HUM00143541: The effect of high intensity interval training and surgical weight loss on distal symmetric polyneuropathy outcomes

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Protocol Version Date: July 8, 2020

2. SPECIFIC AIMS

Distal symmetric polyneuropathy (DSP) affects nearly 15% of Americans over the age of 40. The prevalence in diabetes, the most common cause, is ~30%, and in the morbidly obese is 23%. Patients with DSP have lower quality of life, more pain, and fall more frequently than those without this disease. In diabetes, lower extremity amputations are 2.6 times more likely in those with DSP. Despite the substantial morbidity associated with this highly prevalent condition, DSP patients have limited therapeutic options. Some medications can reduce pain, but they are not disease modifying treatments. For patients with diabetes, better glucose control results in a large risk reduction of DSP in type 1 diabetes, but large, randomized trials failed to show a statistically significant risk reduction in type 2 diabetes. For obese patients, no proven treatments are available. Therefore, there is a *critical need* to develop disease modifying treatments for DSP patients with diabetes and/or obesity.

Multiple studies reveal an association between the metabolic syndrome and DSP. We further defined the contributions of individual metabolic syndrome components in three separate epidemiologic studies, including our ongoing NIH K23 study. While diabetes is the best established risk factor for DSP, our data support obesity and pre-diabetes as important metabolic drivers of nerve injury. The most effective therapies for obesity and pre-diabetes are exercise and/or weight loss. While the combination of these two vastly different interventions is likely optimal, each requires a life-altering change in behavior. Therefore, the *primary objective of this application* is to determine the independent and combined effects of exercise and surgical weight loss on DSP outcomes. Our *central hypothesis* is that exercise and surgical weight loss will both improve DSP outcomes, but the effect of exercise will be significantly larger. The *rationale* for this hypothesis is our preliminary data revealing stable DSP outcomes 2 years after diet-induced weight loss (the natural history is worse DSP outcomes over time), while other groups show a substantial improvement in DSP outcomes after exercise in subjects with minimal weight loss. Limitations of previous work include the lack of a comparison group for the intervention. Likewise, the effect of surgical weight loss on DSP has not been rigorously tested.

We propose to study the effects of a randomized exercise intervention on DSP outcomes (Aim 1). We will recruit patients from a Bariatric Surgery clinics. Patients will be recruited after their visit with the physician's assistant and/or surgeon. We will stratify randomization of the exercise intervention by those that do and those that do not have bariatric surgery to allow evaluation of the effect of surgical weight loss on DSP outcomes (Aim 2). For the exercise intervention, we will employ the *innovative* high intensity interval training (HIIT) regimen, which our group has shown improves metabolic outcomes and has high compliance. For weight loss, bariatric surgery results in larger and more sustained weight loss compared to dietary approaches, making this an ideal intervention to investigate. Similar to HIIT, surgical weight loss improves metabolic outcomes and has high compliance.

Aim 1: Examine the efficacy of a HIIT exercise regimen on DSP outcomes (randomized). We hypothesize that HIIT will improve DSP outcomes compared with routine exercise counseling. To test this hypothesis, we will randomize patients approved for bariatric surgery clinic 1:1 to HIIT versus routine exercise counseling. The HIIT regimen will consist of 3 sessions/week (2 supervised and 1 unsupervised) for 24 months. Routine exercise counseling is the current real world practice in the bariatric surgery clinic. The primary DSP outcome will be intraepidermal nerve fiber density (IENFD) of the proximal thigh measured at baseline, 3, 12, and 24 months. Linear mixed models will determine the longitudinal effect of HIIT on DSP while accounting for repeated measures. Importantly, we will capture a sensitive, quantitative DSP measure (IENFD), determine if an intervention effect exists, and link the measures to meaningful patient-oriented outcomes such as DSP-related pain (Short form McGill), function (Berg Balance Scale), and quality of life (NeuroQOL).

Aim 2: Explore the impact of surgical weight loss on DSP outcomes (non-randomized). We hypothesize that bariatric surgery patients will have better DSP outcomes compared to patients without surgery, but the effect will be less than the effect of HIIT. To test this hypothesis, we will stratify the HIIT randomization in Aim 1 1:1 for those that have and those that do not have surgery. This will allow us to explore the effect of surgical weight loss on DSP outcomes without requiring an expensive, randomized, surgical intervention trial. Linear mixed models will determine the longitudinal effect of surgical weight loss on DSP. We will also assess the interaction between HIIT and surgical weight loss to evaluate the combined effect of these two interventions.

Impact: We will determine whether HIIT and/or surgical weight loss are promising disease modifying treatments for DSP. If successful, our phase 2 trial will form the basis of a large, pivotal phase 3 trial of exercise and/or weight loss for the treatment of DSP. Either would be the first disease modifying therapy for this common, highly morbid condition.

3. RESEARCH STRATEGY

A. Significance

A1. Distal symmetric polyneuropathy (DSP) is common, disabling, and untreatable. Peripheral neuropathy affects more than 2% of the population, with DSP representing the leading neuropathy subtype.[1] The prevalence of DSP increases dramatically in older populations to ~15% in those over the age of 40.[2] In a nationally representative Medicare population, DSP patients reported a significantly lower quality of life, increased falls, and more pain than a propensity score-matched control group.[3] DSP patients were also more likely to have trouble with sleep, activities of daily living, depression, and fatigue. Diabetes is the most common cause of DSP, accounting for 32-53% of all cases.[4-6] In the U.S., DSP from diabetes is a significant public health burden, serving as the leading cause of diabetes-related hospital admissions and non-traumatic amputations. Unfortunately, few treatments exist for DSP patients with diabetes and/or obesity. An American Academy of Neurology systematic review found that several treatments decrease pain in this population.[7] Disappointingly, none are disease modifying therapies. While improved glucose control is a potential disease modifying therapy, the results are quite disparate in patients with type 1 and type 2 diabetes. In type 1 diabetes, enhanced glucose control dramatically reduces the incidence of DSP (78% relative risk reduction).[8, 9] Conversely, in type 2 diabetes, enhanced glucose control only slightly reduces the risk of developing DSP (5-9% relative risk reduction), which is not statistically significant in a meta-analysis or any individual study.[10, 11] This discrepancy highlights the difference between these two conditions and emphasizes that many patients with type 2 diabetes develop DSP despite adequate glucose control. Therefore, factors besides hyperglycemia are likely involved in the pathophysiology of DSP in type 2 diabetes. Our recent work, in conjunction with past studies, provides evidence to support the metabolic syndrome (MetS) and its individual components (obesity, pre-diabetes or diabetes, low high-density lipoprotein (HDL) cholesterol, elevated triglycerides, and elevated blood pressure) as important risk factors for DSP. Given the current absence of disease modifying therapies for DSP, there is a critical need to identify new interventions.

A2. MetS is associated with DSP, with diabetes, pre-diabetes, and obesity representing the primary drivers. Multiple studies demonstrate an association between the MetS and DSP.[12-16] However, until recently, the precise metabolic drivers within this syndrome were unknown. In our ongoing NIH K23 study (NS-079417), we performed comprehensive metabolic and DSP phenotyping in 102 obese subjects and 53 lean controls.[17] We found that diabetes and obesity were independently associated with DSP (**Table 1**). Furthermore, pre-diabetes approached statistical significance, with an odds ratio similar to that of diabetes. The prevalence of DSP increased from 3.8% in lean controls to 11.1% in normoglycemic obese controls, indicating that the effect of obesity alone is substantial. The prevalence further increased to 29% and 34.6% in those with pre-diabetes and diabetes, respectively. The main difference between those with diabetes and pre-diabetes was the severity of DSP. We also demonstrated that DSP in this obese population resulted in worse nerve conduction studies (NCS), lower intraepidermal nerve fiber density (IENFD), worse DSP-specific quality of life, and higher pain scores compared with obese patients without DSP. These findings highlight the severity of DSP in this obese population. Even the obese patients without DSP had statistically significant lower IENFD than lean controls. Given that these obese participants also had lower DSP-specific quality of life and higher pain scores, the lower IENFD is likely clinically relevant even in those not meeting a clinical definition of DSP. In addition to this study in middle aged obese individuals, we also addressed similar questions in an elderly, population-based cohort (N=2,382) from the Health, Aging, and Body Composition Study.[18] We found that diabetes and obesity were the metabolic syndrome components most often associated with our primary and secondary DSP outcomes. Moreover, we demonstrated that DSP is more common as the number of metabolic syndrome components increases, independent of glycemic status. Finally, our unpublished data from a Chinese population based cohort (N=4,001) also demonstrates that diabetes and obesity are independently associated with DSP. Furthermore, the prevalence of

