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Supervised exercise improves cutaneous reinnervation capacity in metabolic syndrome patients

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Abstract

Objective—Unmyelinated cutaneous axons are vulnerable to physical and metabolic injury, but also capable of rapid regeneration. This balance may help determine risk for peripheral neuropathy associated with diabetes or Metabolic Syndrome. Capsaicin application for 48 hours induces cutaneous fibers to die back into the dermis. Re-growth can be monitored by serial skin biopsies to determine intraepidermal nerve fiber density (IENFD). We used this capsaicin axotomy technique to examine the effects of exercise on cutaneous regenerative capacity in the setting of metabolic syndrome.

Methods—Baseline ankle IENFD and 30 day cutaneous regeneration after thigh capsaicin axotomy were compared for participants with type 2 diabetes (35) or metabolic syndrome (32) without symptoms or exam evidence of neuropathy. 36 participants (17 with metabolic syndrome) then joined twice weekly observed exercise and lifestyle counseling. Axotomy regeneration was repeated in month four during this intervention.

Results—Baseline distal leg IENFD was significantly reduced for both metabolic syndrome and diabetic groups. With exercise, participants significantly improved exercise capacity and lower extremity power. Following exercise, 30 day reinnervation rate improved (0.051 $\pm$ 0.027 fibers/mm/day before versus 0.072 $\pm$ 0.030, p = 0.002). Those who achieved improvement in more Metabolic Syndrome features experienced a greater degree of 30 day reinnervation (p < 0.012).

Interpretation—Metabolic Syndrome was associated with reduced baseline IENFD and cutaneous regeneration capacity comparable to that seen in diabetes. Exercise induced improvement in metabolic syndrome features increased cutaneous regenerative capacity. The results underscore the potential benefit to peripheral nerve function of a behavioral modification approach to metabolic improvement.

Keywords

peripheral neuropathy; diabetes; obesity; Metabolic Syndrome

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Introduction

Skin is a dynamic environment in which axons are continuously degenerating and regenerating.¹ Small diameter lightly myelinated and unmyelinated axons in the epidermis are particularly vulnerable to injury, mechanical, toxic or metabolic injury, but they also have greater regenerative capacity than large myelinated axons.² We and others have shown that Metabolic Syndrome and its component insulin resistance, dyslipidemia and obesity are associated with increased risk for distal symmetric sensory polyneuropathy with preferential small fiber loss and neuropathic pain.^{3–6} Animal models of non-diabetic obesity demonstrate similar selective injury to distal small fibers.^{7–10}

In a simplistic sense, neuropathy occurs when the rate of axon loss exceeds regenerative capacity. Careful histological analysis of sural nerve tissue from patients with early diabetic neuropathy shows areas of regeneration (“regenerative clusters”) among unmyelinated nerves¹¹, although compensatory regeneration is outpaced by progressive axon loss. Thus, unmyelinated epidermal axons may be more vulnerable to metabolic injury but more capable of regeneration^{2, 12}. This characteristic may make very early diabetic or prediabetic neuropathy particularly amenable to treatments that enhance regeneration. We hypothesize that Metabolic Syndrome and its component obesity and hypertriglyceridemia impair regenerative capacity and thus independently contribute to neuropathy pathogenesis.

Capsaicin selectively destroys Transient Receptor Potential cation channel V1-positive unmyelinated C-fibers.¹³ Its topical application for 48 hours produces uniform, reproducible cutaneous denervation. Regeneration can be assessed through serial 3 mm punch biopsies to measure intraepidermal nerve fiber density (IENFD). Previous studies using this technique demonstrate reduced regeneration capacity in diabetic patients.¹⁴ Those with neuropathy have even greater reductions in regeneration rate. This technique has been used in clinical trials.^{15, 16} In the current study, a modified capsaicin axotomy technique was used to examine the opposing effects of Metabolic Syndrome and exercise on nerve regeneration in patients without neuropathy symptoms. The study had two objectives: (1) determine if exercise improved nerve regenerative capacity and (2) explore the potential utility of a sequential capsaicin axotomy regeneration model as a novel trial design.

