Chapter 7

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

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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
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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.
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. Merrel et al. did not train their different stomal sphincter designs. Instead, five months after sphincter creation they simply measured sphincter function. Konsten et al. reproduced the same training protocol used for anal dynamic graciloplasty. 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 $\mu$sec 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 ($22^\circ$ 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
- Revised training protocol Group B (n=4)
  - n=3 good results
  - n=1 malignancy

Part II
- Nerve cuff electrodes Group C (n=8)
  - n=5 failure
  - n=2 displaced cuff * 0 weeks
  - n=2 broken lead wire * 0 weeks
  - n=1 kinked cuff * 0 weeks
  - n=1 investigation 8 weeks
  - n=2 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.
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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 pulsedwidth 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>
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<th>Period (weeks)</th>
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<th>4-6</th>
<th>6-8</th>
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<td>25</td>
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<tr>
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<td>100</td>
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<tr>
<td>Pulse width (msec)</td>
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* Duty cycle is the % of time over 24 hours during which the muscle is stimulated.
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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>
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<td>Pulse width (msec)</td>
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* 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.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.
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Part II. Direct Nerve Stimulation

The long-term effects of using direct nerve stimulation in applications as diaphragm pacing, dynamic cardiomyoplasty and sacral nerve stimulation in the treatment of patients with bladder dysfunction have been promising. By electrical stimulation of the phrenic nerves full-time ventilatory support was accomplished in 13 patients. 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 et al. compared direct nerve (bi-polar nerve cuff electrodes) stimulation with intramuscular stimulation. 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 et al. continuously stimulated the sacral (S3) nerve as a treatment for urge incontinence. Immediately after implantation they started to stimulate with 210 µsec, 10-15 Hz and 2.7 ± 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) and previous chronic study (Part I. Intramuscular stimulation) we used temporary myocardial pacing lead electrodes (model 6500, single lead, Medtronic, Fourmies, France, surface area: 8 mm²). 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


Chapter 8

Analysis of Fiber Type Transformation and Morphologic Changes of Small Bowel in Chronic Electrically Stimulated Canine Rectus Abdominis Muscle Stomal Sphincters

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Introduction

Present day research examining the transformation of skeletal muscle through electrical stimulation uses similar concepts to those observed by Salmons et al., who in 1981 used electrical stimulation to transform the metabolic characteristics of skeletal muscle, enabling it to perform more work over longer periods through aerobic metabolism. They showed that skeletal muscle fibers could be transformed from fast twitch (type II) fatigue-prone fibers to slow twitch (type I) fatigue-resistant fibers through training with low-frequency electrical stimulation. Applications involving the training of the graciloplasty and gluteoplasty muscular flaps with low frequency stimulation have improved upon their original design by prolonging the mechanical circumferential force generated by these skeletal muscle flaps making them more clinically useful.

The majority of research dedicated to the functional, metabolic and histologic changes which take place in skeletal muscle has been observed in in-vivo skeletal muscle experiments in small animals. In these models, the skeletal muscle remains at its optimal resting length allowing the actin and myosin filaments to fully interact, generating the most effective amount of force per square area, and thus maximizing the work capacity of the muscle. Additionally, the vascular supply to these skeletal muscle flaps is usually undisturbed with preservation of the muscles main vascular pedicle and its perforators. Understandably, the ability to maintain the optimal resting length of the skeletal muscle may influence the fiber type transformation process. Experimental cardiomyoplasty animal models and human cardiomyoplasty cadaver studies have shown that the complications associated with this procedure may be attributed to the inability to maintain the resting length of the latissimus dorsi muscle after it is wrapped around the heart, along with secondary ischemic muscular changes and the loss of flap innervation.

Other animal models using the graciloplasty have experienced similar problems with respect to disruption of the muscles resting length and distal muscular flap necrosis. One of the potential problems encountered with a functional dynamic skeletal muscle stomal sphincter is the formation of ischemic strictures within the bowel encircled by the muscle flap. Chung et al., have shown in a chronic dog model that ischemia of the bowel around colostomies leads to ischemic bowel strictures. Other investigators have shown that acute ischemic influences on the bowel result in morphologic changes in the bowel layers including loss of the mucosa and a decrement in the muscular layers.