Table 1: The association of metabolic syndrome components with DSP

Variable	OR (95%CI)
Age	1.09 (1.02,1.16)*
Male (reference female)	0.70 (0.12,4.00)
Height (5 cm)	1.12 (0.75,1.69)
Glycemic status Pre-diabetes Diabetes (reference normal)	 3.82 (0.95,15.41) 4.90 (1.06,22.63)*
Waist circumference (5 cm)	1.24 (1.00,1.55)*
SBP (10 mm Hg)	0.98 (0.66,1.46)
Triglycerides (50 mg/dL)	1.03 (0.72,1.48)
HDL (10 mg/dL)	1.31 (0.74,2.32)

Table 2: Prevalence of DSP in a Chinese cohort using three different definitions

DSP measure	Total	Normoglycemia	Pre-diabetes	Diabetes	P value
MNSI Examination	272 (6.8%)	48 (3.3%)	110 (6.3%)	114 (15.1%)	<0.01
MNSI Questionnaire	93 (2.3%)	18 (1.2%)	29 (1.7%)	46 (6.1%)	<0.01
Monofilament	252 (6.3%)	55 (3.7%)	128 (7.3%)	69 (9.1%)	<0.01

DSP increases significantly in those with pre-diabetes and in those with diabetes (**Table 2**). In this patient population, the number of metabolic syndrome components, independent of glycemic status, is also associated with DSP. Another group has reported similar findings in a separate area in China.[19, 20] Taking our three studies together, the main metabolic drivers of DSP are highly likely to be diabetes, obesity, pre-diabetes, and the number of metabolic syndrome components. Proposed interventions should target these metabolic factors.

A3. Weight loss and exercise are the most widely accepted treatments for obesity and pre-diabetes. The 2013 American College of Cardiology/American Heart Association guideline for the management of overweight and obesity in adults recommends diet, lifestyle intervention including increasing physical activity, and bariatric surgery.[21] Notably, this guideline does not recommend pharmacologic therapy for obesity.[22] While pharmacologic therapies reduce weight,[23] the discontinuation rates for these medications are quite high, the evidence is only for short term outcomes, and most patients regain weight after stopping these therapies.[24] Therefore, diet-induced weight loss, surgical weight loss, and exercise are the most widely accepted treatments of obesity. In terms of pre-diabetes, the Diabetes Prevention Program trial demonstrated that an intensive lifestyle intervention was superior to metformin and also superior to standard lifestyle recommendations in the prevention of diabetes.[25] The intensive lifestyle intervention consisted of a diet with a goal of 7% weight loss and moderate intensity exercise for 150 min per wk. Therefore, a combination of weight loss and exercise is the most widely accepted treatment of pre-diabetes.

For our proposed study, we chose to determine the effect of exercise and surgical weight loss on DSP outcomes based on our preliminary data and previously published studies (**see C1.2.1**). For the exercise intervention, we will employ the innovative high intensity interval training (HIIT) regimen because of the high compliance rate, particularly in those with obesity (**see C1.2.2**). In addition to the HIIT intervention, we have the opportunity to explore the effect of surgical weight loss on DSP outcomes by recruiting patients from bariatric surgery clinics, including those who do and those who do not have surgery (**see C2.2.1**). We chose to recruit a bariatric surgery population rather than a medical weight loss population because surgical weight loss yields robust and sustained weight loss, even when following patients for as long as 10 yr after surgery, whereas medical weight loss is often temporary.[26]

A4. Significance. This study is *highly significant* because: 1) either HIIT or surgical weight loss would provide the first disease modifying therapy for DSP other than glucose control for those with diabetes; 2) these interventions would be the first available treatments for obese DSP patients without diabetes; 3) the results will lead to a phase 3 study to determine the best treatment of DSP so that physicians will no longer have to recommend two disparate life altering interventions at once; 4) the study will provide data on the risks and benefits of bariatric surgery for DSP; and 5) a positive study would provide further evidence to support diabetes, pre-diabetes, and obesity as the main metabolic drivers of DSP. Furthermore, while weight loss and exercise are currently recommended for all patients with obesity and/or diabetes, much remains to be learned about the specifics of each of these interventions and their effects on DSP. First, few obese subjects participate in the recommended diet and exercise regimens; therefore, effective interventions that have high compliance are needed. Unfortunately, we do not even know which of these two life altering interventions (weight loss and/or exercise) to focus on. Second, we do not know how aggressive to be in pursuing weight loss, such as when to recommend bariatric surgery for patients at risk for DSP. Third, the effect of HIIT on DSP is unknown, and our results will potentially lead to more specific exercise recommendations. Finally, our consultants will be able concentrate on the underlying mechanisms of exercise and/or weight loss on preventing DSP depending on which is clinically effective. Importantly, our research will eventually lead to more specific recommendations rather than the current broad statements regarding weight loss and exercise.

B. Innovation

The proposed trial is *highly innovative* and represents a paradigm shift in current treatment strategies for the prevention and treatment of DSP for patients with and without diabetes. We strongly contend that the diabetes research community must move beyond glucose control alone to more comprehensive treatment approaches for diabetic complications. Moreover, a successful trial would bring us one step closer to a desperately needed intervention for patients without diabetes, including the large obese population, to prevent and improve DSP. The *innovation* in this proposal is high and includes: 1) an exercise regimen (HIIT) that is time efficient, yields improved metabolic health even after short term participation, and is better tolerated with higher compliance in an obese population than conventional exercise; 2) a study design that allows comparisons between exercise, bariatric surgery, and the combination of these interventions without the need for an expensive, randomized surgical intervention phase 2 trial; 3) a main outcome measure (IENFD), which allows sensitive and objective

quantification of nerve fiber loss and regeneration over time, with true potential to capture early changes of DSP; 4) comprehensive patient-oriented outcomes, including multiple measures of DSP-related pain, daily function, and DSP-specific quality of life outcomes; 5) the use of a non-invasive measure of DSP, corneal confocal microscopy (CCM), to compare with the primary outcome of this trial, IENFD, to inform future clinical trials (**see C.3.4.2**); and 6) an analysis plan that investigates the potential modifying effects of sex on DSP outcomes, the differential effect of sleeve gastrectomy versus Rou-en-Y gastric bypass on DSP outcomes, and the weight loss independent effects of bariatric surgery by incorporating early outcome measures.

C. Approach

Figure 1: Clinical trial design summary for Aims 1 and 2.

The clinical trial design summary for Aims 1 and 2 is shown in **Figure 1**. All patients will be recruited from a Bariatric Surgery Clinic, including those that do and those that do not have surgery. We will randomize patients 1:1 to either the HIIT exercise regimen or routine exercise counseling. Randomization will be stratified 1:1 for those that do and those that do not have surgery. We will also stratify by glycemic status to limit confounding. Randomization will be performed using permuted blocks, with a block size known only to the statistician. We will perform longitudinal analyses using linear mixed models to study the evolution of IENFD from baseline to 2 yr (4 measures in total). We will compare the effect of HIIT, bariatric surgery, and the combination of HIIT and surgery on all outcomes.

C1. Aim 1: Examine the efficacy of a HIIT exercise regimen on DSP outcomes (randomized)

C1.1. Introduction: There is an urgent need for a disease modifying therapy for the prevention and treatment of DSP. Currently, physicians only have neuropathic pain medications and glucose control to offer patients with DSP.[7, 27] However, glucose control alone is not effective at preventing DSP and many patients with diabetes develop DSP despite good glucose control.[27] Similarly, no disease modifying interventions exist for the increasing population with pre-diabetes and/or obesity. Thus, it is essential to identify new interventions that can prevent and treat DSP. To date, uncontrolled studies support improvement in the natural history of DSP decline over time after an exercise intervention.[28-30] The next step is to determine whether a randomized, intensive, exercise intervention improves DSP outcomes compared to routine exercise counseling.

