Chapter 7

Fatigue Resistance in Rectus Abdominis Stomal Sphincters: Functional Results of Two Chronic Studies

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Published in part in:
Introduction

**Muscle Fatigue: The Critical Issue**

Only a few attempts have been made in applying dynamic myoplasty to the problem of stomal incontinence. In 1982 Cavina et al. did the first clinical attempt of using an internal oblique muscle flap for stomal sphincter construction. Later this was followed by two other attempts by Merrel et al. and Konsten et al. in animal experimental studies. All of these attempts failed. Muscle denervation atrophy and muscle fatigue were cited as the major reasons for failure. Based on the cause of these failures we performed a human cadaver study aimed at designing a stomal sphincter from a rectus abdominis muscle (RAM) island flap, taking special care not to denervate the muscle. After accomplishing our goal in this anatomical study we then performed an acute functional study in a dog model. In this study we demonstrated that, when electrically stimulated the RAM stomal sphincter was capable of generating pressures sufficient to maintain stomal continence. Our final set of experiments were designed to investigate methods of minimizing muscle fatigue.

**Past and Current Strategies for Minimizing Muscle Fatigue**

Skeletal muscle is incapable of maintaining a long-term, sustained contraction consistent with stomal continence, without becoming fatigued. In the early days of anal graciloplasty Pickrell et al. introduced a technique that consisted of wrapping the gracilis muscle around the anal canal and relying on voluntarily adduction of the leg to contract the muscle and provide continence. These attempts failed due to gracilis muscle fatigue and resulted in anal incontinence.

In 1981, Salmons et al. demonstrated that chronic low frequency electrical stimulation causes normally fatigable skeletal muscle to undergo a series of morphologic, physiologic, and biochemical changes, resulting in its transformation into nonfatigable muscle. Following Salmons crucial work, a number of investigators in Europe rejuvenated the anal graciloplasty operation by Pickrell et al., specifically, Hallan et al. and Williams and colleagues in England, Baeten and coworkers in The Netherlands and Cavina et al. in Italy. They introduced training regimens for the neo-sphincters with the goal of minimizing muscle fatigue. The results between the different centers vary, however, the addition of electrical stimulation training protocols significantly improved the overall outcomes. Good results were reported in up to 78 percent of patients who have undergone dynamic graciloplasty with continence for both liquid and solid stool. More recently, a multicenter trial (128 graciloplasty patients) showed that overall, 66% of the patients achieved and maintained a successful outcome over the
follow-up period. By etiology, these proportions were 71%, 50% and 66% for patients with acquired fecal incontinence, congenital incontinence, and total anal reconstruction, respectively. Experienced centers had better outcomes and lower complication rates than inexperienced centers. The most common complications described were technical problems with the muscle wrap and with muscle stimulation, perineal infection and infection of the stimulator and leads.

Both in anal and urinary dynamic graciloplasty the ultimate goal is continence. Of the various methods proposed for minimizing muscle fatigue in anal dynamic graciloplasty procedures (training, sequential stimulation and feedback-control), to date only muscle training has been used clinically. Since training the gracilis muscle in these procedures seemed to work for treating anal incontinence, we designed two chronic studies to test whether training would effectively minimize rectus abdominis muscle fatigue in our stomal sphincter application. We tested whether training the RAM sphincter would render it resistant to fatigue and thus provide long-term stomal continence.