In-situ dynamic flap models such as our island-flap stomal sphincter offer insight in the chronic adaptive changes of the muscle fiber type transformation process caused by chronic electrical stimulation and into
potential problems, which may arise from the dynamic physiologic changes, which take place in the small bowel, encircled by the sphincter. Therefore the purpose of this study was to: 1) determine if fiber type transformation from fatigue-prone (type II) muscle fibers to fatigue-resistant (type I) muscle fibers could be demonstrated in our chronic canine stomal sphincter model where the rectus abdominis muscle (RAM) was used to create a functional stomal sphincter, 2) assess if there was any relationship between the degree of muscle fiber type transformation and the continence times among the two different training protocols as used in Chapter 7, Part I. Intramuscular stimulation, 3) examine the long-term effects of the training regimens on the skeletal muscle fibers through histologic and volume-metric analysis over a flap stimulation period of 12 weeks, and 4) to examine the morphologic characteristics which could take place in the layers of the small bowel wall encircled by our dynamic sphincter.

Materials & Methods

In the eight male mongrel dogs (23-25 kg), as used in Chapter 7, Part I. Intramuscular stimulation, a RAM island-flap stomal sphincter was created, as previously described, by wrapping the left RAM flap around a blind loop of distal ileum (TV-loop), which was no longer in continuity with the rest of the small bowel. Both ends of the blind loop of distal ileum were matured as ileostomies on either side of the animal’s abdominal wall. The island-flap sphincter remained on the left side of the abdomen and was stimulated with two intramuscular electrodes placed around the flap’s intercostal nerve entry point. The sphincters were trained over 12 weeks using two different training protocols as previously described in Chapter 7, Part I. Intramuscular stimulation (Table 1, 2). Muscle biopsies were obtained pre- and post-training from the RAM sphincter. Fiber types within the RAM were assessed with monoclonal antibodies directed against the fast isoforms of myosin. Fiber volume-metric data were obtained after immunohistochemical analysis of the stained fibers with computer scanning software. Fiber histology was examined after staining with Eosin & Hematoxylin and Mason Trichrome stains. Every two weeks sphincter function (continence time) was assessed by its ability to stop the flow of saline (duration of time (minutes)) at an intraluminal TV-loop pressure of 60 mm Hg. At the completion of the study, the right control stoma, the left RAM sphincter-stoma complex and a mid portion of the small bowel wall within the TV-loop were harvested for histologic assessment.
Quantification of Fiber Types and Histology of Skeletal Muscle
Preoperative biopsies were obtained from the untrained RAM for histology and histochemical analysis. The study was terminated at 14 weeks, which was felt to be an adequate time frame to permit fiber type transformation to take place. Post-mortem biopsy samples were collected from the skeletal muscle sphincter 1 cm from the electrode entry point. The untrained and trained RAM biopsy samples for histochemical analysis were frozen in liquid nitrogen and stored at -70°C. At the time of processing, samples were cut in 10 µm sections in a cryostat and incubated with a monoclonal antibody MY-32 (Sigma Chemical Co., St Louis, MO) against the mouse major histocompatibility complex type II. Specimen cross sections were photographed under an Olympus CKZ inverted microscope (Jacob Instrument Corp., Shawnee Mission, KS) at high magnification (100x). A clear plastic grid (5 x 15) cm was placed over the microphotograph prints and the fiber types were counted by two independent examiners. Fibers which fell on the outer grid marks were included as part of the specimen. At least 450 fibers were counted per specimen.

Specimens for histological analysis were fixed in 4% phosphate buffered paraformaldehyde (pH 7.4), paraffin embedded cross sections of 6 µm thickness were cut on a microtome in a cryostat. Specimens were then placed on cover slips and stained with Hematoxylin & Eosin and Mason Trichrome stains.

Volume-metric measurements were obtained after scanning the histochemical fiber prints stained with the monoclonal antibody MY-32 against the fast isoform of myosin into a computer. Sigma Scan Pro Software® Version 5.0 (SPSS Science Co., Chicago, IL) was used to calculate the area within individual muscle fibers.