C1.2. Justification & feasibility:

C1.2.1 Justification for the hypothesis that exercise is likely to improve DSP outcomes: Three uncontrolled studies have shown the potential for exercise to improve DSP outcomes with lifestyle interventions that primarily focused on exercise without significant weight loss.[28-31] One study followed 32 patients with DSP caused by impaired glucose tolerance and measured IENFD in skin biopsies at the proximal thigh after 12 mo of a lifestyle intervention.[30] The intervention consisted of individualized diet and exercise counseling; however, the BMI of participants only decreased by an average of 1.1 kg/m^2. Despite the minimal weight loss, IENFD levels significantly increased by 1.4 fibers/mm, in spite of the known natural history of decline over time. The improvement in IENFD also significantly correlated with decreased neuropathic pain, which indicates that this IENFD increase is likely clinically relevant to patients. Similarly, another study followed 36 patients with diabetes and/or the metabolic syndrome and determined the cutaneous nerve regenerative capacity (measured by IENFD) after 4 months of a lifestyle intervention.[29] The intervention was 30-90 min of supervised exercise twice weekly that was supplemented with home exercise. Dietary counseling was only provided twice

and the BMI decreased by an average of only 0.11 kg/m^2. Following the exercise intervention, the cutaneous nerve regenerative capacity increased from 0.051 to 0.072 fibers/mm/day (p=0.002). Notably, those with improvements in more metabolic syndrome components had a greater increase in cutaneous nerve regenerative capacity (p<0.012). A third study followed 17 patients with DSP caused by diabetes before and after 10 wk of aerobic and strengthening exercise.[28] They found that intraepidermal nerve fiber branching at the proximal thigh was significantly improved after exercise (0.11 branch nodes/fiber, p=0.008) despite no change in BMI. Furthermore, pain and neuropathic symptoms were significantly reduced. IENFD at the proximal thigh also improved, although this result did not quite meet statistical significance (1.68 fibers/mm, p=0.09). All of these studies show the promise of exercise regimens to improve IENFD outcomes without significant weight loss, but importantly none had a control group. Finally, a small randomized trial of a mixture of patients with type 1 and type 2 diabetes revealed improvements in some NCS parameters and vibration perception thresholds after 4 yr of an aerobic exercise regimen.[31] BMI changed only a little over the 4 yr, and IENFD was not measured in this study. Limitations of this study include the lack of designated primary and secondary outcomes, no blinded outcome assessments, inclusion of type 1 and type 2 diabetes, unequal randomization of the population, and no patient oriented outcomes. Of note, exercise interventions in mouse and rat models of obesity and diabetes also support the positive effects of exercise on DSP.[32, 33]

In addition to the above exercise studies on DSP outcomes, our current NIH K23 study is following 130 obese participants for 2 yr following a diet-induced weight loss intervention with limited exercise counseling.[17] Our preliminary data reveal that IENFD increased by only 0.2 fibers/mm at both the distal leg and proximal thigh despite an average improvement in BMI of 3.67 kg/m^2. This is in contrast to the two previously mentioned exercise trials demonstrating IENFD improvements of 1.4 and 1.68 fibers/mm after 1 yr and 10wk respectively.[30] Taken together, these studies support our hypothesis that exercise is the most likely intervention to improve DSP outcomes. Given the natural history of IENFD decline over time, diet-induced weight loss is also likely to improve DSP outcomes, but not to the same degree as exercise. Importantly, the effect of surgical weight loss on IENFD is unknown.

C1.2.2 Justification for high intensity interval training (HIIT): The ideal exercise regimen would **improve metabolic outcomes** and have **high compliance** in the short and long term. We believe that HIIT is that regimen. Co-investigator Jeff Horowitz, PhD, and consultant Jonathan Little, PhD have been involved in 5 studies in obese populations that support an improvement in metabolic outcomes and/or high compliance with HIIT. Specifically, one study involved obese adults (n=11; body mass index (BMI): 32±1 kg/m^2) completing either a 2-wk program of HIIT (10 x 1 min @ 90% heart rate (HR) max; n=5) or moderate-intensity continuous training (MICT) (50 min @ 60-70% HR max). They measured fasting blood glucose and insulin concentration 3 d after the last exercise training session to calculate Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) as an assessment of whole-body insulin resistance. Three days after the last training session, HOMA-IR was lower than pre-training values in all HIIT subjects, but not in the MICT group. As a result, the reduction in HOMA-IR was significantly greater in HIIT vs. MICT (p<0.01). These findings agree with several previous studies that used more direct assessments of insulin sensitivity (e.g., clamp, glucose tolerance tests (GTT)) to demonstrate that, unlike conventional exercise training, HIIT induces a persistent improvement in insulin sensitivity.[34-37] Similarly, another study enrolled type 2 diabetic patients (n=8; BMI= 32±6 kg/m^2; 2h GTT >200 mg/dl) in a 2-wk HIIT protocol, with each session identical to the HIIT regimen proposed in this application (10 x 1 min at ~90%; 25 min total exercise time).[38] All participants completed the entire HIIT protocol without any complications. After 2 wk of HIIT (without weight loss and measurements made 3 d after exercise), hyperglycemia was reduced by nearly 15% as measured by 24 hr blood glucose concentration. Perhaps more importantly, HIIT reduced post-prandial blood glucose concentrations by ~30%, which was highlighted by a marked suppression in blood glucose concentration after meals as measured by a continuous glucose monitor. A third study randomized 53 participants with diabetes to 2 different dietary interventions or placebo.[39] All participants underwent 12 weeks of HIIT. The mean BMI in this population was 35±7. Continuous glucose monitoring, HA1C, percent body fat, lean mass, VO2peak, and endothelial function were all improved over time after HIIT. Importantly, 96.2% of participants completed all HIIT sessions in 12±1 weeks. Another study involved 10 overweight or obese individuals performing HIIT and continuous moderate intensity exercise seven days apart to compare the effects of these regimens on post-prandial glucose.[40] The mean BMI in this population was 36±7. HIIT had greater and longer lasting effects on post-prandial glucose compared with continuous moderate intensity exercise. All participants were able to complete the HIIT exercise sessions without difficulty. These data indicate that HIIT can indeed lead to meaningful improvements in metabolic health, and that HIIT is safe and well-tolerated in obese and type 2 diabetic patients. Notably, Dr. Little's group

also has data supporting that compliance to HIIT is higher than conventional regimens. Specifically, his group randomized 32 obese, pre-diabetic men and women (BMI: 33.1±7.7 kg/m^2; HbA1C: 5.7±0.1%) to either HIIT (10 x 1 min @ 90% HR max; n=15) or MICT (50 min @ 60-70% HR max; n=17).[41] Subjects self-selected their mode of training (e.g., walk/run, elliptical machine, stairs, bicycle). After a 2-wk familiarization period of supervised training, subjects continued their assigned exercise program on their own, 3 times per wk for 4 wk. Compliance was monitored using accelerometers and training log books. They found that compliance in this obese, pre-diabetic population was higher in those with HIIT compared with MICT (89±3% vs. 71±8%, p=0.05). Along with the metabolic improvements noted above, the improved compliance in those performing HIIT compared to MICT is a major reason for choosing this as the exercise intervention. Of note, all five of the above studies were well tolerated in obese participants (total N=91) including 34 with BMI>35, 23 of these with BMI>40. Furthermore, a recent meta-analysis identified 9 additional studies using HIIT as an intervention in obese populations with a total of 112 participants.[42] Two of these studies had mean BMIs above 35 (35.7 and 36.6).[43, 44] The lowest compliance in these 9 studies was 71% with most reporting 80% compliance or better.[42, 45] Overall, these data support HIIT as an innovative, safe, and well tolerated intervention to improve metabolic outcomes with high compliance even in those with very high BMI.