Training Protocols in Other FES Applications

Like in dynamic myoplasty the importance of stimulation frequency and stimulation amount for the transformation of fatigue prone muscle to fatigue resistant muscle is a fast-twitch muscle fibers into slow-twitch muscle fibers has been a matter of discussion in other FES (Functional Electrical Stimulation) applications as described in Chapter 3. Pette et al. compared the effect of continuous low-frequency stimulation (10 Hz) and low-frequency stimulation for only 8 hours a day (intermittent stimulation) of the tibialis anterior and extensor digitorum longus muscle in a rabbit model. Changes in fiber type were observed after intermittent stimulation periods exceeding 40 days or continuous stimulation periods longer than 20 days. It was evident that the changes in contraction properties toward slow-twitch muscle fiber type are found both in intermittent and continuous stimulated fast muscles, although the changes proceed faster during continuous stimulation. Hudlicka et al. used an equal number of stimuli per minute, as in low-frequency continuous stimulation experiments, but delivered them in short bursts of high frequency (40 Hz). Although there were some quantitative differences between the contractile properties of the fast muscles subjected to these bursts of high-frequency stimulation and those subjected to conventional low frequency stimulation, the principle outcome was the same, that is, a conversion to slow-twitch characteristics.

There is evidence that the transformation resulting from stimulation at higher frequencies within the physiological range is similar if the same aggregate number of impulses is delivered to the muscle. On the other hand, burst stimulation of this type tends to be more effective in preserving muscle mass.
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and force-generating capacity. The latter is very important for sphincter function. However, burst stimulation can’t be used for the problem of stomal incontinence since sustained contraction is needed.

Training Protocols used in Dynamic Graciloplasty

Different training protocols for anal dynamic graciloplasty have been described in the literature. Williams et al. not only used a protocol with an intermittent pattern of stimulation (10-25 Hz, on-time 4-10 sec, off-time 1-24 sec) but also a protocol with a continuous pattern of stimulation (2 Hz). Seccia et al. did the same but used a stimulation frequency of 20 Hz and an on-off ratio of 2:4 for the intermittent stimulation. For their continuous stimulation protocol (training protocol for eight weeks, with increasing duty cycle every two weeks) they used a frequency of 25 Hz. Besides these differences with Williams et al., Seccia uses direct nerve stimulation in their intermittent stimulated group and intramuscular electrodes in their continuously stimulated group. Williams et al. mention in their study that it was impossible from their study to say whether continuous or intermittent stimulation is the best way to convert the muscle because there were too many variables between the two groups. Seccia et al. should have mentioned this as well since they have at least two substantially different variables (different electrodes with different training protocols). Konsten et al. and Janknegt et al. used an intermittent stimulation protocol for their gracilis neo-sphincter for anal and urinary incontinence respectively. The only difference between their protocols was the duty cycle (percentage of time that the sphincter is on). Although most of the training protocols for dynamic graciloplasty last 8 weeks or longer, shorter training protocols have been investigated. Rosen et al. demonstrated in an animal model that the functional efficiency of a training protocol for a sartorius muscle lasting 5 weeks was as good as the one lasting 8 weeks when using a stimulation frequency of 20 Hz. From all these studies we may conclude that of the different training protocols tested, all seem to work for both the gracilis and sartorius muscle.

One of the many factors that influence the training of muscle is the type and placement of the electrode being used. In the past, both intramuscular and perineural placement of the electrodes have been used by the pioneer centers of anal dynamic graciloplasty with equally good results. In spite of this, the tendency nowadays is to use intramuscular rather than perineural placement of the electrodes. Therefore we initially have used intramuscular electrodes in the acute functional study (Chapter 6) and accordingly in the subsequent chronic study (Part I. Intramuscular stimulation). However, the question rose which training protocol would work for our application.
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Part I. Intramuscular Stimulation

Clinically Used Dynamic Graciloplasty Protocol for Training of the RAM Stomal Sphincter

While reviewing the literature a specific training protocol for dynamic myoplasty for stomal incontinence has not been outlined. Cavina et al. only described the electrical stimulation parameters they used but did not describe how long they trained their muscle sphincter and which training protocol they used.1 Merrel et al. did not train their different stomal sphincter designs. Instead, five months after sphincter creation they simply measured sphincter function.2 Konsten et al. reproduced the same training protocol used for anal dynamic graciloplasty.3 Since this training protocol specified by the FDA for clinical anal dynamic graciloplasty proved to work, we felt justified to use this training protocol for our rectus abdominis island flap stomal sphincter with a slight modification in the pulsewidth. Our pilot studies showed us that a pulsewidth of 270 µsec generated a better contraction (higher magnitude of force while using a lower voltage) in our rectus abdominis muscle sphincter. The purpose of the first chronic study (Part I, Fig. 1) was to define a training protocol that could generate 4 hours of stomal continence for an intraluminal bowel pressure of 60 mm Hg. The first training protocol that was tested was the same protocol used clinically in anal dynamic graciloplasty. In case it was found to be inadequate another protocol had to be designed and tested.