Histology/Morphometric Measurements of Small Bowel
The island-flap stomal sphincter complex, mid-portion of the small bowel within the Thiry-Vella loop and contralateral (control) stoma were obtained after the training protocol was completed at the time of sacrifice. The tissue specimens were fixed in formalin and paraffin embedded cross sections of 6 µm thickness were cut on a microtome in a cryostat then placed on a glass slide, cover-slipped and stained with Hematoxylin & Eosin and Trichrome stains. Histologic specimens were examined under the supervision of a Board certified pathologist (DA) to determine if architectural changes were present in the specimens. An ocular micrometer was used to quantitate the thickness of the intestinal wall components: mucosa, muscularis mucosa, submucosa, circular and longitudinal muscularis propria.
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Statistical Analysis
Study design was implemented with a pair of groups. A paired t test was used to compare the fiber-type transformation, fiber volume and bowel layer thickness morphology between untrained and trained skeletal muscle. Data are represented as mean ± SEM. Significance was attributed to P values < 0.05.

Results
The percentage of type I and II muscle fiber types was determined immunohistochemically (Fig. 1). After continuous training over 12 weeks using training protocol A, the percentage of type I muscle fibers increased from a mean of 30.8 % ± 1.8 to 73.8 % ± 10.4, while the type II fibers decreased from 69.3 % ± 1.8 to 26.3 % ± 10.4. Training protocol B resulted in an increase of type I muscle fiber transformation from a mean of 39.3 % ± 2.3 to 77.3 % ± 6.8 with a concomitant decrement of type II muscle fibers from 60.8 % ± 2.3 to 22.8 % ± 6.8 (Table 1). Fiber diameters and volume were reduced after the skeletal muscle was trained. Continuous stimulation using training protocol A and B resulted in a decrement of 32% of the total

| Table 1  Percent Fiber Type Transformation in Untrained and Trained RAM Island-Flap Stomal Sphincters |
|--------------------------------------------------|--------------------------------------------------|
| Protocol A Untrained RAM (n=4) | Protocol A Trained RAM (n=4) | Protocol B Untrained RAM (n=4) | Protocol B Trained RAM (n=4) |
| % Type I Fibers | 30.8 ± 1.8* | 73.8 ± 10.4* | 39.3 ± 2.3* | 77.3 ± 6.8* |
| % Type II Fibers | 69.3 ± 1.8* | 7.3 ± 10.4* | 60.8 ± 2.3* | 22.8 ± 6.8* |

*p < 0.05 (untrained vs. trained); Data are expressed as percentage of specimen fiber types ± SEM.

<table>
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<th>Table 2  Volume-metric Measurements of Untrained and Trained RAM Island-Flap Stomal Sphincters</th>
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<tr>
<td>Fiber Volume (Pixel units)</td>
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<td>----------------------------</td>
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<tr>
<td>758.6 ± 17.6*</td>
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*p < 0.01 (untrained vs. trained); Data are presented as mean ± SEM.
fiber area as shown in Table 2. There was a statistically significant difference between the fiber volumes in pixel units between the untrained and trained muscle fibers (P < .001).

The fiber morphology between the untrained and trained skeletal muscle is depicted in Figure 2. In the untrained skeletal muscle specimens, the fibers are polygonal in shape with very little connective tissue or fat in between the fibers. After training, the fiber size (diameter) decreased, the general appearance of the fibers varied little without structural changes and there was

**Figure 1**

Immunohistochemistry of untrained and trained skeletal muscle fibers stained with an antibody against the fast forms of myosin. **A**) Untrained RAM fibers, fibers are predominately of the fast fiber type. **B**) Trained RAM fibers obtained from the island-flap sphincter after 3 months of training, the majority of fibers are of the slow fiber type.
Figure 2
Histology of skeletal muscle fibers (magnification 100x, Bar 0.05 mm). 

A) H&E stain of untrained rectus abdominis skeletal muscle, note large fiber diameter with peripheral cell body nuclei. 

B) H&E stain of rectus abdominis skeletal muscle sphincter biopsy after 3 months of training, fiber diameter is smaller than untrained muscle, nuclei appear crowded due to smaller fiber diameter. 