Methods

Participants with Metabolic Syndrome, evenly divided between those with and without type 2 diabetes, were recruited from University of Utah Neurology, Diabetes and Health Network (UUHN) Clinics. Metabolic syndrome was based on the National Cholesterol Education Program Adult Treatment Panel (ATP)-III criteria¹⁷ with modification of the obesity criterion to accept BMI >30 kg/m² to define obesity, as stipulated in the World Health Organization criteria.¹⁸ This decision was based on the challenges of reproducibly assessing waist circumference in obese individuals due to pannus. Potential participants were also identified directly by querying UUHN and Salt Lake City Veterans Administration Medical Center electronic medical and billing records for ICD-9 codes, BMI and selected laboratory values consistent with obesity, hyperglycemia, and dyslipidemia. A list of potential

participants was returned to the referring physician for approval, after which a letter describing the study was sent to the identified patients.

The University Institutional Review Board approved the study, and all participants provided written informed consent. Participants were 21 to 70 years old at study enrollment. BMI, waist circumference, blood pressure, fasting glucose, hemoglobin A1c, lipids, and if necessary a two hour glucose tolerance test following a 75 gram dextrose load, were performed at screening to confirm metabolic status. Use of lipid lowering or antihypertensive agents were accepted as evidence of dyslipidemia (one metabolic syndrome criterion only) and hypertension respectively. This method acknowledges the change in vital signs and laboratory values produced by these medications, and is an accepted means of enumerating metabolic syndrome features in a research setting.^{4, 19}

Previous studies have demonstrated neuropathy is associated with reduced cutaneous regeneration.¹⁴ In this study, potential participants with symptoms or exam evidence of neuropathy were excluded in order to specifically examine if Metabolic Syndrome alone was sufficient to reduce regeneration rate. At screening participants were asked about exclusionary symptoms of distal foot sensory loss, numbness or neuropathic pain, and completed a validated neuropathy questionnaire, the Michigan Neuropathy Screening Instrument (MNSI).²⁰ Two validated neuropathy examination scales, the Utah Early Neuropathy Scale (UENS)²¹ and Michigan Diabetic Neuropathy Scale (MDNS)²⁰ were performed to exclude asymptomatic neuropathy. Participants with neuropathy symptoms or signs were excluded. Participants taking coumadin were excluded because of bleeding risk. Pregnant women were excluded because the dramatic metabolic changes associated with pregnancy would interfere with interpretation of testing. Participants meeting all enrollment criteria completed the study procedures as below:

Capsaicin axotomy and skin biopsies

A detailed description of the capsaicin axotomy technique has been published,¹⁴ and was modified by leaving the capsaicin patch in place for 48 hours, as described below. Using previously described methods, 3 mm punch skin biopsies were sectioned into 50 micron thick sections, and stained with PGP 9.5, a pan-axonal marker that binds to the C terminus of ubiquitin hydrolase.²² IENFD was quantitatively assessed using established criteria by a blinded technician.

At baseline, a 3 mm punch biopsy was obtained from the left distal leg (unless the left leg could not be used due to injury or skin condition) 10 cm proximal to the medial malleolus. Biopsies for the capsaicin axotomy experiment were taken from standardized locations in the same distal/proximal location along the thigh. A baseline biopsy was taken 7 cm proximal to the middle of the superior border of the patella. Following the initial biopsy, a 5×5 cm gauze bandage containing 1.8 g of 0.1% capsaicin was applied 5 cm lateral to the baseline thigh biopsy site. The midportion of the gauze was aligned proximally with the biopsy location, covered with a clear protective overbandage, and allowed to remain in place for 48 hours. Participants were asked to return at the same time of day their patch had been placed to minimize variability in patch duration. A photo of the thigh with the patch applied was obtained to guide future biopsies. Participants were instructed not to remove the patch