Materials & Methods

Animal Care

Eight Mongrel dogs weighing approximately 25 kg were used in this study. The animals were fed commercial dog diet and provided water ad libitum. This study was performed in the American Association of Laboratory Animal Care (AALAC) approved Research and Resource Center at the University of Louisville Health Science Center. Prior to the experiment, animals were housed in separate cages at a controlled temperature (22°C) and with a 12-hour light/dark cycle daily. They were given Enrofloxacin (5.0 mg/kg body weight, intramuscular, Baytril®, Bayer, Kansas) 30 min before and daily for 5 days after surgery. Animals were preoperatively medicated with Atropine (subcutaneously, 1 ml/10 kg) and anesthetized with intravenous Pentothal (6-12 mg/kg). Following anesthesia these animals were intubated and ventilated with a 2% Isoflurane/ 94% oxygen/ 4% nitrous oxide gas mixture (1 liter/min/kg) to maintain a surgical plain of anesthesia. Dogs were euthanized with an overdose (10 ml, intravenous) of Beuthanasia (390 mg pentobarbital sodium and 50 mg phenytoin sodium per ml) at the end of the study period.
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Figure 1
In the first chronic study (Part I) two different training protocols, Anal Dynamic Graciloplasty (Group A) vs. Revised (Group B), were tested while using intramuscular electrodes. This study revealed that the revised training protocol was far superior. Since fiber recruitment is 100% and stimulation voltage is lower when using nerve cuff electrodes we tested the revised training protocol in combination with nerve cuff electrodes in a second study (Part II).

Flow Chart Chronic Studies

Part I
- Intramuscular electrodes
  - n=8
  - Anal Dynamic Graciloplasty training protocol
    - Group A (n=4)
    - n=4 bad results
    - n=3 good results
    - n=1 malignancy

Part II
- Nerve cuff electrodes
  - Group C (n=8)
  - n=5 failure
  - n=3 good results

- n=2 displaced cuff
  - * 0 weeks
- n=1 kinked cuff
  - * 0 weeks
- n=2 broken lead wire
  - * 0 weeks
  - * 2 weeks
- n=1 investigation
  - 8 weeks
- n=1 broken lead wire
  - * 10 weeks
  - * 14 weeks
The studies were performed under Xylazine (intravenous, 2-3ml/20kg body weight) sedation.

Surgical Procedure
All surgical procedures were performed with accurate maintenance of fluid balance (Lactated Ringer’s, 5% Dextrose 5 ml/h/kg), heart rate and temperature and a sterile surgical technique was used.

With the dog in the supine position, a longitudinal median abdominal incision was made to locate the left RAM. The anterior rectus fascia was incised paramedian. The rectus abdominis muscle was elevated while preserving the integrity of the posterior fascial sheath. Marking sutures were placed at the tendinous insertion and most caudal intersection, so that the muscle could be extended to its original length after detaching it from its distal insertion. The two most caudal nerves and the vascular pedicle were dissected free.

These two nerves were stimulated directly by a bipolar stimulating electrode cuff (model 4080, Medtronic, Inc., Minneapolis, MN). The part of the muscle that contracted when stimulating the most caudal nerve was tailored (4 cm width) into the final flap. The RAM was then transected 13 cm from the pubic symphysis leaving the most caudal nerve and the deep inferior epigastric artery and veins intact. Finally the RAM flap was made into an island flap by dissecting it from its insertion on the pubic symphysis.