Table 3: Population Demographics

	Bariatric (n=71)	WMP (n=125)
Age, mean (SD)	45.5 (11.1)	52.8 (10.9)
Female	53 (74.7%)	68 (55.3%)
White	56 (78.9%)	110 (90.9%)
Black	13 (18.3%)	9 (7.4%)
BMI, mean (SD)	45.7 (6.4)	41.4 (6.4)
Waist circumference, cm, mean (SD)	123.4 (17.4)	122.4 (15.5)
SBP, mean (SD)	128.0 (15.0)	130.0 (13.3)
DBP, mean (SD)	73.1 (11.4)	66.5 (10.8)
Diabetes	25 (35.2%)	30 (24.0%)
Pre-diabetes	26 (36.6%)	45 (36.0%)
Normoglycemia	20 (28.2%)	50 (40.0%)
HDL, mean (SD)	45.6 (13.6)	46.0 (11.5)
Triglycerides, mean (SD)	120.5 (58.7)	155.0 (80.8)

C1.2.3 Feasibility of the study design: We have demonstrated the ability to recruit, retain, and measure extensive DSP outcomes in two cohorts of obese patients. For our NIH K23 study, we have enrolled 125 obese patients and 90 age- and gender-matched lean controls from the Weight Management Program (WMP) at U-M (**Tables 3 and 4**), which has led to a manuscript detailing the metabolic drivers of DSP as detailed in **A2**.[17]

As part of an ongoing study funded by a 1 yr pilot grant (2P30-DK020572), principal investigator (PI) Brian Callaghan, MD, MS, Assistant Professor of Neurology, and co-investigator Justin Dimick, MD, MPH, the Zuidema Professor of Surgery, completed comprehensive metabolic and DSP phenotyping on 71 participants (up from 43 previously) from U-M's bariatric surgery clinic (**Tables 3 and 4**). They also recruited an additional 28 participants that are in the process of completing study measures. Of the 40 patients who completed bariatric surgery and have been enrolled in the study for over 6 mo, 37 (93%) had additional testing at 6 mo, demonstrating our ability to effectively retain subjects once they have entered our study. Notably, the metabolic phenotyping and neurologic outcomes in these studies are comparable to those in the current proposal, including skin biopsies to measure IENFD at the proximal thigh and distal leg (**Table 5**). We are also currently recruiting patients from the U-M bariatric surgery clinic who have decided not to have surgery. Hence, recruiting the non-surgical population is not likely to be a barrier given our experience with this

Table 4: Prevalence of DSP

	Normoglycemia	Pre-diabetes	Diabetes	Total
IWMC	6 (12.0%)	11 (24.4%)	9 (30.0%)	26 (20.8%)
Bariatric	1 (5.0%)	2 (7.7%)	7 (28.0%)	10 (14.1%)
Total	7 (10.0%)	13 (18.3%)	16 (29.1%)	36 (18.4%)

Table 5: Extensive DSP outcome measures in the bariatric surgery cohort compared with lean controls

Variable	Lean Without DSP	Obese without DSP	Obese with DSP
IENFD leg (fibers/mm)	14.7 (7.0)	9.1 (7.3)	5.4 (4.3)
IENFD thigh (fibers/mm)	26.1 (8.1)	18.2 (12.7)	15.3 (5.4)
Sural amplitude (uV)	21.4 (5.7)	10.9 (5.5)	7.1 (4.3)
Sural PL (ms)	4.1 (0.4)	3.8 (0.4)	4.0 (0.3)
Peroneal amplitude (mV)	5.7 (2.5)	5.6 (2.4)	3.2 (2.1)
Peroneal DML (ms)	5.0 (0.9)	4.7 (0.7)	5.6 (1.1)
Peroneal CV (m/s)	43.3 (9.2)	45.7 (6.3)	39.5 (3.1)
Peroneal F response (ms)	65.9 (85.5)	47.5 (8.2)	55.3 (5.5)
Tibial amplitude (mV)	13.8 (5.7)	9.2 (4.9)	4.1 (3.4)
Tibial DML (ms)	7.6 (10.2)	4.6 (0.9)	5.4 (0.7)
Tibial F response (ms)	50.1 (4.9)	50.4 (5.9)	58.2 (4.2)
UENS	0.2 (0.6)	1.5 (3.0)	9.5 (5.2)
MNSI Questionnaire	0.4 (0.7)	2.7 (2.6)	6.6 (3.4)
MNSI Examination	0.1 (0.6)	0.7 (1.1)	2.1 (1.4)
Sudoscan feet	75.7 (14.3)	70.5 (12.5)	54.5 (21.9)
Sudoscan hands	64.4 (13.4)	65.3 (14.9)	45.8 (18.2)
SAS	3.0 (5.1)	5.6 (4.5)	10.3 (6.2)
Neuro-QOL	1.7 (0.9)	2.6 (1.1)	3.1 (0.9)
McGill Pain score	1.0 (3.5)	5.0 (6.6)	10.8 (7.2)
VAS Pain score	5.5 (18.6)	26.3 (28.7)	55.0 (28.4)

population to date. Of note, of the 480 patients that consider bariatric surgery each year at the U-M clinic, 55% undergo a surgical operation. Therefore, a large non-surgical population exists as well (N=216). For the exercise intervention, Drs. Horowitz and Little have a tremendous history of successful completion of clinical research projects including HIIT and other exercise trials.[46-50]

C2. Aim 2: Explore the impact of surgical weight loss on DSP outcomes (non-randomized)

C2.1. Introduction: Similar to previous data on exercise interventions on DSP outcomes, one uncontrolled study revealed improved DSP outcomes after bariatric surgery (Roux-en-Y gastric bypass) compared with the known natural history of decline.[51] The next step is to investigate the potential impact of bariatric surgery on DSP outcomes compared to those without surgery. Our innovative study design allows exploration of the effect of surgical weight loss on DSP outcomes without an expensive, randomized, surgical intervention trial.

C2.2. Justification & feasibility:

C2.2.1 Justification for bariatric surgery: Similar to the ideal exercise regimen, the best weight loss strategy would **improve metabolic outcomes** and have **high compliance**. Just like HIIT, bariatric surgery meets those criteria. Mounting evidence supports bariatric surgery for improvements in metabolic syndrome components including diabetes, hypertension, and dyslipidemia. In terms of diabetes, a prospective, longitudinal study of 28,616 obese diabetic patients undergoing a variety of surgical approaches revealed remission or improvement of diabetes in 44-83% of participants.[52] These results have been duplicated in four randomized controlled trials of surgery compared with medical management or lifestyle interventions.[53-56] Most of these trials looked at diabetes outcomes at 1-2 yr, but data at 3-5 yr is also promising.[57, 58] All four trials revealed large effect sizes regardless of the type of surgery employed. Data to support a reduction in hypertension are less robust compared with diabetes. A large prospective, longitudinal study found resolution or remission of hypertension ranging from 44-79% depending on the surgical procedure.[52] However, a randomized trial of surgery compared with lifestyle and medical modifications demonstrated similar large reductions in systolic blood pressure at 1 year.[54] Whether the difference in long term compliance in these groups would result in different long term hypertension outcomes is unclear. Data on dyslipidemia are similar to that for hypertension. The same large prospective, longitudinal study found remission of dyslipidemia ranging from 33-66% at 1 year depending on the surgical procedure performed.[52] No randomized data demonstrating a reduction in dyslipidemia are available to provide higher levels of evidence. In terms of reducing obesity, bariatric surgery is incredibly effective and the effect is long lasting. Patients lose between 45-85% of their excess weight, with maximum weight loss between 1-2 yr after the procedure.[59, 60] In addition, surgery results in longstanding weight loss that is sustained up to 10 yr after the procedure.[26] Medical weight loss with dietary or pharmacologic interventions is often successful in the short term, but in contrast to surgery, it is difficult to sustain. The lack of concern regarding compliance is one of the main reasons why studying a bariatric surgery population is preferred to studying a medical weight loss population. In addition to the data supporting the effect of bariatric surgery on improving metabolic outcomes and the inherently high compliance, one small study supports a possible effect on DSP outcomes. Specifically, a prospective cohort study of 20 patients with type 2 diabetes undergoing a Roux-en-Y gastric bypass revealed that the 12 patients with pre-operative DSP had significant improvements in DSP outcomes measures 6 mo after surgery. Of note, no control group was provided and outcome measures were not masked to the intervention. Despite these limitations in study design, these results are encouraging for a possible positive effect on DSP outcomes after bariatric surgery.[51]

C3. Clinical trial methodology

C3.1. Target population: We will enroll 35 patients into each arm of the study (HIIT/no surgery, HIIT/surgery, routine exercise/no surgery, routine exercise/surgery) for a total of 140 patients.

***Inclusion criteria*:**

1) Attending a bariatric surgery clinic;
2) At least 40 years of age
3) BMI > 35 with one comorbid condition present or BMI > 40 without comorbid conditions present;
4) willing and capable to sign the IRB-approved consent form and cooperate with the medical procedures for the study duration;
5) willing to accept random treatment assignment to HIIT or routine exercise counseling;

***Exclusion criteria*:**

1) History of DSP from causes other than diabetes and/or the metabolic syndrome as determined through medical history, family history, history of medications, occupational history, history of exposure to toxins, physical and neurological examinations;

2) use of warfarin, heparin, or other anticoagulants, which would increase the risk of complications from skin biopsy;
3) contraindication to HIIT participation including a failed exercise stress test;
4) participation in an experimental medication trial within 3 mo of starting the study;
5) undergoing therapy for malignant disease other than basal-cell or squamous-cell skin cancer;
6) medical or psychiatric reason for not being a surgical candidate;
7) requiring a walking assist device;
8) currently smoking;
9) weight over 450 pounds.