and to avoid water immersion. After 48 hours, a repeat 3 mm punch biopsy for IENFD measurement was obtained at the same distal-to-proximal level as the baseline biopsy, 2 cm within the capsaicin patch area. A biopsy was repeated 30 days (+/- 7) later at a location 1 cm lateral to the 48 hour location. Regeneration was measured in two ways; as a fraction of baseline IENFD, and as a cutaneous reinnervation rate calculated as change in IENFD per day compared to 48 hour IENFD. Capsaicin patches and 3mm punch skin biopsy were well tolerated with no reports of significant pain, infection or bleeding.

Exercise intervention

Participants who agreed to join the exercise intervention repeated the MNSI, MDNS and UENS. Nerve conduction studies (NCS) were performed after bringing the tested limbs (usually left, as above) to between 32 and 34 degree C using a Viasys Viking NCS machine (Natus, Middleton WI). Sural and radial sensory responses, tibial and peroneal motor responses, proximal conduction velocities and F-responses were obtained by the Investigators or an American Association of Electrodiagnostic Technicians certified technician.

Participants consenting to the second study phase underwent six months of intensive supervised exercise and exercise counseling with goals modeled on the Diabetes Prevention Program²³; increasing moderate aerobic exercise to at least 150 minutes a week, and normalizing BMI or losing 7% of baseline body weight. The protocol consisted of twice weekly individualized exercise training supervised by a PhD trained Physical Therapist (RM), and administered by exercise physiologists at the Utah Skeletal Muscle Exercise Research Facility. Prior to starting the program, cardiovascular history was reviewed, and each potential exercise participant underwent a standard clinical exercise treadmill test (ETT) supervised by a University cardiologist. Potential exercise participants found to have poor exercise tolerance or ischemic changes on ETT were excluded. Prior to exercise, a Six Minute Walk test was performed over a standardized course.²⁴ Quadriceps isometric peak force (in Newtons) was measured using a KinCom dynamometer (KinCom 500H, Isokinetic International: Chattanooga, TN) and lower extremity extension power (in Watts) was measured using the Nottingham Power Rig (Nottingham, UK).²⁵ Subjects participated in 30–90 minutes of supervised exercise twice weekly, which was supplemented by assigned home exercise, for a total of six months. Exercise consisted of a balanced mix of aerobic and resistance training tailored to the participant's baseline fitness and any orthopedic or pulmonary limitations. Participants were taught to perform home exercise at a moderate exertion level, 11–14 on the Borg "Rate Perceived Exertion" scale,²⁶ and to record exercise in a diary as a counseling aid.

A Masters trained dietician provided individualized nutritional counseling at the start of the intervention based on a baseline three day food diary. Participants were provided with a curriculum of dietary educational material developed for the Diabetes Prevention Program. Dietary counseling was reinforced after three months of intervention.

After three and six months of exercise training, blood pressure, BMI, hemoglobin A1c, fasting glucose and fasting lipids were repeated. Beginning at three months after exercise and nutritional counseling initiation the capsaicin axotomy procedure was repeated on the

contralateral thigh. Clinical scales, NCS, and distal leg biopsy for IENFD were repeated at the completion of the six month counseling and exercise program.

Statistical Methods

Student's t tests were used to compare neuropathy measures, including the rate of reinnervation between participants with and without diabetes. Pearson's correlation coefficients were used to evaluate relationships between Metabolic Syndrome features, neuropathy endpoints and regeneration rate. Paired Student's t tests were used to compare regeneration rate before and after the supervised exercise and counseling program, the primary endpoint of this study. An independent samples median test (Kruskal Wallis Test) was used to compare the change in regeneration rate between participants who achieved improvement in increasing numbers of metabolic features.