Two intramuscular stimulation electrodes (temporary myocardial pacing lead electrodes, model 6500, single lead, Medtronic, Fourmies, France) were used for electrical stimulation of the sphincter. The leads of these electrodes were insulated with silicone tubing filled with silicone (Factor II Inc., Lakeside, AZ) in order to get a watertight seal. Before implantation these electrodes together with a pulse generator (Itrel III, Medtronic Inc., Minneapolis, MN) were immersed for at least 10 minutes in a saline antibiotic solution (Gentamycin 10ml/0.2l). Thereafter the electrodes were placed 1 cm cranial and 1 cm caudal from the nerve entry into the muscle flap after having determined the optimal electrode placement with EMG-electrodes.

The peritoneum was opened to gain access to the distal ileum. An approximately 20-cm segment of distal ileum, Thiry-Vella loop (TV-loop), was isolated. Intestinal continuity was restored by a hand sewn double layer end-to-end anastomosis using 3-0 Vicryl for the mucosa and 4-0 silk for the submucosa and serosa. Thereafter, the RAM island flap was snugly wrapped around the distal end of the TV-loop in which a rubber stent (diameter, 1.0 cm) was placed. The ventral side of the flap became the interior surface of the sphincter. The flap was sutured with Dexon (3-0) mattress sutures to create a sphincter (Fig. 2).

A stoma of the distal end of the TV-loop including the sphincter was matured in the left lower quadrant of the abdominal wall. In the right lower quadrant a conventional stoma of the proximal end of the TV-loop through the RAM was
made (Fig. 2). A Marlex mesh (Davol Inc., Cranston, RI) was tethered over the suture line and around the stoma sphincter to reinforce the abdominal wall. The insulated stimulation electrodes were tunneled to the left flank and connected to a subcutaneously placed pulse generator as previously described. After positioning the stimulator in the subcutaneous tissue, all wounds were closed in layers.

**Figure 2**
Rectus abdominis canine island-flap sphincter design, Thiry-Vella loop and contralateral (control) stoma. (Left) Line drawing representation. The island-flap is created by wrapping the RAM around a blind loop of distal ileum while preserving the deep inferior epigastric pedicle and the most caudal intercostal nerve. (Right) Intraoperative photo of the dynamic RAM island-flap stomal sphincter. The sphincter’s intramuscular electrodes are depicted protruding from the muscle; the vascular pedicle is preserved near the inferior border of the flap.
Training Protocols

A) Training Protocol as Used in Anal Dynamic Graciloplasty

Sphincter Training and Functional Measurements
To allow time for postoperative edema to subside, allow the muscle to become fixed in its new position and the wounds to heal, training was not begun for two weeks after surgery. Initially in 4 dogs (group A) a training protocol (protocol A) was applied as is clinically used in anal dynamic graciloplasty in our hospital (Table 1).

Sphincter function was evaluated every two weeks up to 14 weeks after surgery. During the training period of eight weeks the stimulation voltage was increased, if required, until an intraluminal stomal pressure of 80 mm Hg was measured. The intraluminal stomal sphincter pressure was measured using a microtransducer catheter (Millar®, Millar Instruments, Houston, TX), connected to a computer-based data acquisition system (CED 1401 PLUS interface and a 1902 signal amplifier, Cambridge Electronic Devices, U.K.).

The function of the sphincter was investigated by its ability to stop the flow of saline through the TV-loop while stimulated at a frequency of 25 Hz and a pulswidth of 270 µsec. The proximal (right sided) conventional stoma of the TV-loop was intubated with a latex 22 Fr. Foley® catheter (Bard Urological Co., Covington, GA) and the balloon was inflated to achieve a watertight seal. A y-connector was attached to the Foley catheter. Through one branch of the y-connector the microtransducer catheter was entered to monitor the intraluminal TV-loop pressure. The other branch was connected to a saline infusion system (Fig. 3). The TV-loop was perfused with saline by gravity-induced flow up to a loop pressure of 60 mm Hg. The time from the commencement of stimulation to the return of flow of saline through the TV-

