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WASHINGTON UNIVERSITY IN ST. LOUIS
Interdisciplinary Program in Movement Science

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SKELETAL MUSCLE IMPAIRMENTS IN PEOPLE WITH DIABETES AND
PERIPHERAL NEUROPATHY

by

Lori Jeanne Tuttle

A dissertation presented to the
Graduate School of Arts and Sciences
of Washington University in
partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

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2011

Abstract of the Dissertation

Skeletal Muscle Impairments in People with Diabetes and Peripheral Neuropathy

by

Lori Jeanne Tuttle

Doctor of Philosophy in Movement Science

Washington University in St. Louis, 2011

Dr. Michael J. Mueller, Chairperson

Diabetes mellitus (DM) is a chronic disease that affects almost 24 million people and has large socioeconomic costs. Of these 24 million people, 60-70 % will develop some form of nervous system impairment, including peripheral neuropathy (PN). It is important to understand muscle structure and impairments in people with DM+PN in order to determine appropriate interventions to limit the disability that is associated with DM+PN. The objectives of this research are to describe skeletal muscle structure in people with DM+PN, to determine whether neuropathic muscle structure and composition are associated with movement impairments and functional limitations, and whether neuropathic muscle can be modulated by activity level and/or an exercise intervention.

In Chapter 2, we examine whether intermuscular adipose tissue (IMAT) volumes are different between groups with DM, DM+PN, and a group without DM or PN (NoDMPN). We report that there is no difference in IMAT volumes in these groups, but that increased IMAT is associated with poorer physical performance and the DM+PN

group had the lowest measures of physical performance. In Chapter 3, we examine whether activity level in people with DM+PN is associated with IMAT. We report that activity level is inversely associated with IMAT volumes. In Chapter 4, we examine whether an exercise intervention for people with DM+PN is able to improve function and change IMAT and muscle volumes. We report that our duration-based exercise program was successful in increasing 6 minute walk distance, but there was no change in IMAT or muscle volumes. In Chapter 5, we provide a case report detailing an exercise intervention for a specific individual with DM+PN who was able to increase his average activity level and shows improvement in muscle performance and physical function.

Overall, our results suggest that people with DM+PN have lower levels of physical performance than their peers and increased IMAT is associated with poor physical performance. Increased activity levels are associated with decreased IMAT volume. People with DM+PN are able to safely increase their walking distance following an exercise intervention, but we did not see a change in muscle composition. Additional research is needed to determine the specific roles of IMAT in skeletal muscle and function.

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Dedication

For my Mom, who wanted me to have a career and not just a job.

and

For my Dad, who reminds me to laugh at myself.

TABLE OF CONTENTS

ABSTRACT OF THE DISSERTATION.....ii
ACKNOWLEDGEMENTS.....iv
TABLE OF CONTENTS.....vii
LIST OF TABLES.....viii
LIST OF FIGURES.....ix

CHAPTER 1: BACKGROUND AND SIGNIFICANCE.....1
 BODY.....2
 REFERENCES.....12

**CHAPTER 2: INTERMUSCULAR ADIPOSE TISSUE IS ASSOCIATED WITH
POOR FUNCTIONAL PERFORMANCE AND IS MUSCLE SPECIFIC16**
 ABSTRACT.....17
 BODY.....19
 REFERENCES.....37

**CHAPTER 3: LOWER PHYSICAL ACTIVITY IS ASSOCIATED WITH
HIGHER INTERMUSCULAR ADIPOSE TISSUE IN PEOPLE WITH TYPE 2
DIABETES AND PERIPHERAL NEUROPATHY.....39**
 ABSTRACT.....40
 BODY.....42
 REFERENCES.....58

**CHAPTER 4: PEOPLE WITH DIABETES AND PERIPHERAL NEUROPATHY
ARE ABLE TO EXERCISE SAFELY AND INCREASE SIX MINUTE WALK
DISTANCE.....61**
 ABSTRACT.....62
 BODY.....63
 REFERENCES.....79

**CHAPTER 5: A WEIGHT-BEARING EXERCISE PROGRAM FOR PEOPLE
WITH DIABETES AND PERIPHERAL NEUROPATHY: A CASE
REPORT.....82**
 ABSTRACT.....83
 BODY84
 REFERENCES.....101

CHAPTER 6: SUMMARY.....104
 BODY.....105

APPENDIX A111
 BODY.....112
 REFERENCES.....121

APPENDIX B.....123
 BODY.....124
 REFERENCES.....129

APPENDIX C.....130

LIST OF TABLES

CHAPTER 2	
Table 1.....	31
Table 2.....	32
Table 3.....	34
Table 4.....	35
CHAPTER 3	
Table 1.....	54
Table 2.....	55
Table 3.....	56
CHAPTER 4	
Table 1.....	77
Table 2.....	78
CHAPTER 5	
Table 1.....	98
Table 2.....	99
APPENDIX A	
Table 1.....	115
Table 2.....	116

LIST OF FIGURES

CHAPTER 1	
Figure 1.....	11
CHAPTER 2	
Figure 1.....	36
CHAPTER 3	
Figure 1.....	57
CHAPTER 5	
Figure 1.....	100
APPENDIX A	
Figure 1.....	118
Figure 2.....	119
Figure 3.....	120
APPENDIX B	
Figure 1.....	126
Figure 2.....	127
Figure 3.....	128

Chapter 1:
Background and Significance

Diabetes mellitus is a chronic disease that affects almost 24 million people (diagnosed and undiagnosed), results in significant physical disability, and creates large socio-economic costs.¹ The effects of diabetes mellitus on body composition and muscle structure and impairments may explain the impaired physical function associated with diabetes mellitus. Peripheral neuropathy is a frequent complication of diabetes mellitus and is associated with lower extremity sensory disturbances, muscle weakness, and functional deficits. People with diabetes and peripheral neuropathy display an abnormal infiltration of fat in the intra- and extra-cellular compartments of their lower extremity musculature that is not noted in their peers.^{2,3, 4,5} The extent to which neuropathy in particular contributes to this fatty infiltration is unknown. It is also unknown whether fatty infiltration in the muscle contributes to muscle weakness and if so, whether the fat infiltration and muscle weakness can be reversed or attenuated through exercise or other interventions. Magnetic resonance imaging (MRI) has the potential to increase our understanding of muscle composition and dysfunction and to help optimize rehabilitation strategies. The mechanism for decreased physical function in people with diabetes is important for physical therapists and other health care providers to understand so that the optimal rehabilitation strategies for the recovery of muscle function can be determined in order to reverse or prevent further disability. In this introductory chapter, I will briefly overview muscle structure and impairments in DM and PN, the relationship of IMAT to physical function, and the effect of exercise on IMAT.