C) Trichrome stain of trained rectus abdominis muscle sphincter wrap around small bowel, note minimal fibro-fatty deposition between atrophied skeletal muscle fibers.
Analysis of Fiber Type Transformation and Small Bowel Morphology

Table 3  Morphometric Measurements of Bowel Layers

<table>
<thead>
<tr>
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<th>Stoma</th>
<th>Stomal Sphincter</th>
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<tr>
<td>Mucosa</td>
<td>1.04 ± .09</td>
<td>1.16 ± .14</td>
</tr>
<tr>
<td>Muscularis Mucosa</td>
<td>.08 ± .01</td>
<td>.08 ± .01</td>
</tr>
<tr>
<td>Submucosa</td>
<td>.43 ± .08</td>
<td>.30 ± .06</td>
</tr>
<tr>
<td>Circular Muscle</td>
<td>.63 ± .09</td>
<td>.57 ± .07</td>
</tr>
<tr>
<td>Longitudinal Muscle</td>
<td>.25 ± .03</td>
<td>.25 ± .04</td>
</tr>
</tbody>
</table>

* Data are expressed as mean thickness of bowel layers (mm) ± SEM, n = 7.

Figure 3
Small bowel canine biopsies, (magnification x100, Bar 0.10 mm).

A) H&E stain of small bowel biopsy from mid-portion of Thiry-Vella loop with undisturbed bowel layers. B) H&E stain of small bowel biopsy encircled by dynamic sphincter with healthy mucosa and unimpressive changes in the remaining bowel layers. C) Trichrome stain of small bowel encircled by dynamic sphincter without evidence of gross fibrosis of the small bowel layers. D) H&E stain of contra-lateral stoma small bowel layers.
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a minor increase in the endomysial tissue between the fibers. Microscopically there was no evidence of damage to the fibers or degenerative changes. The continence time for the skeletal muscle sphincters measured in minutes (mean ± SEM) for training protocol A averaged 1.3 ± 0.5 minutes at 10 weeks in contrast to the revised training protocol B which averaged 229 ± 10 minutes over the same time period, (P < 0.05). In Figure 4, Chapter 7, a comparison of RAM sphincter continence time (hours) between Group A and Group B at different time-points (weeks) after start of electrical stimulation are depicted. Data are presented as mean ± SEM. (*) p< 0.05 vs. Group A

Morphometric analysis of the individual bowel layers within the Thiry-Vella loop and the sphincter were similar in thickness (p > 0.05) when measured with a micrometer (Table 3). Histologically, the layers of the bowel which were encircled by our dynamic stomal sphincter including the mucosa, muscularis mucosa, submucosa, and muscularis propria were without architectural changes including neither ulcerations nor necrosis. Trichrome stains did not reveal any evidence of fibrosis within the bowel layers (Fig. 3).

Discussion

The dynamic RAM island-flap sphincter designed in this experiment for stomal continence relies upon the fatigue-resistant properties associated with type I skeletal muscle fibers. These fibers have been shown in previous experiments to contain a greater percentage of mitochondria, rely more heavily on oxidative metabolism, contain a higher capillary density, and have reduced glycolytic activity. The above skeletal muscle properties are advantageous with respect to promoting fatigue-resistance when increased demands are placed on skeletal muscle. Our previous experimental work with our dynamic island-flap stomal sphincter created from the RAM showed that the RAM in the canine consists predominately of fatigue-prone (type II) fibers (approximately 65%) with the remaining fibers of the fatigue-resistant type (type I) in origin.

After studying many other skeletal muscle dynamic flap designs we felt that the maximal work potential of our flap could be achieved by: 1. Maintaining the innervation of the flap enabling it to be adequately trained. Previous experiments using a free gracilis muscle flap to achieve stomal continence in a canine model were largely unsuccessful due to flap denervation atrophy which lead to poor flap performance. This concept is also appreciated by other investigators who have attributed the variable results of the cardiomyoplasty to loss of the flap’s innervation distally which impaired the work performance of such flaps. 2. The maintenance of adequate flap perfusion by preservation of the arterial supply and venous drainage to all regions of the flap. Prior research on cardiomyoplasty and graciloplasty have shown that poor perfusion
Analysis of Fiber Type Transformation and Small Bowel Morphology

to the distal part of the flap has a detrimental effect on flap performance and fiber phenotypic changes.\textsuperscript{23,24} 3. Achieving adequate stretch of the flap’s muscular fibers to attain physiologic overlap of the actin and myosin filaments thus optimizing the skeletal muscle’s contractile characteristics. Williams and associates have shown that muscle stretched to its optimal resting length along with physiologic stimulation results in the least amount of connective tissue deposition within the skeletal muscle and leads to improved muscular work capacity and performance.\textsuperscript{25,26}