Table 1  Training Protocol A
<table>
<thead>
<tr>
<th>Period (weeks)</th>
<th>0-2</th>
<th>2-4</th>
<th>4-6</th>
<th>6-8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>On time (sec)</td>
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<td>0.2</td>
<td>0.4</td>
<td>1.0</td>
<td>4 hours</td>
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<tr>
<td>Off time (sec)</td>
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<td>1.0</td>
<td>0.7</td>
<td>0.5</td>
<td>15 min</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
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<td>25</td>
<td>25</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Duty cycle (%)</td>
<td>8</td>
<td>17</td>
<td>36</td>
<td>67</td>
<td>100</td>
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<td>Pulse width (msec)</td>
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<td>270</td>
<td>270</td>
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<td>270</td>
</tr>
</tbody>
</table>

* Duty cycle is the % of time over 24 hours during which the muscle is stimulated.
loop by visual control and by registration of a drop in pressure on the computer screen was defined as the continence time.

Results

The results after 8 weeks of training were disappointing with a maximal continence time of only 5 min and 56 sec in one dog after two weeks of electrical stimulation. In the three other dogs of group A, continence times were less at different time-points. The mean continence times ± SEM of group A at 0, 2, 4, 6, 8 and 10 weeks of electrical stimulation were 1.2 ± 0.6 min, 3.6 ± 0.9 min, 2.4 ± 0.6 min, 1.5 ± 0.3 min, 1.6 ± 0.6 min, and 1.3 ± 0.5 min, respectively. Because of these unacceptable results we reduced the

Figure 3

Experimental set-up. The contralateral stoma is intubated with a latex Foley® catheter. With gravity induced flow the stomal sphincter is able to retard flow through its lumen by generating a pressure gradient of greater than 60 mm Hg.
follow-up time from the initially 14 weeks after the operation to 12 weeks (= 10 weeks after start of stimulation).

Discussion

Although the training regimen as used in anal dynamic graciloplasty worked for that application, it did not work for our application. Failure, however, of our stomal sphincter to maintain long-term stomal continence could be attributed to other factors. Malfunctioning could be caused by the stimulation equipment (electrodes, pulse generator) used or related to the muscle sphincter itself (rectus abdominis muscle instead of gracilis muscle). The latter could be a possibility since so far training of a rectus abdominis muscle by means of electrical stimulation has not been attempted clinically. Therefore we chose first to test another stimulation protocol since if that one would work the other causes of failure could be ruled out.

B) Revised Training Protocol

Requirements Training Protocol for Stomal Continence

A stomal sphincter should be able to sustain a contraction for at least 4 hours for an intraluminal pressure of 60 mm Hg. It is not known how much the force will decrease by training a muscle. Maintaining its contraction force is possible by adjusting the stimulation voltage or current, within the limits of what the stimulated tissue can sustain. However, that can’t be done unlimited because at a certain stage it will lead to muscle or nerve damage. Therefore the balance has to be found between the stimulation frequency and the stimulation voltage or current. Another aspect of stimulation that has to be considered is stimulating the muscle for a part of the day or during the whole day. Relaxation of the muscle for a part of the day is less damaging and more physiologic, being comparable with what is done by athletes in endurance training. In addition it is known from stimulation experiments that a regimen in which periods of activity alternate with periods of rest generates a response, which differs in the time course of its component changes from that elicited by continuous activity. For example, after 4 weeks of low-frequency stimulation applied for only 8 hours per day, metabolic changes are well advanced, whereas changes in myosin synthesis, which would be evident after a similar period, are not detectable until a much later stage.

Considering the above-mentioned requirements for a stomal sphincter, a more physiologic training protocol (Table 2) was developed through a collaborative effort with the Cleveland Functional Electrical Stimulation (FES) group. This revised training protocol (protocol B) was applied in four other dogs (group B). The differences between this revised training protocol and the one
clinically used in anal dynamic graciloplasty is that the ‘on’ and ‘off’ time of the sphincter is longer (seconds instead of tenths of a second), that in the beginning of the training protocol the sphincter is stimulated for a certain amount of hours during the day (12 hours (week 0-2) followed by 18 hours (week 2-4) instead of 24 hours) and that the sphincter is trained at a lower stimulation frequency (14 Hz instead of 25 Hz).