Muscle Structure and Impairments in Diabetes and Peripheral Neuropathy

The incidence and prevalence of diabetes mellitus and complications related to diabetes mellitus are reaching epidemic levels.¹ Comorbidities and conditions that are often related to diabetes such as obesity,^{6,7} heart disease,^{6,7,8} stroke,⁷ and arthritis,⁷ have been identified as contributors to disability in people with diabetes.^{9,10} The effects of diabetes mellitus on body composition (muscle adaptations in particular) as well as muscle function are now being examined as contributors to the impaired physical function that is associated with diabetes.⁹⁻¹¹

Sixty to 70 percent of individuals with diabetes mellitus have mild to severe forms of nervous system disease.¹ Individuals with diabetes and peripheral neuropathy develop sensory loss as well as muscle weakness and motor dysfunction.¹² Andersen et al.¹³ reported strength losses of ~21% in the ankle musculature of individuals with diabetes mellitus. The severity of peripheral neuropathy has been associated with muscle weakness in type 1 and type 2 diabetes mellitus.¹⁴ In another study, Andersen et al.¹² used magnetic resonance imaging (MRI) to demonstrate that muscle atrophy underlies motor weakness of the distal extremities in individuals with diabetes mellitus and peripheral neuropathy. However, the effect of diabetes mellitus and peripheral neuropathy on muscle quality remains a topic of interest because most individuals with diabetes mellitus do not display noticeable muscle atrophy despite muscle weakness compared to individuals without diabetes mellitus. The Health, Aging, and Body Composition study verified that arm and leg muscle mass do not accurately predict muscle strength for older adults with diabetes mellitus.⁹ They studied 485 older adults with diabetes mellitus that had larger arm and leg muscle mass than those persons without diabetes and found that diabetes is associated with lower strength and muscle

quality (defined as muscle strength per unit regional muscle mass, assessed by DXA), and may contribute to the development of disability in older adults with diabetes mellitus.⁹ Another Health, Aging, and Body Composition study was the first to report data on how muscle mass and strength in people with diabetes mellitus change over time.¹⁰ These data showed that individuals with diabetes mellitus experience a greater loss of muscle strength and quality than people without diabetes mellitus.¹⁰ Despite the high prevalence of diabetes and peripheral neuropathy and their potentially devastating effects on strength and function, little is known about neuropathic skeletal muscles and their impact on function or their ability to adapt to an exercise program.

Goodpaster et al.⁵ showed that accumulation of adipose tissue (measured by computed tomography) within the fascia surrounding skeletal muscle in the thigh is associated with insulin resistance. Goodpaster et al.² performed a subsequent study to examine whether skeletal muscle fat is characteristic of elderly individuals with diabetes, hyperinsulinemia, and impaired glucose tolerance. Subcutaneous thigh fat was similar, however intermuscular adipose tissue (IMAT) was higher in the people with diabetes and impaired glucose tolerance.² Intermuscular adipose tissue (IMAT) is defined as the visible adipose tissue beneath muscle fascia and between muscle groups (Figure 1).^{15,16} IMAT has been linked to insulin resistance, and has been described as a unique adipose tissue depot that is similar to visceral adipose tissue in its risks for metabolic impairment.^{2,4,17} They concluded that people with increased amounts of IMAT may be at increased risk for metabolic disease, including diabetes mellitus.²

Because adipose tissue is associated with metabolic impairments, decreased muscle function, and diabetes, investigators pursued the use of MRI methods to quantify

the amount of lipid within the muscle.^{17, 18,19} MRI allows for non-invasive, quantitative, and reliable measurements of quantifying IMAT.¹⁸ In addition, the inherent contrast between different tissue types within the body when viewed via MRI make MRI an excellent technique for morphometric measures of skeletal muscle.

Relationship of IMAT to physical function

In our previous work, we have shown that a group with obesity, diabetes, and peripheral neuropathy had significantly greater amounts of IMAT in the calf compared to a non-obese control group and that this IMAT was associated with poor physical performance.¹¹ It is unclear to what extent obesity contributes to this accumulation of IMAT in skeletal muscle and to what extent the independent factors of diabetes and peripheral neuropathy contribute to the amount of IMAT. In addition, the relationship between IMAT and physical performance is not clear in these groups. Previous investigators^{4,17} have shown that IMAT is linked to insulin resistance and that denervation²⁰ can also contribute to fat infiltration in muscle, but it is unclear whether obesity alone accounts for a significant portion of IMAT infiltration.

It is also unknown whether IMAT accumulation is muscle or muscle compartment specific, i.e. whether one muscle or a group of muscles tends to have more IMAT than others. Different muscles in the calf have different amounts of fast twitch and slow twitch fibers. For example, the gastrocnemius muscle is considered largely a fast twitch muscle and is used more for large force production, while the soleus muscle is considered more slow twitch and is a postural muscle that is better suited for using lipids

as a fuel source.²¹ It is unknown whether different “types” of muscles are more affected by IMAT infiltration in the presence of diabetes, diabetes and neuropathy, and obesity.

Increased physical activity has been shown to decrease pain and improve balance in people with diabetes and peripheral neuropathy.^{22,23} Improved physical activity is particularly important for people with diabetes, not only for improved glucose control and cardiovascular fitness, but also because people with diabetes are two times more likely to have limitations in physical mobility and this likelihood for physical mobility limitations increases in the presence of peripheral neuropathy.²³ The mechanisms for physical mobility limitations in diabetes are unclear. IMAT has been linked to immobilization in healthy young adults²⁴ and has been shown to be reduced with exercise in a population with diabetes and without peripheral neuropathy,²⁵ indicating that physical activity may be a potent modifier of IMAT content in skeletal muscle. IMAT has also been shown to be higher in the affected limb in people with chronic stroke²⁶ and spinal cord injury.²⁷ IMAT has been shown to be higher in rotator cuff injury with nerve involvement²⁰ indicating that nerve dysfunction (such as peripheral neuropathy) could impact IMAT.

Effect of Exercise on IMAT

The benefits of exercise to help control diabetes mellitus and reduce subsequent complications are well known and accepted.^{28,29} Besides improved diabetes control and lower mortality rates, walking and increased activity appear to improve complications from peripheral neuropathy.²² The evidence of the benefits of exercise for people with diabetes has led the American Diabetes Association (ADA) and the American College of

Sports Medicine (ACSM) to recommend that adults with diabetes participate in at least 30 minutes per day of moderate-intensity physical activity, but the effects of this exercise program are untested in people with diabetes and peripheral neuropathy.³⁰

IMAT in the thigh has been shown to reduce in size in response to increases in activity and exercise programs in an older population³¹ as well as in a group with diabetes, but no peripheral neuropathy,²⁵ but it is unclear whether an exercise program can impact IMAT in the calf in a population with diabetes and peripheral neuropathy. Although people with diabetes and peripheral neuropathy clearly have lower extremity impairments, little evidence exists to determine if these impairments can improve with exercise. Studies investigating the effect of strengthening exercise have reported only modest improvements^{32,33} and a Cochrane review of the literature concluded that there is inadequate evidence to evaluate the effect of strengthening exercise for people with diabetes and peripheral neuropathy.³⁴

While there are a number of studies to support the beneficial effects of exercise in people with diabetes, there is little information about how exercise will affect the muscle of people with diabetes and peripheral neuropathy. Previous studies have demonstrated that IMAT levels are increased in the presence of insulin resistance and diabetes, but it is unclear whether this adipose tissue can adapt to exercise in the presence of diabetes and peripheral neuropathy. A greater knowledge is needed to determine the muscular impairments that exist in people with diabetes and peripheral neuropathy and whether or not these impairments can be modified by an exercise intervention.

Mueller and Maluf³⁵ have proposed a Physical Stress Theory for rehabilitation speculating that essentially all biological tissues adapt to the stresses placed upon them, provided the stresses are not above a threshold that would cause injury. Based on this theory, neuropathic muscle and skin should be able to adapt to increased activity and an exercise program. It is not clear what the appropriate magnitude and duration of activity and exercise is necessary and safe to cause adaptation in neuropathic muscle and skin.

PRIMARY PURPOSE: The objectives of this research are to describe skeletal muscle structure in people with diabetes and peripheral neuropathy, to determine whether neuropathic muscle structure and composition are associated with movement impairments and functional limitations, and whether neuropathic muscle can be modulated by activity level and/or an exercise intervention.