More recently, investigators have attempted to preserve the relative portion of type IIA intermediate muscle fibers (a subset of type II fibers) which have superior forceful contractile qualities when compared to type I fibers along with acceptable fatigue-resistance properties.\textsuperscript{9,27} Although there was no appreciable difference between the amount of type I fibers transformed using the two different training regimens, the continence times were dramatically different between the two groups when the sphincter was stimulated to contract against an intraluminal bowel pressure of 60 mm Hg. Training protocol A with a mean continence time of 1.3 ± 0.5 minutes was inferior to protocol B’s mean continence time of 229 ± 10 minutes after 10 weeks of training (P < 0.05). One possible explanation for the difference between the two protocol’s continence times is that the relative proportion of type IIB and IIA fibers might have been different between the two training protocols with a higher percentage of type IIA fibers in the animals trained with protocol B. Our monoclonal antibody stain was unable to differentiate between these two types of fibers. Type IIA fibers which exhibit superior fatigue-resistance and adequate contractile properties when compared to type IIB fibers have been shown to be a stable inducible phenotype. The conversion and maintenance of type IIA fibers is highly dependent on the training protocol used.\textsuperscript{9,27} Lower frequency stimulation was found to play a significant role in fiber type conversion and maintenance of type IIA fibers in a study by Sutherland et al.\textsuperscript{27}

In our experimental training protocol B we used an initial lower frequency of 14 Hz during the early stimulation period over 8 weeks when compared to training protocol A with its 25 Hz frequency. This may have influenced the relative proportion of type IIA fibers between the two groups. An additional explanation may be attributed to the relative amount of change and stability of the enzymatic cellular processes present in the muscular fibers oxidative metabolism. Kwong and associates have shown that the inducible enzymes necessary for aerobic metabolism are variable and may proceed the fiber transformation process in skeletal muscle fibers.\textsuperscript{28} The ability of skeletal muscle to maintain a contraction in an efficient manner after transformation of the skeletal muscle’s fiber type is complete, may depend on the relative proportion of key enzymes in the citric acid cycle, fatty acid oxidation pathway and respiratory chain.\textsuperscript{11} Perhaps in our study, both stimulation
protocols were able to transform the phenotypic characteristics of the muscle through changes in the myosin filaments, while protocol B was superior at producing and maintaining a greater proportion of those enzymes needed to generate a more efficient contraction.

The volume-metric data suggests that training resulted in a decrement of muscle fiber size by 32%. The reduction in fiber size may be beneficial through the reduction in the diffusion distance between the capillaries and the cells mitochondria, thus potentially increasing the oxidative metabolic capacity of the muscle. The training protocols used in this experiment were adequate at transforming the fibers from type II to type I fibers, but perhaps unable to maintain the relative percentage of the intermediate type IIA fibers which are generally larger in diameter when compared to type I fibers. Duan et al., have shown that fiber diameter may be influenced by the duration of stimulation and rest used in the training protocols. Intermittent stimulation with more frequent rest may result in fibers more characteristic of type IIA fibers which tend to retain their size and more closely resemble the fast-twitch muscle fibers in diameter, while preserving the oxidative fatigue-resistant properties of type I skeletal muscle.