Statistical Analysis
Analysis of continence time between groups of animals in protocol A and B were assessed using analysis of variance (ANOVA) for repeated measurements followed by post-hoc-t-tests to determine differences at various time intervals. Results are represented as mean ± SEM. Significance was attributed to \( p \) values < 0.05.

Results
After several weeks of training the sphincters with the revised training protocol encouraging data were obtained. In three dogs of group B a continence time of 4 hours with an intraluminal bowel pressure of 60 mm Hg was reached after 8-10 weeks of electrical stimulation (Fig. 4). One of these three dogs was followed for a longer time. After 16 weeks of stimulation a continence time of 5 hours and 34 minutes was reached. One dog in group B had to be withdrawn from the study after 6 weeks due to the discovery of a visceral sarcoma. Although the numbers in the groups were low, a statistical significant difference in continence time between group A and B at 8 and 10 weeks after electrical stimulation was found (Fig. 4).

### Table 2 Training Protocol B

<table>
<thead>
<tr>
<th>Period (weeks)</th>
<th>0-2</th>
<th>2-4</th>
<th>4-6</th>
<th>6-8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>On time (sec)</td>
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<td>10</td>
<td>10</td>
<td>30</td>
<td>4 hours</td>
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<td>Off time (sec)</td>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>15 min</td>
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<tr>
<td>Frequency (Hz)</td>
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<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Duty cycle* (%)</td>
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<td>18</td>
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<td>24</td>
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<td>Pulse width (msec)</td>
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<td>270</td>
<td>270</td>
<td>270</td>
<td>270</td>
</tr>
</tbody>
</table>

* Duty cycle is the amount of hours during which the muscle is stimulated.
Discussion

After a thorough literature search we found that different training regimens were used in anal dynamic graciloplasty that reached the same endpoint and that seemed to be equally good. In dynamic graciloplasty no animal experimental studies in which different protocols have been tested and compared in the same study have been performed. However, in dynamic cardiomyoplasty this research has been done. Badylak et al. compared the effectiveness of 3 different methods of electrical stimulation for creating fatigue resistance while monitoring the pumping ability of the latissimus dorsi muscle in dogs. They found that one of the training protocols was better in terms of speed of fiber conversion and increased fatigue resistance without the loss of muscle strength. It must be taken into account that this was a relatively short study (6 weeks) and that data that are obtained from clinical studies in general last more then 3 months. The question rises why different training protocols seem to work equally well for anal dynamic graciloplasty and not for dynamic myoplasty for stomal continence? In none of the anal dynamic graciloplasty articles a clear reason is given why they use different protocols.

Figure 4
Comparison of rectus abdominis muscle sphincter Continence Time (hours) between Group A and B at different time points (weeks) after start of electrical stimulation. Data are presented as mean ± SEM (p < 0.05 vs. Group A). The dashed line represents the goal of 4 hours of stomal continence.
Since the stomal sphincter has to maintain a pressure that is able to withhold an intraluminal bowel pressure of 60 mm Hg it is required to adjust the stimulation voltage to a higher level during the training period. This is justified since it is known that the peakforce decreases by training a muscle. However, after the training period we still had to adjust the voltage in order to reach the goal pressure. We attribute this to the fact that prolonged stimulation results in fibrosis. This finding is found by other investigators too.\textsuperscript{16}

Another way of stimulating the muscle is by direct nerve stimulation using nerve-based electrodes. In general a lower stimulation voltage is needed to elicit a muscle contraction and all the muscle fibers that are innervated by the nerve can be recruited. Besides, the use of a lower stimulation voltage lengthens the life span of the implanted pulse generator. Thus, direct nerve stimulation is theoretically a more efficient way of stimulation. Another advantage of direct nerve stimulation is that placement of a nerve cuff electrode is less complicated than an intramuscular electrode and consequently shortens the operation time. Therefore the next logical step was to test the effect of direct nerve stimulation in combination with the revised training protocol and evaluate if this would render even more favorable results.
Chapter 7