SPECIFIC AIMS AND HYPOTHESES

Specific Aim 1: (Chapter 2)

To determine differences in muscle structure and composition (IMAT, muscle volume) in specific calf muscles (gastrocnemius, soleus) and calf compartments (anterior, lateral, deep) between people with diabetes and peripheral neuropathy (DM+PN), people with diabetes and no peripheral neuropathy (DM), and people without diabetes and without peripheral neuropathy (NoDMPN). Additionally, the relationship between calf muscle structure and composition and measures of muscle performance (peak torque) and physical function (PPT, 6 minute walk test, stair power) will be examined.

Hypothesis 1: People with DM+PN and people with DM will have greater amounts of IMAT, and similar calf muscle volumes compared to the NoDMPN

group and the DM+PN group will display the largest amounts of IMAT of all 3 groups.

Hypothesis 2: The soleus muscle will display more IMAT than the gastrocnemius muscle in people with DM+PN and people with DM.

Hypothesis 3: IMAT will be negatively correlated with peak torque and measures of physical function.

Specific Aim 2: (Chapter 3)

To characterize activity levels (average daily step count) in people with DM+PN and to determine relationships between activity level and IMAT volume in the calf, calf muscle volume, muscle performance, physical function, and glucose control.

Hypothesis 1: People with DM+PN will take around 5,000 steps per day based on prior observations and reports.

Hypothesis 2: Higher average daily step count will be associated with lower IMAT volume, larger muscle volume, improved glucose control (lower HbA1c levels), higher muscle performance, and higher physical function.

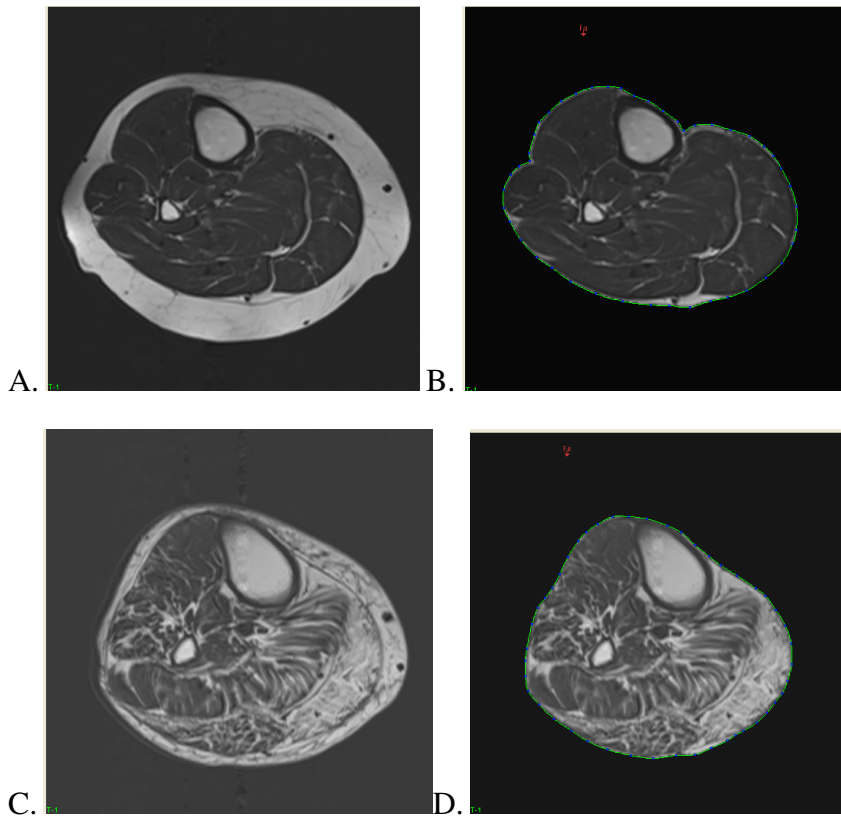
Specific Aim 3: (Chapters 4&5)

To determine the effect of a three month progressive exercise program on calf muscle structure and composition (IMAT, muscle volume), muscle performance (peak torque), and functional limitations (Physical Performance Test, 6 minute walk test) in people with DM+PN who complete the 3 months of training (EX) and compare these outcomes to a group of people with DM+PN who do not complete the training (CON).

Hypothesis 1: The EX group will display decreased IMAT in calf muscles and increased calf muscle volume after a progressive exercise program compared to the CON group.

Hypothesis 2: The EX group will display increased dorsiflexor and plantarflexor peak torque and improved measures of functional limitation after a progressive exercise program compared to the CON group.

Figure 1. Panels A and C are cross sections of the calf via MRI. Notice that subcutaneous adipose tissue and intermuscular adipose tissue are intact. Panels B and D are the same cross sections with the subcutaneous adipose tissue removed leaving the intermuscular adipose tissue and calf muscle. Panels A and B are images from a person with diabetes and panels C and D are images from a person with diabetes and peripheral neuropathy.



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Chapter 2

Intermuscular Adipose Tissue is Associated with Poor Functional Performance and is Muscle Specific

This chapter is in review:

Tuttle LJ, Sinacore DR, Mueller MJ. Intermuscular Adipose Tissue is Associated with Poor Functional Performance and is Muscle Specific. *J Diabetes Complications*.

ABSTRACT

Purpose: People with obesity, diabetes, and peripheral neuropathy have high levels of intermuscular adipose tissue (IMAT) volume which has been inversely related to physical function. The primary purpose of this study was to determine if physical function and calf IMAT are different between groups with diabetes mellitus (DM), DM and peripheral neuropathy (DM+PN) and a group without DM or PN (NoDMPN).

Methods: We studied 45 people; 11 with type 2 diabetes, 24 with diabetes and peripheral neuropathy, and 10 age and BMI matched people without diabetes and without peripheral neuropathy and measured physical function (6 minute walk, Physical Performance Test, stair power), muscle performance and muscle morphology (calf muscle volume, calf IMAT).

Results: There were group differences for measures of physical performance: six minute walk distance, physical performance test, and stair power ($P < 0.05$). There were no differences between groups in measures of calf muscle or calf IMAT volumes. Calf IMAT infiltration was muscle and compartment specific. The gastrocnemius muscle had a higher ratio of IMAT/Muscle volume than any other muscle or compartment [0.286(0.37) (gastrocnemius) vs. 0.131(0.13) (soleus) vs. 0.154(0.16) (anterior compartment)]. Calf IMAT was inversely related to physical performance on the 6 minute walk test ($r = -0.47$), and the physical performance test ($r = -0.36$). IMAT/Muscle volume was inversely related to physical performance (PPT $r = -0.44$, 6MW $r = -0.48$, Stair Power $r = -0.30$).

Conclusions: Calf IMAT volume, diabetes and peripheral neuropathy are associated with poor physical performance and calf IMAT may be muscle or compartment specific.

Key Words: physical performance, peripheral neuropathy, Intermuscular Adipose Tissue (IMAT)

Introduction

Previous research has shown that a group of people with obesity, diabetes, and peripheral neuropathy had significantly greater amounts of intermuscular adipose tissue (IMAT) in the calf compared to a non-obese control group and that this calf IMAT was associated with poor physical performance (Hilton et al., 2008). IMAT is defined as the visible adipose tissue beneath muscle fascia and between muscle groups (Lawler et al., 2003 and Ruan et al. 2007). The unique contributions of obesity, diabetes, and diabetes and peripheral neuropathy to the amount of IMAT in skeletal muscle are not clear. In addition, the relationship between calf IMAT and physical performance is not clear in these groups. IMAT in the thigh has been linked to insulin resistance, and has been described as a unique adipose tissue depot that is similar to visceral adipose tissue in its risks for metabolic impairment (Goodpaster et al., 2003, Goodpaster et al., 2000 and Gallagher et al., 2005). Previous investigators (Goodpaster et al. 2000 and Gallagher et al., 2005) have shown that IMAT is linked to insulin resistance and that denervation (Rowshan et al., 2010) can also contribute to fat infiltration in muscle, but it is unclear how the combination of diabetes, peripheral neuropathy and obesity impact IMAT infiltration.