Histologic analysis of the sphincters also correlates with the volume-metric data results both of which showed a decrease in the fiber diameter resulting in less distance between the fiber's nuclei. The Mason Trichrome stain did not show significant accumulation of fibrotic tissue between the skeletal muscle fibers. Atrophy was apparent without obvious damage to the skeletal muscle fibers. The lack of significant fibrosis between the skeletal muscle fibers may be attributed to the degree of stretch found within the island-flap's wrap and the generous blood supply provided by the deep inferior epigastric artery. Oakley et al., demonstrated in a latissimus dorsi muscle goat model that loss of the flap's resting tension alone seemed to contribute substantially to the degree of muscular flap damage and connective tissue infiltration. When the flap was subjected to a loss of its resting length along with partial devascularization and constant training, the added effect of all three parameters resulted in a higher degree of muscular flap damage with fat and connective tissue infiltration. Similar architectural characteristics have been observed in the latissimus dorsi muscles of cardiomyoplasty patients on post mortem exam with replacement of muscle fibers by fibrofatty tissue. Our model allows the skeletal muscle fibers to remain stretched around the bowel circumferentially. This is unlike the graciloplasty in which the muscle is wrapped around the bowel in a twisted configuration, which may influence the contractile characteristic of the wrap and the degree of fiber stretch and ultimately the deposition of collagen between the fibers.
With prolonged external compression of an intestinal stoma it is reasonable to assume that the sphincter has the potential to cause ischemic trauma to the small bowel that it encircles. Several investigators have noted the influence of an ischemic insult on bowel. Cohen et al., \textsuperscript{31} has shown in a canine model early changes in the bowel layers including hemorrhage into the mucosa, congestion of the mucosa and submucosa with subsequent mucosal sloughing with balloon occlusion of the superior mesenteric artery over 3 hours. Chung and colleagues\textsuperscript{17} have investigated and determined in a canine model that bowel ischemia is proportional to the length of bowel devascularized and this ischemic insult influences the anastomotic diameter of colostomies. In their model the ischemic event lead to changes in the architecture of the bowel including fibrosis of the muscularis and submucosal layers. In our stomal sphincter model the terminal ileal vascular arcade is ligated to accommodate the skeletal muscle wrap thus the bowel within our sphincter must be supplied by intramural collateral flow. Armstrong and associates\textsuperscript{32} have shown in a canine model that ligating the vascular arcade over 4 cm of bowel (as in our model) results in a decrement of blood flow from 32.4 ml/100 gm/min to 15.6 ml/100 gm/min or intramural blood flow falls with the square root of the distance from the inflow source. We suspect that flow to the bowel in our model may be lessened initially. This influenced our decision to begin the stimulation protocol 2 weeks after the creation of our sphincter. However, with chronic flap stimulation we felt that our sphincter could potentially have an adverse effect on the bowel from prolonged compressive flap ischemia. Morphometric and histologic data of the bowel layers did not identify any gross differences between the control bowel and bowel encircled in the stomal sphincters. The thickness of the bowel layers was similar between the two groups. The mucosal layer, which is the most susceptible to ischemic changes,\textsuperscript{31} remained healthy in the sphincter group. Bonakdarpour and associates\textsuperscript{33} have shown in a semi-chronic canine model that when the bowel was subject to an ischemic insult afflicting a 65-105 cm segment of bowel over a 2-3 week period varying degrees of mucosal ulceration and necrosis occurs. In our sphincter model the convoluted bowel architecture of the mucosa was preserved with the normal compliment of crypts and secretory (goblet) cells. There was no evidence of mucosal necrosis or ulceration. The muscularis mucosa was unchanged between the control and sphincter groups and there was no evidence of muscular atrophy within this layer. The submucosal layer which is naturally composed of connective tissue and has been shown in numerous studies to become fibrotic with chronic ischemic insults was also histologically unchanged between the two animal groups in our study.\textsuperscript{17,33} The largest smooth muscle layer of the bowel, the muscularis propria includes the circular and longitudinal muscles. Previous studies in dogs have shown that the cross sectional surface area and thickness of the circular and longitudinal
muscles of the small bowel is parabolic with the greatest thickness of these layers occurring in the proximal and distal segments of the bowel.\textsuperscript{14} We also were unable to detect any significant differences in the thickness within these layers between the control and sphincter groups. These layers may be more resistant to ischemic insults because of the relative thickness of these tissue layers and their close proximity to the mesenteric collateral blood flow. Also, the parallel orientation of the island-flap's muscular sphincter probably exerts a more even distribution of pressure over the bowel lessening the risk of ischemic damage to the bowel wall. This is unlike the gamma wrap of the conventional graciloplasty procedure for fecal incontinence which has been found to produce colonic fistulas from the force exerted by the gracilis tendon along the colonic wall and bowel ischemia.\textsuperscript{15,16}