Part II. Direct Nerve Stimulation

The long-term effects of using direct nerve stimulation in applications as diaphragm pacing,\textsuperscript{37} dynamic cardiomyoplasty\textsuperscript{38} and sacral nerve stimulation in the treatment of patients with bladder dysfunction\textsuperscript{39} have been promising. By electrical stimulation of the phrenic nerves full-time ventilatory support was accomplished in 13 patients.\textsuperscript{37} The average follow-up time was 26 months and nerve damage from prolonged electrical stimulation has not been a problem. In an animal model Malek \textit{et al.} compared direct nerve (bi-polar nerve cuff electrodes) stimulation with intramuscular stimulation.\textsuperscript{38} Following electrode implantation the latissimus dorsi muscle was chronically stimulated for two months. Their results indicated a tradeoff between the nerve cuff electrode’s lower threshold, higher recruitment, and lower energy consumption at saturation, and the intramuscular electrode’s greater mechanical stability and better long-term reproducibility. In a clinical study Bosch \textit{et al.} continuously stimulated the sacral (S3) nerve as a treatment for urge incontinence.\textsuperscript{39} Immediately after implantation they started to stimulate with $210 \mu\text{sec}$, 10-15 Hz and $2.7 \pm 0.4$ volts. In the 18 patients with an average follow-up of 29 months they reported significant improvement in several urodynamics parameters. They didn’t have any clinical evidence of nerve damage.

In the acute functional study (Chapter 6)\textsuperscript{5} and previous chronic study\textsuperscript{40} (Part I. Intramuscular stimulation) we used temporary myocardial pacing lead electrodes (model 6500, single lead, Medtronic, Fourmies, France, surface area: 8 mm$^2$). We used these electrodes in an attempt to overcome the problem of partial contraction of the sphincter that we saw in a pilot study in which we used the same wire electrodes used clinically in anal dynamic graciloplasty (model 4300, Medtronic, MN, adjustable length up to 4 cm). This was done by placing these temporary pacing electrodes into the muscle flap near the entry zone of the intercostal nerve. While we could recruit a greater part of the muscle flap with these small electrodes, we still were not able to recruit the entire muscle. Partial muscle flap contraction was evidenced by partial contraction of the sphincter during peroperative stimulation. Even though we were able to achieve our goal of 4 hours of stomal continence when using the intramuscular electrodes, the fact we did not achieve full muscle contraction was less favorable. As described in the last paragraph of Part I, we decided therefore to run an additional chronic functional study and switch to direct nerve stimulation. The main purpose of this chronic study (Part II. Direct nerve stimulation, Fig. 1) was to investigate the ability of nerve cuff electrodes to make the stomal sphincter fatigue-resistant in combination with training while using a lower stimulation voltage.
The final objective of this study was as in the former chronic study: 4 hours of stomal continence for an intraluminal bowel pressure of 60 mm Hg.

Materials & Methods

Surgical Procedure
In another group of 8 dogs (Group C), the RAM sphincter was created as detailed previously with the difference in type of electrode being used. After the sphincter was made a tripolar spiral nerve cuff electrode (Axon Engineering, Cleveland, OH) was carefully placed around the intercostal nerve that innervated the sphincter.

Training Protocol
To allow time for postoperative edema to subside, stimulation of the nerve was not begun for two weeks after implantation. After determining the fusion frequency (minimal frequency that results in a fused tetanic contraction), the required voltage in order to maintain continence for 60 mm Hg of bowel (TV-loop) pressure (80 mm Hg of sphincter pressure) and the continence time of an untrained stomal sphincter, the revised training protocol as previously described (Table 2) was started.