It is also unknown whether calf IMAT accumulation is muscle or muscle compartment specific, i.e. whether one muscle or a group of muscles tends to have more IMAT than others. Muscles in the calf have different distributions of fast twitch and slow twitch fibers. For example, the gastrocnemius muscle is considered predominantly a fast twitch muscle and is used more for large force production, while the soleus muscle is considered more slow twitch and is a postural muscle that is better suited for using lipids

as a fuel source (Edgerton et al., 1975). It is unknown whether muscles with different “predominance” of fiber types are targeted by IMAT infiltration in the presence of diabetes, diabetes and neuropathy, and obesity.

Therefore, the purposes of this study were a) to determine differences in physical performance measures between people with diabetes mellitus (DM), people with diabetes mellitus and peripheral neuropathy (DM+PN), and an age- and BMI- matched group without diabetes and without peripheral neuropathy (NoDMPN); b) to determine the relationship between calf muscle structure and composition, measures of muscle performance (peak torque, average power) and physical performance (PPT, 6 minute walk test, stair power); and c) to determine if the volume of IMAT present is different between individual muscles and muscle compartments. We hypothesized that the groups with DM and DM+PN would have greater volumes of IMAT in the calf, and similar calf muscle volumes compared to the NoDMPN group, and that the DM+PN group would display the largest volume of calf IMAT of all 3 groups. Additionally, we hypothesized that calf IMAT volume would be inversely correlated with measures of physical performance and that the soleus muscle would display more IMAT than the gastrocnemius muscle and all other calf compartments across the three groups due to the predominance of slow twitch fibers and higher lipid oxidation capacity in the soleus muscle.

Methods and Procedures

Participants

Forty-five subjects participated in this study—11 people with type 2 diabetes [5men/6 women; aged 56(9); BMI 35.5(6.4)], 24 people with diabetes and peripheral

neuropathy [15 men/9 women; aged 64(13); BMI 32.6(6.3)], and 10 people without diabetes and without peripheral neuropathy [4 men/6 women; aged 64(9); BMI 32.9(4.6)]. The groups were matched for age and BMI. Participants were recruited from the Washington University School of Medicine Diabetes Clinic, Washington University's Volunteers for Health, the Center for Community Based Research, and from diabetes clinics in the surrounding St. Louis community. This study is part of a larger study investigating the effect of exercise for people with diabetes and peripheral neuropathy. Participant characteristics are listed in Table 1. Participants were excluded if they weighed more than 300 pounds (equipment weight limit), had a history of severe foot deformity or amputation, any co-morbidity or medications that would interfere with exercise (such as severe rheumatoid arthritis, peripheral arterial disease (absent pulses), dialysis, or current cancer treatment). Participants provided written informed consent. This study was approved by the Human Research Protection Office at Washington University in St. Louis.

Assessments

Peripheral Neuropathy

Presence of peripheral neuropathy was determined based on an inability to feel the 5.07 Semmes-Weinstein monofilament on at least one point on the plantar surface of the foot, and on a vibration perception threshold greater than 25V as measured with a biothesiometer applied to the plantar surface of the great toe (Maluf et al., 2003 and Diamond et al., 1989).

Six Minute Walk Test

All participants performed the six minute walk test (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002) which was validated previously in obese adults (Beriault et al., 2009). The participants walked back and forth in a hallway between 2 cones that were placed 100 feet apart. The participants were instructed that the goal was to walk as far as possible in 6 minutes. Six minute walk distance was recorded as total distance walked (in feet).

Physical Performance Test (PPT)

The modified 9-item PPT was used to assess physical performance in all participants. This test is designed to mimic activities of daily living and the 9-item PPT has been shown to correlate well with disability and frailty (Brown et al., 2000, Binder et al., 1999, and Brown et al., 2000). The 9-item PPT includes placing a book on a shelf, putting a lab coat on and taking it off, picking up a coin from the floor, a 25 foot walk down and back at a fast speed, turning in a 360 degree circle, simulated eating, writing a sentence, climbing a flight of stairs with 10 steps, and sit to stand 5 times from a low chair. Each of the items is scored on a scale of 0-4 based on the time it takes to complete the task. Each task is performed twice and the average time is used to determine the 0-4 score. A maximum score is indicated by a score of 36.

Stair Power Measure

Stair power (in watts) was calculated based on the time it took each participant to climb a flight of 10 stairs as part of the PPT (average of 2 trials) using the following formula:

$$\text{Stair Power} = 3.171 * \text{Weight(kg)} / \text{time(sec) Climb avg} * 1/.1383 * 1/.7378$$

Where 3.171=Distance Traveled (m)

Climb avg= average time to climb a flight of 10 stairs (sec);

1/.1383=Conversion (kgm/s to ftlbs/s);

1/.7378=Conversion (ftlbs/s to Watts)

Subjects were allowed to touch a handrail for balance, but not for pulling or pushing to ascend the stairs.

Intermuscular Adipose Tissue (IMAT)

Calf intermuscular adipose tissue volumes were quantified using MRI on the right leg of each participant. The MRI scans were performed with the participant in a supine position with a Siemens CP extremity coil placed over the right calf muscle. The MRI measurements were performed with a 3.0-Tesla superconducting magnet with a pulse sequence of TE=12 milliseconds, TR=1,500 milliseconds, matrix=256x256; both a fat saturated and a non fat saturated image were collected (Hilton et al., 2008). Thirty transverse slices were collected beginning at the joint space of the knee and proceeding distally. The slices were 7mm thick with no interslice gap. Nine consecutive slices were selected to calculate muscle and IMAT volumes. Volumes were quantified using a PC workstation and custom Matlab software. The software uses voxel brightness to distinguish between muscle and adipose tissues. The subcutaneous adipose tissue was removed from each image by drawing a line along the deep fascial plane surrounding the calf muscle so that only the fat within and between the muscles (IMAT) was remaining. The software uses edge detection algorithms to assist the user in separating the subcutaneous fat from the muscle as well as separating individual muscles and muscle compartments. In the calf, the muscle was divided into 1) the anterior compartment, 2) the lateral compartment, 3) the deep compartment, 4) the gastrocnemius muscle, and 5) the soleus muscle. Figure 1. Calf IMAT and calf muscle volumes are reported in cm³. An additional variable, IMAT per muscle volume was also used in analysis. Based on

test-retest reliability of 21 subjects, the error in measuring muscle volume is less than 1% and less than 2% for measuring fat volumes on average in any muscle or compartment.

Ankle Dorsiflexion and Plantarflexion Peak Torque and Power

Concentric isokinetic ankle dorsiflexor and plantarflexor peak torque and power were assessed using a Biodex Multijoint System 3 Pro isokinetic dynamometer. The tests were performed at angular velocities of 60°/s. The average power at 60°/s was determined by the time-averaged integrated area under the curve at the constant velocity of movement in the available range of motion (Hilton et al., 2008). All participants were given 3 practice trials to ensure they were comfortable with the test. The mean values for peak torque and average power were calculated for 3 trials.