Screening Visit: Patients will complete a medical history review during their screening visit to determine subject eligibility. As outlined by the University of Michigan clinical care guidelines, subjects will complete a Covid-19 test prior to the stress test. A results will be provided to the study team. Patients will complete an exercise stress test as part of the screening procedures. The %HR the stress test will start with will be the patient's resting heart rate, typically below 100. The %HR max will vary per patient. This is calculated by 220-age. It is possible that this value will not be met but it is also possible that the predicted %max HR will be exceed. The Cornell protocol will be utilized for this study. The stress test will be performed on a treadmill. Only patients that have a normal exercise stress test will be allowed to participate in this study. To mitigate the risks associated with the exercise stress test, the test will be overseen by medical professionals that are ACLS certified including a physician, nurse and/or exercise physiologist. Patients with an abnormal EKG will be referred to their primary care physician and not cleared for participation

Justification of the target population: We are focusing on an obese population because we have built a collaborative obesity-DSP team with 5 years of experience recruiting and studying this population. Focusing on an obese population, rather than a diabetic population, increases the number of people affected by our results. Furthermore, our observational data revealing a high prevalence of DSP in obese participants without diabetes highlights that DSP is an important issue for all obese patients.[61] Limiting recruitment to the U-M's bariatric surgery clinic allows us to evaluate the effect of both HIIT and bariatric surgery on DSP outcomes. Selection bias will be reduced by requiring that patients have started the bariatric surgery process by seeing a physician assistant or bariatric surgeon. Patients will also be referred to a dietician. The limited generalizability to other populations can be addressed in subsequent studies.

C3.2. Interventions:

C3.2.1 HIIT: Training sessions during the 24-mo will be supervised by exercise physiologists and trainers. Dr. Horowitz, the director of the Human Phenotyping Core (HPC) of the U-M's Nutrition and Obesity Research Center will provide oversight of this aspect of the study given his vast expertise on the impact of HIIT and other exercise interventions on metabolic outcomes.[46-50] The exercise modality will be self-selected (e.g., walk/run (outdoors or treadmill), elliptical machine, stair climbing, cycling), and subjects will be encouraged to regularly vary the exercise modality to reduce risk of overuse injury and to enhance the variety of the exercise program.

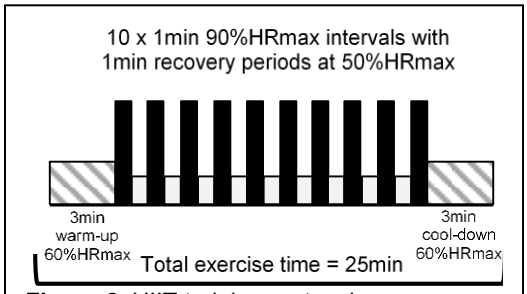

Figure 2. HIIT training protocol.

Subjects receiving the surgery intervention will start the HIIT regimen no sooner than 4 wk post-operatively to provide an adequate time to heal from the procedure. All subjects will first undergo a familiarization program for 1 week where they all will perform conventional exercise (steady-state at 65% HR max for 25 min/session). After this 1-week familiarization period, subjects will begin the HIIT regimen. The first session of the "ramp-up" period will entail 19 min of conventional exercise at 65% HR max, followed immediately by 2 x 1 min intervals at 90% HR max with a 1 min active recovery period between intervals at 50% HR max, and a 3 min cool down at 60% HR max after the second interval. After that first ramp up training session, 1 additional high intensity interval will be added to each training session, so by the beginning of week 3 of HIIT training, subjects will reach the full HIIT training protocol of a total of 10 x 1 min intervals at 90% HR max (**Figure 2**). They will continue this training protocol, 3 sessions/wk (2 supervised and 1 unsupervised), for 24-mo. Subjects who may feel challenged by the rate of increase in the number of intervals during this ramp up period will be allowed to increase the number of intervals at a slower pace, but all subjects must reach the full HIIT protocol during week 4 of HIIT training. Of note, our team regularly has patients with obesity and/or diabetes progress to the full protocol HIIT within 3 weeks, demonstrating the feasibility of this training approach in our population.[38, 41, 62, 63]

Participant safety and well-being during the exercise interventions: Caution is warranted when initiating an exercise training study in sedentary obese adults, and additional concern is needed when participants are required to exercise at high intensity. We take this issue seriously and extreme caution will be taken throughout the project to greatly reduce risk associated with the exercise regimen. Patients will be provided medical oversight and any injuries, and/or medical concerns that may arise in our patients during the course of this project will be address by Dr. Callaghan, the co-investigators, the patient's Bariatric Surgery Team, and the patient's primary care physician. It is important to note, however, that several studies have now been published demonstrating that the HIIT protocol in this project can be used safely in patients with obesity, cardiovascular disease, and type 2 diabetes.[64-66] Furthermore, another study demonstrated the safety of exercise interventions in those with DSP from type 2 diabetes with no serious or unanticipated adverse events reported.[67] Notably, all patients will complete an exercise stress test and exercise will be supervised for the full 24 mo of the HIIT intervention.

Exercise compliance: In addition to the 2 supervised sessions per week, subjects will be asked to complete 1 unsupervised session. To improve compliance in both types of sessions, patients will receive monthly phone calls from research staff to discuss potential solutions to barriers. Subjects will be provided with a wearable physical activity monitor with a visible heart rate display. Importantly, the data from these devices are downloaded wirelessly to a computer and/or mobile device, which then automatically updates their physical activity profile on their personal web-page. Our research study coordinator will receive permission to access each subject's physical activity profile, enabling us to assess their compliance remotely. The data reported on the website includes a 24-hr profile of heart rate and calories expended, allowing us to clearly distinguish compliance to the HIIT regimen. We will assess each subjects' physical activity profile on a weekly basis and record how many prescribed exercise sessions were successfully completed. If participants do not have access to the appropriate technology, we will make alternative arrangements such as providing them with an inexpensive mobile device with the necessary technology, providing a recordable heart rate monitor to wear during exercise, or collecting training logs.

C3.2.2 Routine exercise counseling: Patients receive counseling regarding exercise as a routine part of their participation in the bariatric surgery clinic. Specifically, they are counseled to participate in 60 min of aerobic exercise daily in addition to 2-3 non-consecutive days of strength training workouts every wk. Patients are encouraged to contact the bariatric conditioning program, obtain a gym membership, purchase exercise equipment, join a walking group, and/or sign up for fitness classes (employer or city parks and recreation).

C3.2.3 Bariatric surgery: Patients will undergo the surgical procedure as recommended by their surgeon as part of their routine care at a bariatric clinic. Our preliminary data indicates that 85% of patients had a sleeve gastrectomy, 15% had a Rou-en-Y gastric bypass at the University of Michigan, and none had an adjustable gastric band. We expect a similar distribution of procedures as part of this proposed study. Patients undergoing sleeve gastrectomy and Roux-en-Y gastric bypass have similar weight loss and time to stabilization, which limits potential confounding by type of surgical procedure.[59, 60] Specifically, weight loss is typically 60-80 pounds at 6 mo, and 100-120 pounds at one year with stabilization at 1-1.5 yr. In contrast, adjustable gastric band procedures result in a 45-55 pound weight loss at 6 mo with stabilization at 2 yr, but this procedure is not performed at U-M. Of note, as part of their routine post-operative care, patients are supplemented with a multivitamin, sublingual B12, and calcium. Furthermore, patients have laboratory monitoring including comprehensive metabolic panel, complete blood count, B12, TSH, ferritin, and vitamin D. The prophylactic supplementation and laboratory monitoring mitigate the potential for nutritional deficiencies to lead to nerve injury and subsequent DSP. The strength of this approach is that it mirrors current practice in the bariatric surgery clinic; therefore increasing generalizability. Furthermore, when neurologic complications occur after bariatric surgery, a specific nutritional deficiency other than B12 deficiency is often not found.[68]

C3.3. Metabolic phenotyping: These tests will aid in the appropriate description of the population, allow for adjustment of potential confounding as needed, and may provide further data in support of the metabolic factors that improve when IENFD increases over time.