Conclusion

In conclusion, it is possible to achieve fiber type transformation from fatigue-prone (type II) muscle fibers to fatigue-resistant (type I) muscle fibers in this unique canine island-flap model using the RAM to construct a stomal sphincter. To achieve a successful functional sphincter it is critical to maintain the vascular perfusion and neuronal innervation of the flap, the loss of which may contribute to muscular atrophy and poor flap performance. The training protocol used to convert the skeletal muscle sphincter to more favorable fatigue-resistant properties may profoundly influence the contractile characteristics of the flap while minimally affecting the degree of fiber type transformation. Fiber atrophy, which was seen in this study, is common when skeletal muscle is transformed from type II to type I fibers. Histologically minimal fibro-fatty deposition between the muscle fibers was seen. In addition, our dynamic island-flap sphincter constructed from the RAM and trained over a 3-month period does not result in ischemic damage to the bowel wall that it encircles. There were no significant architectural differences in the layers of the bowel wall encircled by the stomal sphincter, which suggests that the collateral intramural perfusion was sufficient to maintain the integrity of all the bowel layers.
References

Chapter 8


Chapter 9

Summary & Epilogue
Summary
The key elements to creating a continent fecal stoma are voluntary control and a dynamic muscle sphincter that is resistant to fatigue and that is able to generate contraction pressures great enough to prevent the inadvertent leakage of stool. Additionally, the design of the sphincter muscle must be relatively straightforward, require no special devices, and preferably would not involve microsurgical techniques. A few attempts at creating a continent stomal sphincter using dynamic myoplasty have been reported but to date this remains an illusive goal. Denervation atrophy and early muscle fatigue have plagued all reported attempts to make a continent stoma a reality. It was our goal to see if we could make an abdominal stoma continent using dynamic myoplasty. A multiphase project was undertaken that was designed to solve the critical issues of denervation atrophy and early muscle fatigue.

Before starting with the description of the experimental studies, background information was given on the three research fields relating to this thesis, to better understand and approach the problems encountered in the experimental studies. I. Intestinal stomas and their associated problems, with focus on stomal incontinence and its treatment options (Chapter 2). II. Dynamic myoplasty, its clinical applications and the former attempts in applying dynamic myoplasty to the problem of stomal incontinence (Chapter 3). III. Functional electrical stimulation (FES), including the basic knowledge of physiologic and electrical muscle stimulation and with focus on the problem of muscle fatigue and methods of approaching it (Chapter 4).

To solve the problem of denervation atrophy an anatomic feasibility study was undertaken in fresh human cadavers (Chapter 5). This first study was designed to determine which local muscle could serve as an innervated and well-perfused muscle flap. The rectus abdominis muscle (RAM) was found to be ideal. This muscle has a dominant vascular pedicle in the deep inferior epigastric vessels, its elevation can be performed without dividing the intercostal nerves, and the sphincter can be created without the need for a microsurgical anastomosis. Additionally, the muscle location makes creating a sphincter in the lower abdominal quadrants relatively straightforward. Of the two RAM stoma sphincter designs the island flap was found to be superior to the peninsula flap design.

The next phase of the study was to identify an animal suitable for the development of a model for stoma sphincter design. After reviewing several potential animal models and doing several pilot studies, the dog was found to be appropriate. In an acute canine study, it was determined that the RAM island flap sphincter design used in human cadavers could be applied to the dog (Chapter 6). In this study an end ileostomy was created around which the muscle flap was wrapped. Using an electrical stimulation device, the muscle
was able to be stimulated and to generate peak pressures well above 60 mm Hg (pressure needed to maintain fecal continence in humans). Muscle fatigue was found to be directly proportional to the stimulation frequency and continence was provided at all the tested bowel pressures (30, 65 and 100 mm Hg).

These promising acute functional study results paved the way for the initiation of chronic trials incorporating survival operations in dogs. In the first chronic study (Chapter 7, Part I. Intramuscular stimulation), it was revealed that the sphincter design was fatigue-resistant for 4 hours up to three months post-op with one of the two training protocols tested. Although preliminary, a continence time of up to five and one half-hours has been achieved with a pressure in excess of 60 mm Hg in one animal.