Statistical Methods

Statistical analyses were performed using Systat for windows, version 13.0. An analysis of variance was used to examine the main and interaction effects of group (DM, DM+PN, NoDMPN) and measures of physical performance, calf muscle volume, and calf IMAT (gastrocnemius, soleus, anterior compartment, lateral compartment, deep compartment). Post-hoc t-tests were used to examine differences in groups (DM, DM+PN, NoDMPN) on the variables of physical performance, calf IMAT, and calf muscle volumes as needed based on the results of the ANOVA. Pearson correlations were used to examine the associations between variables. Significance level was set at $P=0.05$.

Results

Group differences were significant for measures of physical performance: six minute walk distance ($P=0.04$), physical performance test ($P=0.001$) and stair power ($P=0.04$). Table 3. The NoDMPN group had a longer six minute walk distance than the DM or DM+PN groups. The PPT scores were highest for the NoDMPN group and lowest for the DM+PN group. Stair power values were highest for the NoDMPN group and lowest for the DM+PN group. There were no group differences between total calf muscle volume, calf IMAT volume, anterior compartment muscle volume, anterior compartment IMAT volume, lateral compartment muscle volume, lateral compartment IMAT volume, deep compartment muscle volume, deep compartment IMAT volume, soleus muscle volume, soleus IMAT volume, gastrocnemius muscle volume, gastrocnemius IMAT volume, and IMAT/Muscle Volume. Table 2. There were group differences in dorsiflexor peak torque and power values with the DM group having higher dorsiflexor peak torque and power than the DM+PN and NoDMPN groups ($P=0.00$), but no group differences in plantarflexor peak torque and power ($P=0.37$).

Across all participants, calf IMAT volume was associated with BMI ($r=0.31$) and IMAT volume was associated with poorer physical performance on the 6 minute walk test ($r=-0.47$) and the physical performance test ($r=-0.36$). IMAT/Muscle Volume was also associated with poor physical performance (PPT $r=-0.44$, 6MW $r=-0.48$). Muscle volume was not strongly associated with 6 minute walk distance or physical performance test score, but was associated with stair power ($r=0.51$). Table 4.

The gastrocnemius muscle had a higher ratio of IMAT/Muscle volume than any other muscle or compartment [0.286(0.37) (gastrocnemius) vs. 0.131(0.13) (soleus) vs.

0.154(0.16) (anterior compartment) vs. 0.181 (deep) vs. 0.187 (lateral) $P=.005$] across all participants.

Discussion

This study is the first to examine the impact of IMAT accumulation on specific calf muscle compartments and muscle groups and relates it to calf muscle performance and individual level physical performance. In addition, this study shows that people with DM and DM+PN have lower physical performance in comparison to a group without DM or PN matched for age and BMI. This study is the first to report that IMAT accumulation may be muscle and compartment specific in people with diabetes, peripheral neuropathy, or obesity.

Interestingly, while there were no measurable differences in muscle morphology between the 3 groups, there was a difference in measures of physical performance between the 3 groups. We found that the NoDMPN (obese) group had higher levels of physical performance than the group with DM and the group with DM+PN. These results could indicate that the pathologies of diabetes and diabetes and neuropathy have a greater impact on physical performance than obesity alone.

Overall, the inverse correlation between calf IMAT volume and physical performance indicates that IMAT accumulation is associated with physical performance decline, but it appears that there are other factors, such as the presence of diabetes and/or neuropathy that are key mediators of physical performance. The ratio of IMAT/Muscle volume was inversely related to measures of muscle performance across all subjects. The ratio of calf IMAT/Muscle volume may be a predictor of physical performance, but the

IMAT/Muscle volume does not differ between those with diabetes and diabetes and neuropathy compared to a NoDMPN, obese group of subjects. These results are consistent with other reports in the literature and suggest measures other than absolute muscle volume or muscle cross-sectional area are needed to completely characterize calf muscle composition and muscle performance (Hilton et al., 2008, Park et al., 2006). These data are also consistent with reports that people with DM or DM+PN have limitations in physical performance and function beyond what is fully explained by muscle changes alone (Park et al., 2006, Anderson et al., 1997). Certainly problems secondary to sensory neuropathy can contribute to these deficits in physical performance (Cavanagh et al., 1992).

We found, contrary to our expectations, that there were no group differences in measures of IMAT volumes, or muscle volumes between groups with DM, a group with DM+PN, and a NoDMPN group matched for age and BMI. These results indicate that diabetes and peripheral neuropathy were not associated with IMAT accumulation in the calf beyond their association with BMI in these groups of subjects. These results were surprising because our previous study indicated that a group with diabetes, peripheral neuropathy and obesity had two times the volume of IMAT compared to a non-obese, non diabetic, non- neuropathic control group (Hilton et al., 2008). We expected that the presence of diabetes or diabetes and neuropathy in addition to obesity would have an additive effect on IMAT volume of the calf muscles. Our previous study had a small sample size (6 in each group) and we reported IMAT volumes that were larger than what we report here. Four of the six subjects with DM+PN in that group were sampled from a patient sample with a history of foot ulcers rather than the community at large, so it is

possible that we were capturing a group with more severe involvement of DM and PN than what we report here.

This is the first study to report that the amount of IMAT/Muscle volume in the calf was muscle specific in these pathologies. The gastrocnemius muscle had the largest ratio of IMAT/Muscle volume of any of the calf muscles and compartments, which was contrary to what we expected. It is possible that individual muscles are affected by IMAT infiltration differently and the gastrocnemius muscle may be preferentially affected by IMAT infiltration. We can only speculate that perhaps those muscles with a predominance of fast-twitch fibers are affected by IMAT accumulation preferentially or sequentially. The plantar flexor muscles are important for ankle stability, walking velocity and cadence (Perry et al. 1993). Furthermore, the gastrocnemius is used more for power and burst type activity compared to the soleus muscle which is most active for postural control. Perhaps the gastrocnemius is more affected by IMAT than the soleus muscle due to a greater reduction in power activities compared to postural activities in these groups. Additional studies are required to determine the underlying mechanisms for the IMAT accumulation in the gastrocnemius muscle and its propensity for having greater fat infiltration than other calf muscles. Understanding the muscle specific distribution of fat and the underlying mechanisms for fat infiltration may lead to enhanced treatment strategies to improve the health of the muscle and individual. For example, Marcus et al. (2008) demonstrated that people with type 2 diabetes were able to improve performance, decrease fat, and increase lean tissue in the thigh muscles after a 16 week exercise program that included both aerobic and high-intensity eccentric exercise training. Perhaps specific rehabilitation strategies that target the gastrocnemius

muscle could alter the fat infiltration and improve deficits in muscle performance and physical performance.

Of note, the NoDMPN group had an average HbA1c value of 5.8 which is indicative of people at risk for developing diabetes (Zhang et al. 2010). This is consistent with other reports in the literature that link IMAT with insulin resistance (Goodpaster et al., 2000 and Gallagher et al., 2005) and this could be a potential indicator of those at risk for developing diabetes. Interestingly, the NoDMPN group had higher levels of physical performance than the DM or DM+PN groups, so perhaps an intervention targeted at minimizing IMAT could diminish risk for developing diabetes and mitigate the functional decline that is associated with diabetes and diabetes and peripheral neuropathy.