Results

67 Participants with Metabolic Syndrome, 35 fulfilling the American Diabetes Association definition of diabetes,²⁷ met inclusion criteria for baseline evaluation and capsaicin axotomy. Mean age was 54.7 ($\pm$ SD 9.8) years, and 58 percent were female. Baseline metabolic and neuropathy characteristics are shown in Table 1. Appropriately, glucose measures were significantly higher for baseline participants with diabetes than for those with Metabolic Syndrome not meeting ADA diabetes criteria. However, other features of Metabolic Syndrome were not different between baseline groups. Participants in the diabetic cohort reported a mean duration of known diabetes of 97 ($\pm$ 57) months.

Age- and sex-specific normal values for distal leg IENFD have been published in an international cooperative study in which the Investigators participated.²² Distal leg IENFD was similarly reduced for both non-diabetic (3.91 $\pm$ 1.74 fibers/mm) and diabetic Metabolic Syndrome participants (3.29 $\pm$ 1.55). Fifty-five percent of nondiabetic and 66% of diabetic participants had reduced distal leg IENFD. Reinnervation rates following capsaicin axotomy at 30 days were similar between the two groups (Table 1). Mean values for NCS measurements were within normal limits, and not significantly different between baseline groups. Baseline IENFD at the distal leg was significantly correlated with baseline fasting glucose (Pearson correlation coefficient 0.358 $p=0.04$), but not with other measures of metabolic function.

Thirty-six Metabolic Syndrome participants (19 with diabetes) entered the second phase of the study. Age, sex ratio, and BMI of Phase 2 participants were not significantly different from those of the baseline Metabolic Syndrome and diabetes groups shown in Table 1. Two participants dropped from the intervention due to injury prior to three months, and two more completed only thirty days of post-intervention reinnervation before dropping from the study, yielding 32 fully evaluable pre- and post-exercise comparisons. For participants as a group, the intervention significantly improved measures of exercise force, power and endurance (Table 2). There was a significant positive correlation between change in lower extremity power and change in distal leg IENFD (Pearson correlation $r=0.529$, $p=0.025$). Features of Metabolic Syndrome also improved over the course of the six-month intervention, though improvement approached statistical significance only for measures of

glucose control, and most of the improvement in glucose measures was driven by diabetic patients. For the diabetic participant subset, HgbA1c declined from 7.4 ± 1.5 to 6.9 ± 1.1 percent.

There was a marked ($>40\%$) and statistically significant improvement in 30 day regeneration rate for lifestyle participants as a group (0.051 ± 0.027 before versus 0.072 ± 0.030 post exercise, $p=0.002$) (Figure 1). 21 Participants improved 30 day reinnervation rate, while 11 failed to improve. There was no significant difference in regeneration rate between diabetic and non-diabetic participants. There was no significant difference in age, sex distribution, or baseline pre- or post-capsaicin IENFD comparing those who improved regeneration rate versus those who did not. For the group as a whole, exam evidence of neuropathy features as measured by both UENS and MDNS significantly improved with the intervention (UENS score 1.49 ± 2.32 before, 0.92 ± 1.79 after intervention). NCS did not change significantly.

In order to examine the relationship between the completeness of post capsaicin degeneration and regenerative capacity, the percentage of baseline IENFD preserved at 48 hours was correlated with the percentage of baseline IENFD recovered at 30 days. There was no difference in 48-hour IENFD residual percentage between baseline and post-exercise trials (24.3 ± 11.2 vs. 23.1 ± 12.7). There was no correlation between the degree of denervation and 30-day regeneration for either capsaicin reinnervation bout (Pearson correlation coefficient 0.254 baseline, and -1.01 post exercise, $p<0.130$ and $P<0.552$).

Prior studies have identified baseline IENFD as a predictor of post-capsaicin regeneration.¹⁵ In this cohort there was a statistically significant positive correlation between baseline IENFD and regeneration rate at baseline ($cc\ 0.362$, $p<0.029$) but not during the exercise epoche ($cc\ 0.264$, $p<0.119$). Conversely there was a negative correlation between baseline thigh IENFD and 30 day regeneration percentage at baseline ($cc\ -0.391$, $p<0.017$) and following exercise ($cc\ -0.458$ $p<0.004$). These mixed results suggest the correlations are due to mathematical effects. Participants with higher baseline IENFD are prone to have a mathematically more robust regeneration rate given the high baseline IENFD, but conversely there may be a ceiling effect to regeneration rate that limits time to recovery to precapsaicin IENFD in those with high baseline IENFD.