Assessment of Stoma Sphincter Function
Sphincter function (continence time) was evaluated every two weeks after surgery, using the fusion frequency. This was done to prevent overstimulation of the nerve. Every two weeks the fusion frequency had to be determined. During the training period of eight weeks the stimulation voltage was increased until an intraluminal stomal pressure of 80 mm Hg or more was measured and continence was achieved for 60 mm Hg of TV-loop pressure.

Results
The sphincters of 4 of the 8 dogs tested did not contract at all at the start of stimulation (0 weeks). The reasons for failure were two nerve cuffs that were displaced from the nerve, one dead nerve because of kinking caused by the nerve cuff electrode, and one broken lead wire of the nerve cuff electrode (Fig. 1). The sphincters of three of the four remaining animals stopped working after 2, 10 and 14 weeks of electrical stimulation (Fig. 1). An autopsy study revealed that the reasons for failure were lead wire breakage of the electrode in all three animals. These results were supported by the fact that all the three nerves of these animals were found to be viable after histological assessment (H&E staining, light microscopy), although there was evidence of
nerve degeneration (vacuolar changes, granulation tissue formation, and compression of the perineural sheet). The sphincter of the dog that was still working after 8 weeks of electrical stimulation was used for investigational purposes. In this dog we found that there was an increase in voltage as a result of increase in resistance. Impedance measurements showed that the increased resistance was a consequence of fibrosis at the level of the contact points of the nerve-cuff electrode and of fibrosis of the nerve.

Functional results could only be obtained from the sphincters of the dogs that were at least working for 8 weeks ($n = 3$). The mean continence times ± SEM of these three dogs at 0, 2, 4, 6 and 8 weeks of stimulation were 1.3 ± 0.4 min, 68.3 ± 10.1 min, 169.6 ± 35.4 min, 240 min, and 240 min, respectively (Fig. 5). One dog of group C reached a continence time of 4 hours (240 min) with an intraluminal bowel pressure of 60 mm Hg after 4 weeks of electrical stimulation while the remaining two reached this point after 6 weeks of electrical stimulation.

**Figure 5**

Rectus abdominis muscle sphincter Continence Time (hours) of Group C ($n = 3$) at different time points (weeks) after start of electrical stimulation. Data are presented as mean ± SEM. The dashed line represents the goal of 4 hours of stomal continence.
Discussion

The functional results of the sphincters of the three dogs that worked for eight weeks or more were promising, leaving the electrode failure aside. However it was not possible to compare the functional results of the study in which we used intramuscular stimulation (Part I.) and this study using direct nerve stimulation due to the fact there were too many differences between the two studies. Nevertheless we did observe that in the direct nerve stimulation study muscle training took a lot less time as evidenced by the fact that 4 hours of sphincter continence was achieved earlier than in the intramuscular stimulation study.

Others have demonstrated that different designs of nerve cuff electrodes resulted in varying stimulation outcomes. A comparative study of 5 different designs of nerve cuff electrodes was undertaken by Loeb et al. to determine their relative merits for stimulating and recording whole nerve activity over extended periods of chronic implantation on peripheral nerves in cats. They found various advantages and shortcomings of the different designs. Only one of these electrodes, when properly installed, showed stable impedances and recruitment thresholds for the duration of 9 weeks. The effect of long-term implantation (307 days) of tripolar split cuff electrodes around peripheral nerves of spinal cord injured patient was investigated by Slot et al. They proved there was no influence on the electrophysiological properties of the nerve.

From our chronic functional study using direct nerve stimulation in combination with the revised training protocol we may conclude that the RAM stomal sphincters are trained faster as opposed to intramuscular stimulation. However, technical difficulties of electrode displacement and lead fracture of the nerve cuff electrode caused poor long-term outcomes. In addition, we may conclude that the results of our two chronic studies indicate a trade-off between the nerve cuff electrode’s higher recruitment, and lower voltage, and the intramuscular electrode’s greater mechanical stability. It is expected that future studies using more sturdy electrode designs will give better functional outcomes in these chronic studies.
Chapter 7

References
Fatigue Resistance: Chronic Studies


Chapter 7