There are limitations that should be considered. First, we have a relatively small sample size. Based on the small effect size between groups, a post-hoc power analysis revealed that we would need to collect data on more than 3600 individuals to determine group differences in total IMAT in the calf with a power of 0.80 and an alpha level at 0.05. Given this power analysis, we believe that total IMAT accumulation in the calf is not due to an additive effect of diabetes and diabetes and neuropathy in this particular sampling of subjects. The magnitude and impact of IMAT accumulation in specific calf muscles or compartments in people with severe diabetes and peripheral neuropathy requires additional investigation. This study is also limited in that we do not have a measure of activity level for each participant, so it is possible that our groups could be different from each other in levels of activity. Since the group with DM+PN was originally recruited for an exercise study, it is possible that we have a selection bias towards people with DM+PN who are higher functioning. In addition, this group only

had 2 people with a history of plantar foot ulcer, so we do not believe these results are generalizable to people with more severe complications and longer durations of diabetes and peripheral neuropathy. Lastly, the correlations between the different variables within and between groups only indicate association and cannot determine cause and effect.

In conclusion, this study found that increased calf IMAT was associated with poorer physical performance and IMAT may target specific muscles and muscle compartments differently, specifically; the gastrocnemius muscle was more affected than other muscles of the calf. The groups with DM and DM+PN had lower measures of physical performance than the NoDMPN group, suggesting that more severe impairment in metabolic pathology along with IMAT accumulation impacts physical performance.

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Disclosure Statement

The authors deny any conflict of interest present in this work.

Table 1. Subject Demographics by Group. Values are means(SD).

Group	N	Gender (M/F)	Age (years)	BMI (kg/m ²)	Weight (lbs)	Diabetes Medication (oral only/insulin &oral)	HbA1c	DM Duration (years)
DM	11	5/6	56(9)	35.5(6.4)	226(33)	6/5	8.1(2.2)	8.1(6.9)
DM+PN	24	15/9	64(13)	32.6(6.3)	217(45)	13/11	7.1(1.3)	12.9(9.0)
NoDMPN	10	4/6	64(9)	32.9(4.6)	213(41)	NA	5.8(0.2)	NA

NA: Not Applicable

Table 2. ANOVA results: muscle morphology measures; all values are means(SD) in cm³.

	DM	DM+PN	No DMPN	<i>P</i> -Values
Muscle Volume	434(72)	407(88)	404(90)	0.65
IMAT Volume	65(36)	70(40)	67(54)	0.94
Anterior Compartment Muscle Volume	69(15)	65(14)	62(9)	0.57
Anterior Compartment IMAT Volume	10(11)	9(5)	7(5)	0.53
Lateral Compartment Muscle Volume	38(9)	37(11)	36(10)	0.85
Lateral Compartment IMAT Volume	5(3)	7(4)	6(5)	0.62
Deep Compartment Muscle Volume	54(13)	60(12)	51(15)	0.15
Deep Compartment IMAT Volume	8(4)	11(5)	9(6)	0.23
Soleus Muscle Volume	130(26)	118(32)	127(28)	0.46
Soleus IMAT Volume	14(8)	15(10)	14(14)	0.97
Gastrocnemius Muscle Volume	142(27)	122(40)	128(31)	0.35
Gastrocnemius IMAT Volume	27(15)	28(21)	31(28)	0.90
IMAT/Muscle Volume	0.158(0.11)	0.193(0.16)	0.144(0.07)	0.58

Anterior Compartment: comprised of tibialis anterior, extensor digitorum longus, and extensor hallucis longus muscles

Lateral Compartment: comprised of peroneus longus and brevis muscles

Deep Compartment: comprised of the tibialis posterior, flexor digitorum longus and flexor hallucis longus muscles

Table 3. ANOVA results: physical performance measures. Values are means(SD)

	DM	DM+PN	No DMPN	<i>P</i> -Values
DFPT (Nm)	15.3(7.5) ^a	4.5(5.3)	5.2(4.3)	0.00
DFPOW (W)	9.8(6.6) ^a	2.2(3.2)	2.3(2.4)	0.00
PFPT (Nm)	48.1(13.0)	51.4(16.5)	58.0(18.6)	0.37
PFPOW (W)	38.7(10.9)	41.9(18.1)	45.9(15.1)	0.38
6MW (feet)	1508(262)	1396(323)	1681(159) ^a	0.04
PPT	31(2.4)	28(4.0)	34(1.5)	0.001*
Stair Power (W)	671(163)	601(226)	808(327)	0.04*

* indicates all 3 groups are different

^a indicates that group is different from other 2 groups

DFPT=dorsiflexor peak torque

DFPOW=dorsiflexor power

PFPT=plantarflexor peak torque

PFPOW=plantarflexor power

6MW=six minute walk distance

PPT=physical performance test (9-item)

Table 4. Correlation Matrix.

	IMAT vol	6MW	PPT	StairPOW	MuscleVol	IMAT/MusVol
BMI	0.31*	-0.18	0.01	0.21	0.49*	0.08
IMAT vol		-0.47*	-0.36*	-0.18	-0.32*	0.93*
6MW			0.79*	0.58*	0.25	-0.48*
PPT				0.60*	0.24	-0.44*
Stair Pow					0.51*	-0.30*
Muscle vol						-0.35*

* indicates significance ($P < 0.05$)

IMAT=intermuscular adipose tissue volume

6MW=six minute walk distance

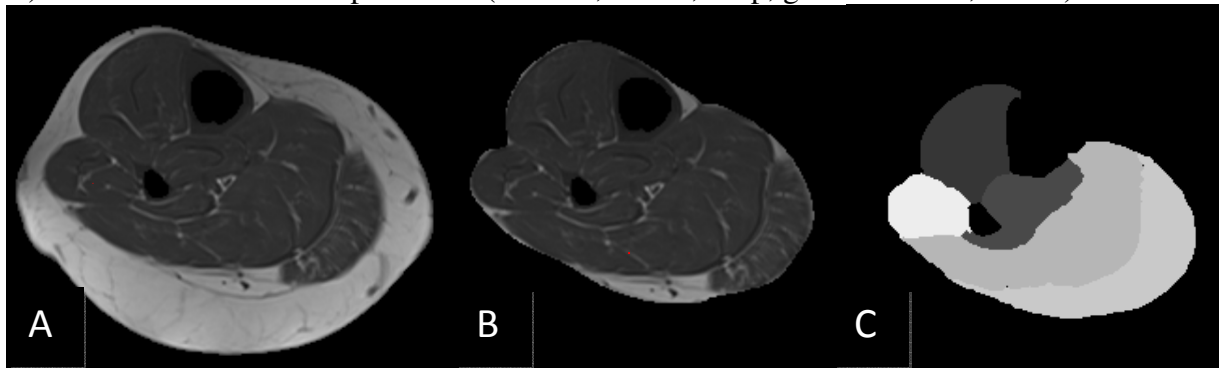
PPT=physical performance test (9-item)

Stair Pow=stair power

Muscle Vol=calf muscle volume

IMAT/MusVol=ratio of IMAT/Muscle Volume in the calf

Figure 1. A) MRI image of calf with bone removed. B) Subcutaneous adipose tissue removed. C) Calf divided into 5 compartments (anterior, lateral, deep, gastrocnemius, soleus)



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Chapter 3

Lower Physical Activity is Associated with Higher Intermuscular Adipose Tissue in People with Type 2 Diabetes and Peripheral Neuropathy

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ABSTRACT

BACKGROUND. Increased lipid accumulation in skeletal muscle has been linked to insulin resistance, impaired muscle performance, and impaired physical function. It is unclear if physical activity is associated with lipid content in skeletal muscle, muscle performance, or overall physical function.

OBJECTIVE. To characterize physical activity levels (average daily step count) in a sample with diabetes and peripheral neuropathy and to determine the relationship between step count, intermuscular adipose tissue volume (IMAT), muscle performance (peak torque, power), and physical function.