The relationship between baseline thigh IENFD and change in regeneration rate and percentage with exercise was examined. There was no correlation between baseline IENFD and change in reinnervation rate ($cc\ -0.051$, $p<0.769$). Examination of scatter plots of baseline IENFD against reinnervation rate and percentage and their change following exercise excluded threshold effects, (i.e. IENFD thresholds beneath which regeneration did not occur or improvement in regeneration capacity did not occur following exercise). There was no significant change in pre-capsaicin IENFD at the distal thigh from baseline to post-exercise (5.35 ± 1.38 vs. 5.50 ± 1.51 fibers/mm $p=0.47$) nor was there a correlation between change in pre-capsaicin distal thigh IENFD and change in regeneration rate following exercise ($cc\ -0.226$, $p=0.19$). These findings indicate that baseline IENFD was not a significant determinant of treatment effect on reinnervation rate.

As anticipated, there was variable compliance. Participants were dichotomized into those who achieved improvement, versus stability or worsening of each metabolic measure. For each measure, there was a higher 30-day reinnervation rate (and percentage, data not shown) for participants who experienced improvement in the measure relative to baseline when compared to those who did not change or worsened (Figure 2). This difference was statistically significant for change in fasting glucose or HgbA1c, but did not achieve statistical significance for other individual Metabolic Syndrome measures. In order to evaluate the relationship between the number of metabolic syndrome features impacted and change in nerve regeneration, the Kurskal Wallis Test (a version of the Mann-Whitney U for use with multiple comparison groups) was used. There was a significant difference in the percentage of baseline IENFD reinnervated when comparing those who improved increasing numbers of metabolic syndrome features ($p < 0.02$). No participant improved all 5 measures (weight, glucose (measured by HgbA1c), HDL, triglycerides and systolic blood pressure) over the six month intervention. Participants who improved 4 metabolic syndrome features had a greater increase in nerve regeneration rate compared to those who improved less than 4 (0.043 ± 0.011 versus 0.015 ± 0.039 fibers/mm/day, $p < 0.004$).

Discussion

Our primary result that participants with Metabolic Syndrome improve cutaneous regeneration rate following intensive lifestyle modification has implications for understanding of early metabolic peripheral nerve injury and utility of the capsaicin axotomy technique as a screen for potential neuropathy treatments. The first phase of the study found that Metabolic Syndrome is associated with reduced distal IENFD compared to age and sex specific normal values, and that participants with Metabolic Syndrome but no diabetes showed a similar reduction in nerve regeneration rate to participants with diabetes. This result adds to accumulated evidence from both human epidemiology and animal studies linking prediabetic insulin resistance obesity and hyperlipidemia with early small fiber predominant neuropathy.^{3, 4, 9, 28–33} This reduced regenerative capacity was present in the absence of neuropathy symptoms.

In this study, a relatively brief but intensive exercise program designed to improve glucose, insulin and lipid metabolism resulted in a clear increase in the ability of cutaneous axons to regenerate following controlled denervation. Previous studies have shown that exercise alone, or in combination with dietary counseling, increases IENFD in patients with neuropathy associated with either prediabetes or early diabetes.^{34, 35} The results underscore the potential benefits and challenges of a behavioral modification approach to metabolic improvement. Less than half of participants lost weight over the six month treatment. While 24 of 32 participants improved at least two Metabolic Syndrome features, no participant improved all five. While the study protocol did not attempt to control changes in medication by the participant's primary care physician that might alter or improve glucose, blood pressure, or lipids, the results suggest that metabolic improvement was, at least in part, the direct result of exercise training and increased fitness. Well controlled human studies have demonstrated exercise training improves insulin sensitivity independent of weight loss, in association with altered distribution of intra-abdominal and intramyocellular lipid pools.^{36–38} Reduction in hemoglobin A1c was the principle identifiable individual metabolic

result of this intervention (suggesting greater insulin sensitivity), and was most strongly correlated with improvement in regenerative capacity.