In addition, a second chronic study (Chapter 7, Part II. Direct nerve stimulation) was undertaken to test whether direct nerve stimulation, as opposed to intramuscular stimulation, would render more favorable results. Although the numbers were too small there was a tendency that the sphincter could be trained faster with direct nerve stimulation. However, electrode failure (displacement and lead fracture) led to a non-functioning sphincter in 63% of the cases when using direct nerve stimulation.

Analysis of fibertype transformation, as described in Chapter 8, revealed that a significant fiber type conversion was achieved in both training protocols (as used in Chapter 7, Part I. Intramuscular stimulation), with a greater than 50% conversion from fatigue-prone (type II) muscle fibers to fatigue-resistant (type I) muscle fibers without evidence of muscle fiber damage or significant fibrosis. The bowel wall within the functional dynamic stomal sphincter did not exhibit any significant architectural changes related to ischemic fibrosis or mucosal damage. This suggests that our anterior abdominal wall dynamic island-flap stomal sphincter, which generates a contractile force over the bowel wall capable of producing enough stomal pressure to achieve fecal continence, is not intrinsically harmful to the bowel that it encircles.
Epilogue

Fecal and urinary stomal continence remains an illusive goal for the hundreds of thousands of individuals who have to live with the loss of bowel and urinary continuity. The exciting results reported here bring us closer to achieving what has been an objective, stomal continence. By combining a local muscle flap design and dynamic myoplasty technology, impressive continence times have been achieved in our chronic dog model. What remains to be determined at this time is the durability of the design with focus on the stimulation electrodes, the ability of the design to function around a functioning end ostomy, what the potential complication rates may be, and who the best candidates are for this technique. These issues are currently being addressed with ongoing trials in our laboratory. By addressing the aforementioned issues, clinical trials in patients may be forthcoming in the near future.

Ultimately the introduction of a physiologic feedback loop would most closely simulate the anal sphincter. With a feedback system (using bowel pressure sensors to indicate the need to increase muscle tone), the sphincter would adjust the magnitude of its contraction according to the amount of backpressure exerted on the stoma. The sphincter would therefore only contract with a force sufficient to maintain continence at any given moment based on the physiologic demands placed on the stoma sphincter. With a feedback system, muscle work would be reduced and the rate of fatigue minimized. Currently there are commercial products for electrical stimulation with feedback capabilities as well as pressure sensors available. Other options to prolong the durability are to use pulse generators in which one can change the stimulus waveforms to ones that are more favorable to use in terms of muscle fatigue and muscle and/or nerve damage. Taking into account that by training the muscle sphincter the time to relaxation increases, one could consider to introduce a resting period of 0.5 seconds every ten seconds of stimulation. The advantage of this is that the generated force will be still sufficient for maintaining continence but at the same time provides the muscle a period of relaxation. Simultaneously the charge built up at the level of the contact points of the electrodes can be reversed. Both proposals could not be investigated in our studies because of limitations of the pulse generator we used.
Samenvatting

De belangrijkste factoren in het creëren van een continent fecaal stoma zijn vrijwillige beheersing en een dynamische sluitspier die onvermoeibaar is en in staat is drukken te leveren die voldoende zijn om het lekken van ontlasting te voorkomen. Tevens moet het ontwerp van de sluitspier relatief eenvoudig zijn, geen extra attributen vereisen en er moeten bij voorkeur geen microchirurgische technieken voor nodig zijn. In het verleden zijn er een paar pogingen gedaan een continent stoma te creëren door middel van de dynamische myoplastiek, echter tot op heden heeft dit niet geleid tot klinische toepassingen. Spier denervatie atrofie en spiervermoeibaarheid worden beschreven als de oorzaken voor het falen. Het was ons doel om een continent stoma te ontwikkelen door gebruik te maken van de dynamische myoplastiek. Een onderzoeksproject met een reeks aan experimenten werd opgesteld om de problemen van denervatie atrofie en spiervermoeibaarheid te bestuderen en zo mogelijk op te lossen.

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