DESIGN. 22 people with diabetes and peripheral neuropathy [15 men/7 women; aged 64.5(12.7); BMI 33.2(6.4)] participated. Average daily step count, HbA1c, modified 9-item Physical Performance Test, 6 minute walk distance, calf intermuscular adipose tissue volume (via MRI), and isokinetic dynamometry of the ankle muscles were measured.

RESULTS. Average daily step count was 7754(4678) steps/day, with a range of 3088 to 20079 steps/day. There were 5 subjects with an average daily step count greater than 10,000. Average IMAT volume was 84(88)cm³. Greater average daily step count was associated with younger age ($r=-0.39$, $P<0.05$) and with lower IMAT volume in the calf, (-0.44 , $P<0.05$). Lower IMAT volume was associated with greater muscle performance ($r=-0.45$) and physical function ($r=-0.43$ - -0.48).

CONCLUSIONS. Average daily step count was inversely related to IMAT and IMAT was inversely related to muscle performance and overall physical function. In addition

we found that people with diabetes, peripheral neuropathy, and without severe foot deformity appear to be able to take a high number of steps per day.

Diabetes mellitus is a chronic disease that affects almost 24 million people, with 60-70% developing nervous system impairments, including peripheral neuropathy.¹ Increased physical activity has been shown to decrease pain and improve balance including in people with diabetes and peripheral neuropathy.² Improved physical activity is particularly important for people with diabetes, not only for improved glucose control and cardiovascular fitness, but also because people with diabetes are two times more likely to have limitations in physical mobility, particularly in the presence of peripheral neuropathy.³ The mechanisms for physical mobility limitations in diabetes are unclear, but previous work has demonstrated that people with diabetes, peripheral neuropathy and obesity have an increased amount of adipose tissue (i.e. fat) in the muscles of the lower extremity that is not noted in their peers and this increased intermuscular adipose tissue is associated with poor physical functional status.⁴ Intermuscular adipose tissue (IMAT) is defined as the visible extra-cellular adipose tissue that is located beneath the muscle fascia and between and within muscle groups.⁵ The mechanism by which IMAT potentially impairs physical function is unclear, but decreased muscle strength and power has been associated with an increased amount of IMAT in the calf and thigh muscles of people with diabetes and peripheral neuropathy thereby suggesting increased IMAT as a mechanism for impaired physical function.^{4,6} IMAT has been linked to immobilization in healthy young adults⁷ and has been shown to be reduced with exercise in a population with diabetes and without peripheral neuropathy⁸ indicating that physical activity may be a potent modifier of IMAT content in skeletal muscle. IMAT has also been shown to be higher in the affected limb in people with chronic stroke⁹ and spinal cord injury¹⁰ and in rotator cuff injury with nerve involvement¹¹ indicating that nerve dysfunction (such as

peripheral neuropathy) could impact IMAT. In addition, IMAT has been associated with insulin resistance,¹² suggesting that IMAT may contribute to or worsen the metabolic impairments of skeletal muscle that accompany diabetes. It is therefore important to determine if IMAT is influenced by physical activity, specifically walking activity and if so, if increases in physical activity are associated with greater muscle performance, glucose control and physical function in people with DM and PN.

The purpose of this study was to characterize activity levels (average daily step count) in people with diabetes and peripheral neuropathy and to determine the relationship between activity level and IMAT volume in the calf, calf muscle volume, muscle performance, physical function, and glucose control. The American Diabetes Association recommends that people take 10,000 steps per day,¹³ but we hypothesized that people with diabetes and peripheral neuropathy would take around 5,000 steps per day based on prior observations and reports.¹⁴⁻¹⁶ We hypothesized that higher average daily step count would be associated with lower IMAT volume, larger muscle volume, improved glucose control (lower HbA1c levels), higher muscle performance, and higher physical function.

METHOD

Participants

Twenty-two subjects with type 2 diabetes and peripheral neuropathy [15 men/7 women; aged 64.5(12.7); BMI 33.2(6.4)] were recruited for this study as part of a larger study involving exercise for people with diabetes and peripheral neuropathy from the Washington University School of Medicine Diabetes Clinic and from diabetes clinics in the surrounding St. Louis area. Participants were excluded if they weighed more than 300

pounds (equipment weight limit), had a history of severe foot deformity or amputation, any co-morbidity or medications that would interfere with exercise (such as severe rheumatoid arthritis, peripheral arterial disease (absent pulses), dialysis, or current cancer treatment). Participants were excluded if they had a current foot ulcer; 2 of the 22 participants had a history of a previous foot ulcer that had been healed for at least 6 months. Participants provided written informed consent. This study was approved by the Human Research Protection Office at Washington University in St. Louis.

Assessments

Peripheral Neuropathy

Presence of peripheral neuropathy was determined based on an inability to feel the 5.07 Semmes-Weinstein monofilament on at least one point on the plantar surface of the foot and based on a vibration perception threshold of greater than 25 volts as measured with a biothesiometer.^{16,17}

Activity Monitoring

For the purposes of this study, activity level refers specifically to average daily step count as recorded by a StepWatch activity monitor.* Participants were given an activity monitor and were instructed to wear the monitor around their ankle during all waking hours. The participants were given the monitor for at least 9 consecutive days calculating an average of 7 days. The first and last days of wear were deleted because a full 24 hours would not be recorded. For a day to be included, the monitor had to be worn for at least 8 hours, and at least 1 weekend day was included in the 7 day average. The device records strides per day and steps per day were calculated by multiplying

* Orthocare Innovations, 840 Research Parkway, Suite 200, Oklahoma City, OK 73104.

strides by 2 (1 stride equals 2 steps). Results of validity tests performed in our laboratory indicate a mean absolute error of 1.1% (SD, 3.1%) for healthy obese subjects and 1.8% (SD, 2.4%) for subjects with diabetes mellitus and peripheral neuropathy.¹⁶

Six Minute Walk Test

All participants performed the six minute walk test¹⁸ as a measure of physical function and walking endurance which was validated previously in obese adults.¹⁹ The participants walked back and forth in a hallway between 2 cones that were placed 100 feet apart. The participants were told that the goal was to walk as far as possible in 6 minutes; the test was not repeated. Six minute walk distance was recorded as total distance walked in feet.

Physical Performance Test (PPT)

The modified 9-item PPT was used to assess physical function in all participants. This test is designed to mimic activities of daily living and the 9-item PPT has been shown to correlate well with disability and frailty.²⁰⁻²² Each of the items is scored on a scale of 0-4 based on the time it takes to complete the task. Each task is performed twice and the average time is used to determine the 0-4 score. A maximum score is indicated by a score of 36.

Intermuscular Adipose Tissue (IMAT)

Intermuscular adipose tissue volumes were quantified using MRI on the right leg of each participant using previously established methods.⁴ The MRI scans were performed with the participant in a supine position with a Siemens CP extremity coil placed over the right calf muscle. The MRI measurements were performed with a 3.0-T

superconducting MRI instrument[†] with a pulse sequence of TE=12 milliseconds, TR=1,500 milliseconds, matrix=256x256. Thirty transverse slices were collected beginning at the joint space of the knee and proceeding distally. The slices were 7mm thick with no interslice gap with voxel size of 0.7x0.7x7mm. The fifteen center consecutive slices were selected to calculate muscle and IMAT volumes. Volumes were quantified using a PC workstation and Analyze Direct software version 9.0[‡] which uses voxel brightness to distinguish between muscle and adipose tissues.⁵ The subcutaneous adipose tissue was removed from each image by drawing a line along the deep fascial plane surrounding the calf muscle so that only the fat within and between the muscles (IMAT) was remaining. On a subset of 10 participants, intraclass correlation coefficient (2,1) values of the same scan by the same observer averaged 0.98 when scans were measured at least 7 days apart.