Marked improvement in insulin or lipid metabolism does not appear to have been a requirement for increased reinnervation rate. While participants who improved many Metabolic Syndrome features showed the greatest gains in reinnervation rate, cutaneous reinnervation increased even for the cohort of participants who improved only one feature. Rather, the prescribed intervention, which focused on exercise, appears to have primarily improved fitness. Analysis of each individual fitness component (Table 2) shows that mean power and force improved significantly, as did Six Minute Walk distance. Unfortunately, a true measure of exercise capacity such as V02Max on an expired gas treadmill exercise test was beyond the means of the study. Nonetheless, this result suggests a salutary effect for exercise, and parallels rodent studies that find C57Bl/6 mice provided running wheel exercise avoid neuropathic pain associated with a high fat diet, even though they show no improvement in Metabolic Syndrome features.³³

Reliance on change in IENFD as the primary endpoint minimizes risk of bias. IENFD is a continuous, quantitative, direct measure of axonal integrity with high between-observer reproducibility.³⁹ IENFD determination by a single technician blinded to the site and experimental condition of the biopsy yields a highly objective and unbiased result. The capsaicin axotomy protocol used in this study differed from that initially developed by Polydefkis *et al*,¹⁴ primarily in that a single capsaicin patch was allowed to remain on the distal thigh for a total of 48 hours rather than being replaced after a day with a second patch. The method reported in this study has the advantages of simplicity and fewer visits for study participants. It also results in less complete epidermal denervation than reported with patch replacement at 24 hours. In this study, mean baseline IENFD within the patch site was 24.1 $\pm$ 11.7 percent of baseline after patch removal. This incomplete denervation does not appear to have influenced results relative to baseline, though it can be expected to have slightly reduced observed reinnervation rate. Reanalysis, excluding results from participants who did not achieve at least 60% denervation, also demonstrates significant improvement in reinnervation rate at 30 days (data not shown). Completeness of denervation was not different between study groups, or following intervention.

Capsaicin axotomy has been used as an outcome measure in a number of pharmaceutical trials^{15, 16}, though to our knowledge this is the first study in which the reported treatment has yielded a significant difference in reinnervation rate. Prior studies used a randomized placebo controlled parallel design, which was limited by significant variability in regeneration rate between individuals. The current method is appealing because it uses each participant as their own control, minimizing variability and enhancing statistical power. As a result, capsaicin axotomy analysis requires fewer subjects for statistical significance than trial methodologies using between-cohort comparisons. Given robust treatment effect was observable at 30 days, this model is also more time efficient than the original 90 day determination published by Polydefkis. Capsaicin axotomy is particularly attractive as a method to perform rapid preliminary evaluations of treatments that may influence distal nerve axon biology. Forty-eight hour application of a single patch, and 30 day rather than 90 day time point would further simplify and speed future trials that employ capsaicin axotomy.

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Figure 1.

Supervised exercise and dietary counseling significantly improved cutaneous reinnervation. (A) Distal thigh (DT) intraepidermal nerve fiber density (IENFD), before capsaicin treatment, was similar at baseline (BL) and following 3 months of exercise (postExerc). 48 hours of capsaicin patch reduced IENFD (B). Following exercise, there was significant improvement in cutaneous reinnervation expressed as a percentage of pre-capsaicin IENFD (B) and reinnervation rate (fibers/mm/day) (C) measured 30 days after completion of capsaicin axotomy. For each figure open markers represent baseline and filled markers represent post-exercise values. Values shown are expressed as mean $\pm$ 1SD, and p values represent t-test for pair wise comparison. N=32.