Phenotypic tests will occur at baseline, 3, 12, and 24 mo, with the exception of the GTT which will only be performed at baseline. The metabolic phenotyping tests include:

1. 2 hr, 75 gram oral GTT with glucose and insulin levels drawn at 0, 30, 60, 90, and 120 min to allow categorization of glycemic status as well as calculation of Oral Glucose Insulin Sensitivity (OGIS). Participants with a known diagnosis of diabetes will not complete an OGTT. Participants with a fasting

glucose >126 mg/dL will not complete the test. Fasting glucose and insulin levels will be drawn at the 3, 12 and 24 month time points;
2. Hemoglobin A1C;
3. Triglyceride, HDL, and low-density lipoprotein **(**LDL) levels from a fasting lipid panel;
4. Systolic and diastolic blood pressure;
5. BMI, waist circumference, and hip to waist ratio. Measurement of leg and thigh circumference at skin biopsy sites and circumferences at NCS stimulation sites will also be documented.
6. Documentation of medications for diabetes, hypertension, hypercholesterolemia, and hypertriglyceridemia;
7. We will obtain plasma, DNA, skin biopsies, subcutaneous adipose tissue and urine samples for potential genomic, metabolomics data, and future research. All samples collected will be stored in the biorepository.

C3.4. Outcome measures: Dr. Callaghan, and consultants Eva Feldman MD, PhD, the DeJong Professor of Neurology and my K23 mentor, and Rodica Busui MD, PhD, Professor of Metabolism, Endocrinology and Diabetes, all have extensive experience with the DSP outcome measures proposed for this study.

C3.4.1 Primary outcome:

IENFD at the proximal thigh as assessed by skin biopsies obtained at baseline, 3, 12, and 24 mo using published protocols will be the primary outcome.[30, 69, 70] IENFD use for this trial was based on important lessons learned from multiple prior failed clinical trials in DSP[71-74] and the Feb 11-12, 2013, FDA-sponsored public workshop entitled: "Clinical Development Programs for Disease-Modifying Agents for Peripheral Neuropathy." The expert consensus of this workshop was: A morphological measure, such as IENFD, will most accurately assess earlier changes consistent with DSP and of nerve fiber regeneration. Traditionally, clinical trials assessed changes in NCS as a primary outcome measure[75]; however, NCS only assess large myelinated nerve fiber function, which in early DSP can be completely normal. Recent data have also described minimal worsening[71] or improvement in NCS[76] in placebo and epidemiological cohorts with little relation to other measures of nerve function in diabetic patients. The smallest nerve fibers are likely the earliest to undergo damage in the natural history of DSP, but are not measured by NCS. Therefore, using NCS will miss the opportunity to identify the earliest phenotypes of disease.

Skin biopsy is a minimally invasive procedure, with less than 1% reporting mild adverse events such as bleeding, swelling, or erythema. It allows morphometric quantification of C-fibers in the epidermis (IENFD).[77, 78] Data from several clinical trials show that decreased IENFD is a robust measure of the clinical signs and symptoms of DSP[77, 79-86] and correlates with a patient's perception of neuropathic pain.[77, 78] Serial measurements of IENFD may be performed to assess the therapeutic efficacy of drugs[77, 78, 87, 88] and/or lifestyle interventions[30] in arresting disease progression and providing pain relief, and a large normative database was recently created by an international consortium.[77, 78, 89] Published guidelines concur that IENFD is the standard for the diagnosis of small fiber sensory neuropathy.[77, 87, 90, 91] Further support for IENFD as an early biomarker is the observation of reduced density in patients with pre-diabetes compared to those with normal glucose tolerance.[30, 83] Finally, several studies report increased IENFD in response to therapeutic interventions that correlate with improvements in the signs and symptoms of DSP.[83-85] IENFD is also endorsed by several societies and authorities in the field as diagnostically efficient at distinguishing patients with early small fiber neuropathy from normal controls, and as a reliable and valid outcome measure to assess DSP in clinical trials.[77, 79, 87, 90, 92] In summary, IENFD is a sensitive and reliable measure of early DSP, its loss directly correlates with increasing DSP severity, is amenable to improvement with appropriate intervention, is safe and easy to perform, and to date represents the gold-standard method for evaluating morphological changes in small nerve fibers.[77, 79, 87, 90, 91] In addition, the PI has used this procedure extensively in two ongoing studies with excellent patient compliance (NS-079417 and 2P30-DK020572). Thus, IENFD was selected as the most appropriate and informative primary endpoint in the current clinical trial.

C3.4.2 Secondary outcomes: Selecting the most appropriate outcome measures is critical in the design of an interventional clinical trial and essential to obtaining clinically relevant information on the true response to the intervention. Thus, we will evaluate changes from baseline to 3, 12, and 24 months in several secondary outcomes:

1. *IENFD at the distal leg*: In addition to the IENFD measure at the proximal thigh, we will also measure

this entity at the distal leg. Previous data support that the proximal thigh measurement is more likely to show change over time after an exercise regimen in a population of patients with pre-diabetes.[30] However, IENFD at the distal leg will provide additional data as to the severity and length-dependence of the nerve injury. Furthermore, measurement at the distal leg may be more sensitive to change in those patients with obesity and normoglycemia, which may have milder nerve injury than those with diabetes.

2. *Corneal confocal microscopy (CCM)*: While IENFD is the current gold-standard for detection of small fiber nerve injury, CCM is an innovative approach that also attempts to measure small fiber nerve injury in the cornea. This procedure is less invasive than the skin biopsy required for IENFD measurement. The benefit of including this secondary outcome measure is that we will be able to compare and contrast the diagnostic characteristics of IENFD to CCM and also determine which measure is more sensitive to change over time. This data will help determine which of these two measurements should be utilized in future intervention studies. Specifically, we will measure nerve fiber density, nerve branch density, and nerve fiber length. A recent meta-analysis revealed that all three of these parameters are reduced in those with diabetic DSP compared to those with diabetes without DSP and to healthy controls.[93] Furthermore, studies have revealed similar diagnostic characteristics for CCM and IENFD for diabetic DSP.[94] CCM parameters also improve after simultaneous pancreas and kidney transplants, even when IENFD remains unchanged, indicating that CCM may be a useful early marker of nerve regeneration.[95] Consultant Roni Shtein, MD, Associate Professor of Ophthalmology and Visual Sciences, has extensive experience utilizing CCM as an outcome measure.
3. *Retinopathy measures:*
    a) 24-2 FDT - measure of retinal ganglion function. Best corrected visual acuity will also be measured.
    b) Retinal Imaging - Retinal Fundus Photography is currently the gold standard for diagnosing and staging diabetic retinopathy.
4. *Electrophysiological measures*:
    a) NCS of the sural, peroneal, and tibial nerves will be obtained following the DCCT/EDIC protocol.[96, 97] Briefly, measurements will be done at the dominant limb, using temperature control.[96, 97] NCS provide a quantitative, reproducible way to evaluate for large fiber function.
    b) Measures of CAN will comprise cardiovascular reflex testing using the DCCT/EDIC protocol (R-R interval ratio during deep breathing, postural change, and Valsalva) as described.[98, 99] These tests are recommended by the Toronto Consensus Panel on Diabetic Neuropathy[100] as the gold-standard for CAN. We will also assess resting HR and HR variability. Consultant Dr. Busui has extensive expertise in performing and interpreting CAN outcomes in clinical trials.
5. *Clinical DSP measures*:
    a) Structured neurological examination following the DCCT/EDIC protocol[96, 97];
    b) Michigan Neuropathy Screening Instrument (MNSI) questionnaire and examination, validated instruments to identify diabetic DSP[103, 104];
    c) Utah Early Neuropathy Scale (UENS), a validated tool to identify small fiber predominant neuropathy such as that found in obese and pre-diabetic individuals[105];
    d) Modified Toronto Neuropathy Score (mTNS), a validated instrument for the detection of mild to moderate diabetic DSP[106]; and
    e) Survey of Autonomic Symptoms (SAS), a validated measure of autonomic symptoms[107]
    f) DNS-score and guidelines, a four-item validated symptom score, with high predictive value to screen for polyneuropathy.
    g) DN4 questionnaire, to assess probability of neuropathic pain.
6. *DSP-specific pain*: Pain will be assessed using the Short Form McGill Pain Questionnaire. This questionnaire is a reliable and validated pain questionnaire that utilizes a visual analogue scale, a 6 point rating scale of pain intensity, and a 4 point rating scale of 15 different neuropathic pain descriptors.[108] Subjects will also be asked to rate their current pain on a numeric rating scale.
7. *Quality of life measures*:
    a) We will use the NeuroQOL, a validated measure of quality of life specific to DSP,[109] which comprises 6 domains (pain, lost/reduced feeling, diffuse sensory-motor symptoms, restrictions in activities of daily living, disruptions in social relationships, and emotional distress) along with an overall measure of DSP impact on quality of life and general quality of life.[109]

b) Questionnaires that ask about the participant's mood and how weight has impacted participant's physical and emotional health. These questionnaires include the EQ-5D, IWQOL-Lite, Godin Leisure-Time Exercise, and a brief physical activity questionnaire.
c) Demographic questionnaire will be administered to participants.

