipod 100; Krenz Electronics, Paris, France). SoftScope and SoftLogger (Computer Instrumentation Ltd, Krenz Electronics) allowed the recording of data on a PC-compatible computer.

The muscle was stimulated via the implanted stimulator and the stimulating electrode. For studying muscle tetanic fusion, electrical stimulation (pulse width, 210 milliseconds; voltage, 3 V) was performed at frequencies of 5, 10, 20, 30, and 40 Hz. For studying muscular fatigability, the muscle was stimulated during 2 minutes by a cyclic electrostimulation protocol: voltage, 3 V; frequency, 40 Hz; and on/off period, 4 seconds/6 seconds.
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 ethyleneglycoltetraacetic 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 stain 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 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 fluorescence 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 (Stat-View; 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

Figure 1. Contractile properties of transposed unstimulated gracilis muscles recorded at 10 (lower curve), 30 (middle curve), and 40 (upper curve) Hz.

Figure 2. Comparison of contractile characteristics of the right transposed unstimulated gracilis muscle (dotted line) and the left transposed stimulated gracilis muscle (thick line). A, Isometric tension response of right and left gracilis muscle during 30- and 20-Hz stimulation, respectively. B, Fatigue resistance of these 2 muscles.
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, was used as model of 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).

### Table: Aldolase (Glycolytic) and ICDH (Oxidative) Specific Activities of the Transposed Gracilis Muscle Stimulated Bilaterally or Unilaterally

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Aldolase, mU/mg</th>
<th>ICDH, mU/mg</th>
<th>Aldolase-ICDH Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SMp, mean ± SD (n = 4)</td>
<td>479 ± 74</td>
<td>237 ± 25</td>
<td>2 ± 0.52</td>
</tr>
<tr>
<td>Control gracilis, mean ± SD (n = 4)</td>
<td>4472 ± 450</td>
<td>82 ± 7.8</td>
<td>55 ± 7.9</td>
</tr>
<tr>
<td>Unstimulated transposed gracilis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 1 Right</td>
<td>4177</td>
<td>82</td>
<td>51</td>
</tr>
<tr>
<td>Left</td>
<td>3327</td>
<td>55.6</td>
<td>60</td>
</tr>
<tr>
<td>Rabbit 2 Right</td>
<td>4108</td>
<td>53</td>
<td>77</td>
</tr>
<tr>
<td>Left</td>
<td>3358</td>
<td>56.5</td>
<td>59</td>
</tr>
<tr>
<td>Rabbit 3 Right</td>
<td>3644</td>
<td>59</td>
<td>62</td>
</tr>
<tr>
<td>Left</td>
<td>3245</td>
<td>74</td>
<td>44</td>
</tr>
<tr>
<td>Transposed gracilis stimulated bilaterally</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 1 Right</td>
<td>2659</td>
<td>61</td>
<td>44</td>
</tr>
<tr>
<td>Left</td>
<td>1911</td>
<td>244</td>
<td>7.8</td>
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<tr>
<td>Rabbit 2 Right</td>
<td>2508</td>
<td>135</td>
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<tr>
<td>Left</td>
<td>1121</td>
<td>323</td>
<td>3.5</td>
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<td>Rabbit 3 Right</td>
<td>2870</td>
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<tr>
<td>Rabbit 4 Right</td>
<td>743</td>
<td>233</td>
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<tr>
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<tr>
<td>Left transposed stimulated gracilis</td>
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<tr>
<td>Rabbit 1 Right</td>
<td>3145</td>
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<td>Left</td>
<td>447</td>
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</tr>
<tr>
<td>Left</td>
<td>280</td>
<td>293</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* Aldolase indicates fructose 1-6 diphosphate aldolase; ICDH, nicotinamide adenine dinucleotide phosphate isocitrate dehydrogenase. Metabolic properties of the muscles are shown by the aldolase-ICDH ratio: a high ratio indicates a glycolytic metabolism and vice versa. Stimulated muscles are compared with transposed unstimulated and control gracilis. The semimembranosus proprius (SMp; 100% of type I fibers) is taken as a model of oxidative muscle. The aldolase-ICDH ratio of control vs unstimulated transposed gracilis is not statistically different. Ratio of unstimulated transposed gracilis vs transposed gracilis stimulated bilaterally and transposed left stimulated gracilis vs right are statistically significant at P<.05 (nonparametric test of Mann-Whitney).
BIOCHEMICAL CHARACTERIZATION
OF MyHC ISOFORMS

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. 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.

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-

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.
worthy that muscle fibers were atrophied in comparison to right unstimulated gracilis samples.

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 ensheathes 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 neangiogenesis, 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 and neangiogenesis-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.

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REFERENCES

**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-clinician 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 clinicians 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 with the occurrence and quality of end-of-life 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 “I don’t like to talk about the care I want if I get very sick.”

**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 involve counseling interventions and health system changes in addition to education. Clinician barriers are likely to identify the following 2 barriers than white patients: “I feel that if I talk about death, it could bring death closer” and “I don’t like to talk about the care I want if I get very sick.”

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