Figure 2.

Thirty-day reinnervation rate (fibers/mm/day) was greater for participants who improved measures of Metabolic Syndrome compared to those who did not. Participants were dichotomized into those who achieved improvement (unfilled bars), versus stability or worsening (filled bars) of each metabolic measure. Reinnervation rate differences associated with improvement in fasting glucose and HgbA1c were statistical significant by between group t-test comparison. N=32. Values are expressed as mean $\pm$ 1SD. Gluc- fasting glucose, HgbA1c- hemoglobin A1c, BMI- body mass index, TGs- triglycerides.

Table 1

Baseline characteristics of Metabolic Syndrome capsaicin axotomy participants

	MS Alone N=32	MS + Diabetes N=35	Comparison p value
<i>Metabolic</i>			
Age	52.5 +/-11.3	53.9+/-9.0	0.708
Fasting glucose	99.1+/-34.7	134.6+/-57.8	0.008
Hemoglobin A1c	5.9+/- 1.1	7.4 +/- 1.4	0.001
Body mass index	35.2 +/-7.3	36.1 +/-12.2	0.723
Systolic BP	123.9 +/-15.3	124.5 +/-14.6	0.875
Cholesterol	180.6 +/-49.6	167.4 +/-38.4	0.242
Triglycerides	203.6 +/-169	205 +/-141	0.979
HDL	38.9+/-7.7	38.8 +/-10.4	0.999
<i>Nerve function</i>			
UENS score	1.25+/-2.44	1.37 +/-1.46	0.140
Sural amplitude	11.3 +/-5.0	10.9 +/-6.49	0.77
Peroneal amplitude	5.3 +/- 2.5	4.8 +/-2.9	0.46
Peroneal CV	46.4 +/- 5.0	44.4 +/- 5.2	0.12
Tibial F-response latency	51.3 +/- 6.4	53.9 +/- 6.2	0.10
<i>Cutaneous nerve fiber density and reinnervation</i>			
IENFD ankle (fibers/mm)	3.91 +/-1.74	3.29 +/-1.55	0.14
IENFD distal thigh	5.47 +/- 1.55	5.15 +/- 1.52	0.32
48 hr IENFD	1.23 +/- 0.72	1.21 +/- 0.79	0.38
48 hr IENFD percent	22.9 +/- 13.6	23.4 +/- 13.2	0.90
30 day IENFD percent	58.3 +/- 18.1	56.9 +/-18.3	0.77
30 day reinnervation rate	0.062 +/- .041	.057 +/- .037	0.55

Table 2

Change in fitness and metabolic function with intervention

N= 32	Baseline	Six month	Comparison p value
<i>Fitness</i>			
Six minute walk distance (M)	446 +/- 168	522 +/- 65	0.017
Mean force (N)	376 +/- 156	440 +/- 184	0.006
Peak force (N)	447 +/- 172	508 +/- 210	0.007
Mean power (Watts)	236 +/- 111	267 +/- 119	0.002
<i>Metabolism</i>			
Fasting glucose	121 +/-51	112 +/-37	0.30
Hemoglobin A1c	6.66 +/- 1.72	6.37 +/- 1.01	0.21
Body mass index	33.87 +/-7.38	33.76 +/- 7.69	0.72
Systolic BP	123.7 +/-16.3	123.0 +/- 13/5	0.69
Total cholesterol	173 +/- 38	165 +/- 36	0.14
Triglycerides	202 +/-180	164 +/- 113	0.16
<i>IENFD (fibers/mm)</i>			
Distal leg	3.8 +/- 1.8	NA	NA
Baseline distal thigh	5.4 +/- 1.4	5.5 +/- 2.5	0.47
48 Hr post capsaicin	1.3 +/- 0.56	1.2 +/-0.75	0.98
48 Hr IENFD percent	24.3 +/-11.2	23.1 +/- 12.7	0.57