8. *Functional scales*:
    a) The Berg Balance Scale, measures balance in 14 separate activities of daily living (the unipedal stance portion has been shown to be particularly reflective of DSP-related mobility loss)[110];
    b) The 8 Foot Up and Go Test, a test of functional mobility that assesses the time needed for a subject to arise from sitting position, walk 8 ft and turn 180 degrees around a cone, and return to sitting[111];
    c) The Modified Falls Efficacy Scale, assessing patient's self-reported ability to perform activities of daily living (e.g. get dressed/ undressed, walking, shopping)[86, 112, 113] The fall survey will also ask "Have you fallen within the last year? If yes, how many falls?"

9. *Qualitative sensory testing:*
    a) Neurothesiometer- quantitative measure of vibration threshold at both toes.

10. *Cognitive impairment:*
    a) NIH Toolbox Cognitive Battery – A 30-minute brief computerized battery comprised of tasks covering several cognitive domains including language, attention, working memory, episodic memory, executive function, and processing speed. The NIH Toolbox Cognitive Battery is a reliable and valid instrument of cognitive function[48].
    b) The Rey Auditory Verbal Learning Test will be presented in addition to the NIH Toolbox. The Rey is presented verbally to the participant and is important as a supplement to the single visual-based short memory task in the NIH Toolbox. The computer format and national standardization of this tool will allow for comparisons to future studies. We will partner with Dr. Bruno Giordani who has expertise in these cognitive assessments.

11. *Urine:* Urine albumin/creatinine ratio and serum creatinine measurements. Urine samples will be stored in the biorepository.

12. *Anthropometric measurements:* Body measurements at different site circumferences will be measured at each study visit including: abdomen, arm, buttocks/hips, calf, forearm, hips/thigh, mid-thigh and waist.

13. *Orthostatic Hypotension:* Blood pressure and heart rate will be assessed after patient is supine for 5 minutes, standing for 1 minute and at 3 minutes standing.

14. *Aerobic Assessment:* All participants enrolled in the study will participate in brief graded exercise test (bike or treadmill) to determine maximal aerobic capacity (VO_2max). Participants randomized to the HIIT cohort will also complete a second VO_2max test after the training intervention to document the change in aerobic fitness. This exercise test is routinely used in clinical research projects to assess aerobic fitness in lean, obese and other clinical populations.

15. *Dual Energy X-ray Absorptiometry*: Body composition will be measured, including body fat, muscle mass and bone density using low-dose x-ray. All participants enrolled in the study will complete a DEXA scan at the beginning and end of the study.

16. *BodPod:* Subjects that exceed the weight limits of the dual energy x-ray absorptiometry may complete the BodPod as an alternative in order to determine body composition. Subjects will complete at baseline and at the end of the study.

C3.5. Visits schedule (Table 6):

Table 6: Study Events Schedule					
Visit Number	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Visit Description	Screening	Baseline	Outcomes	Outcomes	Outcomes
Time point	Before baseline	Day -70 to 0	Month 3	Month 12	Month 24
Visit window	NA	70 days	± 14 d	± 14 d	± 14 d
Informed Consent^/study criteria					
Medical History Review	X				
Stress Test	X				
Randomized to Exercise Protocol		X*			
Laboratory Measures		X	X	X	X
Biorepository Measures					
Plasma		X	X	X	X
Urine		X			X
Subcutaneous Adipose Tissue		X			X
DNA		X			
Skin Biopsies (IENFD)[T]		X	X	X	X
Corneal confocal microscopy		X	X	X	X
FDT-24-2		X	X	X	X
Retinal Imaging		X	X	X	X
Electrophysiologic measures		X			
NCS		X			X
Cardiovascular reflex tests					X
Clinical measures					
Neurologic examination		X			X
MNSI, UENS, mTNS, SAS, DNS, DN4		X	X	X	X
McGill pain questionnaire, NRS		X	X	X	X
Qualitative sensory testing					
Neurotheisiometer		X	X	X	X
Cognitive impairment					
NIH Toolbox		X			X
Rey		X			X
Anthropometric measures^		X	X	X	X
Orthostatic Hypotension Assessment^		X	X	X	X
Quality of Life measures:					
NeuroQol		X	X	X	X
Questionnaires		X	X	X	X
Demographics		X			
Functional Assessments					
Berg Balance Scale^		X	X	X	X
8-foot Get Up and Go Test^		X	X	X	X
Modified Falls Efficacy Scale		X	X	X	X
Aerobic Assessment					
VO2 max	X				X[+]
Dual Energy X-ray Absorptiometry		X			X

*Randomization to exercise protocol to occur after baseline visit
[+]Only participants randomized to the HIIT will complete a second time
[T] Participants can consent to have skin biopsy added to biorepository
^Participants may complete the consent and outcomes virtually prior to the upcoming visit to decrease in-person interaction due to Covid-19. If the outcome cannot be completed successfully it with be completed during the visit.

Data collected for clinical purposes as part of the Adult Bariatric Surgery Program will be reviewed as part of this study and included in the study records.

C3.6. Timeline and milestones: The planned study duration is 5 yr, with 2 yr of subject accrual, 2 yr of subject follow-up, plus 1 yr for analysis and results presentation.

C3.7. Statistical analysis: The four treatment groups (HIIT/no surgery, HIIT/surgery, routine exercise/no surgery, routine exercise/surgery) will be compared with respect to demographic and baseline variables (e.g., age, sex, glycemic status, OGIS, BMI, etc.). If a significant difference is found in any variable, the models described below will be fitted after adjusting for these variables.

C3.7.1 Analysis of primary outcome: The primary outcome is IENFD at the proximal thigh, measured at baseline, 3, 12, and 24 mo. Exploratory analysis will be performed to assess the need for any transformation

on the primary outcome variable. The primary exposure variables are HIIT: yes/no (Aim 1), and bariatric surgery: yes/no (Aim 2). We will adjust for demographic, clinical, and baseline characteristics as covariates in the model. In addition to the main effects of HIIT exercise program and bariatric surgery, we will also examine selected interactions based on *a priori* considerations. In particular, we will focus on the interaction between HIIT and bariatric surgery to assess if the effect of HIIT exercise on IENFD is modified by bariatric surgery. Linear mixed effects regression modeling will be used to determine the relationship between the primary exposure variables and IENFD over time. Longitudinal data analysis approaches such as this offer enhanced statistical power, as subjects are able to serve as their own reference. Furthermore, linear mixed effects regression offers a flexible framework to handle missing data such as due to attrition. Let Y_{it} denote the IENFD of the *i*th patient at the *t*th time point. Then Y_{it} can be modeled using the random intercept model.[114] The random intercept model can be viewed as arising from a two-stage model as follows:

Stage 1: subject-specific trajectory: $Y_{it} = \mu_{0i} + \lambda' Z_i + \underline{\theta' X}_{it} + e_{it}$
Stage 2: between-subjects: $\mu_{0i} = \beta_{00} + \beta_{0i}$
Combining the two stages, we can write $Y_{it} = \beta_{00} + \beta_{0i} + \underline{\theta' X}_{it} + e_{it}$
where β_{00} is the population-averaged intercept, Z_i is the matrix of main effects and interaction corresponding to HIIT exercise and bariatric surgery, $\underline{X}_{it}$ is the matrix of fixed covariates (demographic and baseline characteristics), λ and $\underline{\theta}$ are the vectors of corresponding fixed effects parameters, β_{0i} is the subject-specific random (intercept) effect capturing heterogeneity across subjects due to unmeasured confounders, and e_{it} is the random error characterizing variation due to sources like within-subject fluctuations and measurement error. The random effect β_{0i} is assumed to follow a normal distribution with mean zero and variance σ^2_{sub}, and the e_{it}'s are assumed to be distributed normally with mean **0** and variance covariance matrix R (non-diagonal). Furthermore, we also assume that β_{0i} and e_{it} are independent. Maximum likelihood method will be used to estimate the model parameters. We will use Akaike Information Criterion (AIC) and Schwarz Bayesian Information Criterion (BIC) to guide the selection of the covariance structure. Inference for λ (which is of primary interest) will be based on Wald tests. We will use PROC MIXED in SAS to fit the above model. To explore potential non-linear or threshold effect of treatment exposure on IENFD we will use a nonparametric regression model fitted using the framework of generalized additive models (GAM).[115] Given the exploratory nature of the research proposed here, the GAM models will allow us the flexibility to include a smooth functional term instead of restricting to a linear relationship. The smoothing term is fitted by using penalized regression splines or locally smoothed loess curves, with the degree of smoothness chosen in a data-adaptive way, using cross-validation techniques. We will use the *gam* function in the *mgcv* package available in the R software to fit these non-parametric regression models. We will perform sensitivity analyses to assess how subjects who withdrew affect conclusions of the analysis. An intent-to-treat analysis set (including subjects who are randomized, treated, and have a baseline IENFD) will be used to assess treatment effects, with multiple imputation methods[116] employed to impute outcomes for subjects with missing post-baseline data. Depending on the extent and pattern of missingness, other simpler sensitivity analyses may be used: e.g., subjects completing the study (completers) may be analyzed using a repeated measures analysis of variance model. The model will include the same covariates that are included in the primary analysis.

C3.7.2 Analyses of secondary outcomes: Due to the pitfalls of multiple testing, formal statistical testing won't be performed. For analysis of repeated measures of continuous data (CCM, cardiovascular reflex tests, MNSI, UENS, mTNS, SAS, McGill Pain questionnaire, NeuroQOL, and functional assessments), the longitudinal approaches described above will be employed. Specifically, each outcome will be analyzed separately using a linear mixed effect regression model, with the treatment group as the primary exposure.

C3.7.3 Exploratory analyses: To investigate the potential modifying effects of sex, we will perform an additional analysis that includes an interaction term between the intervention groups and sex. Similarly, we will explore the differential effect of sleeve gastrectomy versus Rou-en-Y gastric bypass by including separate indicator variables for these two different surgical techniques. We will also investigate the potential weight loss independent effects of bariatric surgery by evaluating the earliest time point (3 months). In addition to other metabolic syndrome components, we will study the effect of change in insulin sensitivity as measured by OGIS on our primary outcome. Finally, we will perform an analysis of those who complete more than 80% of supervised exercise sessions to assess the effect of exercise compliance. Importantly, these analyses will be hypothesis generating for future studies.

C3.8. Power calculation: The purpose of this clinical trial is to provide the preliminary data necessary to design a multicenter clinical intervention trial. Therefore, it is not strictly amenable to formal power

considerations. However, there are two assumptions necessary to justify the multicenter trial. The first is that HIIT will cause an increase in IENFD (re-innervation) and the second is that the natural progression of DSP is loss of nerve fibers over time. The primary outcome is an assessment of IENFD at the proximal thigh over 4 time points spanning 24 mo in the four trial arms. To estimate the likely effect sizes of the four interventions, we will rely on previous well-conducted studies, including our ongoing NIH K23 project. For the routine exercise/no surgery group, we estimate that the IENFD will decrease similar to that observed for those with pre-diabetes and DSP. Over 26.7 mo, IENFD at the proximal thigh decreased from 19±5 to 13±4. Given that our population includes patients with normoglycemia, we expect a decline of at least 3 fibers/mm over the 2 yr of our study. For the HIIT/no surgery group, we estimate that the IENFD will increase by 1.4±2.3 fibers/mm based on a study of the effect of exercise by Smith et al.[30] They saw this effect after 1 yr, but we will conservatively estimate that the effect will plateau after the initial yr after the intervention. For routine exercise/surgery group, we estimate that the IENFD will increase by 0.2±7.0 based on our preliminary data on patients undergoing diet-induced weight loss for 2 yr. For the HIIT/surgery group, we estimate that the effect will be additive compared to the two separate interventions. With our expected sample size of 30 patients (allowing for ~15% attrition) in each of the four treatment groups, we will have at least 80% power to detect effect sizes between 0.9 and 1.7 as statistically significant based on a two-sided test with alpha=0.05.

C3.9. Data collection and management: Data report forms will be filled out during each visit, verified, and entered into RedCap. Validation reports will be run, and checked for omissions, improbable values, and data consistency. Distributions of continuous measures will be assessed for symmetry and transformations will be applied as needed. Co-investigator Mousumi Banerjee, PhD, Professor of Biostatistics, Evan Reynolds, graduate student, and Dr. Callaghan will have data access for monitoring and verification.

C3.10. Recruitment and retention plan: Dr. Callaghan has experience recruiting obese participants for two epidemiologic cohorts, his NIH K23 and 2P30-DK020572 grants, that provided the preliminary data for the proposed study. He works closely with Emily Villegas-Umana, RN (project coordinator) and Ericka Chant, MPH (research coordinator) on these projects. Both coordinators have successfully recruited subjects for these studies, developed strategies for retention, and can perform the outcomes measures required for this proposal.

C3.10.1 Recruitment strategies: Patients will be recruited from a bariatric surgery clinic. Recruitment for the study begins at the informational session, where flyers are passed out providing information about the study as well as contact information for the study team. Patients are then recruited one-on-one during their bariatric surgery clinic visits. Patients meet individually with the study coordinator to discuss the study, answer questions, and begin the scheduling process. Patients are then followed as they complete the requirements prior to surgery, and are emailed at different time points to check for interest in participation. The final face-to face interaction for recruitment is during the required nutrition information session. Patients will be consented once they have made a decision whether or not to have surgery. This multi-step approach to recruitment has been effective for our ongoing bariatric surgery study. Compared with the start of the ongoing project, we have added multiple points of face-to-face contact and added a mixed media presentation, which will only increase the feasibility of the current proposal.

C3.10.2 Retention strategies: Patients will be emailed and/or called to schedule their 3, 12, and 24 mo visits approximately 2-3 mo prior to the date. We will also call patients monthly to address any concerns and discuss compliance. This limits the amount of time that the study team is not in contact with the participant, helping to increase patient retention. Patients are mailed out a reminder post-card just prior to the 1-yr and 2-yr mark, thanking them for their participation and reminding them that we will be in contact with them. This patient population has proven to be committed and motivated to participate in our current study, with 94% retention.

C4. Potential problems & alternative approaches for all Aims: We hypothesize that HIIT will improve DSP outcomes. We also believe that the effect of HIIT will be greater than the effect of bariatric surgery. However, our supportive preliminary data are based on a diet-induced weight loss intervention, and the effect of surgical weight loss may be larger. If surgery is as good as or better than HIIT, the results would still lead us toward one of the first disease modifying therapies for DSP. Our study design is also able to look at the combined effects of HIIT and bariatric surgery. If the severity of DSP is less in our population, the IENFD of the proximal thigh may not be the most sensitive DSP outcomes measure; however, we plan to use IENFD of the distal leg as a secondary outcome measure. In contrast to our supportive preliminary data, if the HIIT regimen is difficult to perform for this obese population, we could switch interventions to a conventional exercise regimen, which Dr. Horowitz has extensive experience with in patients with obesity and diabetes.

C5. Biorepository to enable future mechanistic studies: We will obtain fasting plasma DNA, urine, skin

biopsies, and subcutaneous adipose tissue samples. While beyond the scope of this current proposal, this biorepository will allow for future studies to better understand the mechanisms by which HIIT and/or bariatric surgery improve DSP outcomes. Which intervention to pursue will be based on which intervention(s) is/are successful at improving DSP outcomes in this clinical trial. Drs. Horowitz (co-I, HIIT) and O'Rourke (consultant, bariatric surgery) have the expertise to explore mechanisms in humans. As Director of the Obese-Adipose Tissue Research Laboratory, Dr. O'Rourke will help obtain and process the subcutaneous adipose tissue obtained for this proposal. Dr. Feldman (consultant, murine DSP outcomes), in conjunction with Drs. Seeley and Sandoval (consultants, murine bariatric surgery), has the expertise to explore mechanisms in mice. The biorepository will allow translational research from mouse to human and from human to mouse. Our experienced team of investigators and consultants is uniquely positioned to accomplish this goal.

C6. Future directions: This phase 2 study will provide results that will directly inform which intervention or interventions to pursue in a phase 3 study. The phase 3 study will need to be a multi-center study with a patient-oriented primary outcome. This proposal will also help decide which secondary outcomes to include in the pivotal trial after evaluating the longitudinal changes of IENFD, CCM, and NCS over time.

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