Ankle Dorsiflexion and Plantarflexion Peak Torque and Power

Concentric isokinetic ankle dorsiflexor and plantarflexor peak torque and power as measures of muscle performance were assessed using a Biodex Multijoint System 3 Pro isokinetic dynamometer.[§] The tests were performed at angular velocities of 60°/s. The average power at 60°/s was determined by the time-averaged integrated area under the curve at the constant velocity of movement in the available ankle joint range of motion.⁴ All participants were given 3 practice trials to ensure they were comfortable

[†] Siemens Corp, Citicorp Center, 153 E. 53rd St., New York, NY 10022.

^{‡‡} Mayo Clinic, 200 First St. SW, Rochester, MN 55905.

[§] Biodex Medical Systems, 20 Ramsay Road, Shirley, NY 11967.

with the test. The mean values for peak torque and average power were calculated for 3 trials.

Data Analysis

Statistical analyses were performed using Systat for windows, version 13.0. ** Pearson correlation coefficients were used to determine association between variables. Multiple regression analysis was performed with average step count as the dependent variable and IMAT volume, age, BMI, HbA1c, duration of diabetes, muscle performance (dorsiflexion and plantarflexion peak torque and power) and physical performance (six minute walk distance and physical performance test score) as the independent variables. Statistical significance was set at $P < 0.05$. Post hoc, the 22 participants were divided into 3 groups based on step count in an effort to determine if there was something different between people who were taking more steps than the group mean (greater than 8000 steps per day), those who were in our expected range of step count (5000-8000 steps per day), and those who were below the expected step count (less than 5000 steps per day). A one-way ANOVA was performed with age as a covariate to determine differences in IMAT volumes that were due to group differences in activity level. ANOVAs were also performed to determine differences in IMAT volume, muscle volume, muscle performance, PPT and 6 minute walk distance based on group differences in activity level (Table 1). Statistical significance was set at $P < 0.05$.

Results

** Systat Software, Inc., 225 W Washington St., Suite 425, Chicago, IL 60606.

Average daily step count in these 22 participants with diabetes and peripheral neuropathy ranged from 3088 steps per day to 20079 steps per day with an average of 7754(4678) steps per day. Participant characteristics are listed in Table 1. In order to characterize the people who were more active and those who were less active, the 22 participants were categorized into 3 groups based on average step count (less than 5000 steps/day, 5000-8000 steps/day, and greater than 8000 steps per day) (Table 1). The group that was taking less than 5000 steps/day had 3 times the volume of IMAT in their calf muscles compared to the groups that were taking 5000-8000 steps per day and greater than 8000 steps per day (157(127) cm³ vs. 53(27) cm³ vs. 42(22) cm³; $P<0.05$). However, the more active group also was younger than the other 2 groups (Table 1) therefore, a one-way ANCOVA also was performed with IMAT volume as the dependent variable, step count as the independent variable and age as a covariate. Results were unchanged and IMAT volumes remained significantly different ($P=0.03$) between the groups.

A multiple regression analysis was used to determine the variance in average step count accounted for by IMAT volume, age, BMI, HbA1c, diabetes duration, muscle performance, and physical function (Table 2). IMAT accounted for 19% of the variance in average step count ($P=0.04$). The other independent variables did not account for a significant portion of the variance in IMAT in this sample ($P>0.05$).

Activity as indicated by average step count was associated with age ($r=-0.39$) and with IMAT volume ($r=-0.44$), but average step count was not associated with glucose control (as indicated by HbA1c), muscle performance, muscle volume, or physical function ($P>0.05$) (Table 3). IMAT volume was associated with select indicators of

muscle performance (plantarflexion peak torque $r=-0.45$, $P=0.03$) and physical function (PPT $r=-0.43$ $P=0.04$; 6MW $r=-0.48$ $P=0.02$) in addition to an association with average step count ($r=-0.44$ $P=0.04$).

Discussion

This study is the first to demonstrate an inverse relationship between physical activity levels and intermuscular adipose tissue (IMAT) volume in calf muscle in people with diabetes and peripheral neuropathy. We have also demonstrated a significant relationship between increased IMAT volume and lower muscle performance and physical function. This finding is in agreement with previous investigators who found that increased IMAT was associated with lower muscle performance^{4,6,12} suggesting that IMAT may be a potential pathway for intervention to attenuate the impairments associated with diabetes and peripheral neuropathy. However, these data provide only an association between these variables and do not imply causation. More studies are needed to elucidate the role of IMAT in these processes. In addition, these findings lead us to question whether increasing physical activity could decrease IMAT volume and be a way to improve physical function in people with DM and PN. IMAT has also been linked to insulin resistance²³⁻²⁶ in people with metabolic syndrome as well as frank diabetes. The exact relationship between IMAT and insulin resistance is not yet understood, but there is some evidence that weight loss²⁷ and exercise²⁸⁻³⁰ can decrease the amount of IMAT and improve insulin sensitivity. It is unknown whether people with diabetes and peripheral neuropathy demonstrate these same effects on IMAT in response to exercise and weight loss.

We hypothesized that higher average daily step count would be associated with less IMAT volume, larger muscle volume, improved glucose control, higher muscle performance, and higher physical function. This study showed that average daily step count is inversely correlated with IMAT volume, but step count was not correlated with glycemic control, muscle volume, muscle performance or measures of physical function. IMAT volume was inversely correlated with muscle performance (plantarflexor peak torque) and with measures of physical function (PPT score and 6 minute walk distance). These findings suggest that activity could be a modifiable factor that has the potential to impact muscle morphology (IMAT volume) and therefore improve physical function. In fact, the people who were the least active actually had three times the volume of IMAT in the calf as the people who were most active, on average (Table 1). Physical activity has been associated with IMAT in a young healthy population,⁷ but this is the first study reporting that activity is associated with IMAT volume in a population with diabetes and peripheral neuropathy. These results provide evidence for a potential way to impact not only the metabolic disturbance of diabetes but to also improve the functional deficits that have been shown to occur in people with diabetes and peripheral neuropathy. Indeed, Marcus et al.⁸ demonstrated that people with type 2 diabetes were able to improve performance, decrease fat, and increase lean tissue in the thigh after a 16 week exercise program that included both aerobic and eccentric exercise training. Additional studies are needed to determine if more intensive interventions targeted to increase activity or muscle strength can improve muscle and physical function in people with diabetes and peripheral neuropathy. While the relationship between average step count and IMAT volume was significant, there was also great variability in IMAT volume, particularly in

people with a lower step count. As noted in our multiple regression analysis, IMAT only accounted for 19% of the variation in step count and there are many other factors that could be contributing to the variability seen in both IMAT and step count. More studies are needed to be able to determine the variety of factors that impact IMAT volume.

In our previous work⁴ we found that people with diabetes, peripheral neuropathy and obesity had a greater amount of IMAT volume in their calf muscles than their age-matched peers who did not have diabetes or peripheral neuropathy and who were not obese. In our previous study, IMAT volume was related to muscle performance and function. However, we did not have measures of activity level in our previous study. The findings in this study that IMAT volume was associated with muscle performance and function are consistent with our previous results and expand upon this previous work to include activity level as a factor that may influence muscle performance and physical function. The results suggest that activity is associated with muscle morphology and could potentially be a way to improve the impaired physical function that accompanies chronic diabetes.

Based on previous studies, we hypothesized that people with diabetes and peripheral neuropathy would be taking around 5,000 steps per day.^{15,16} We hypothesized this because generally, the expectation is that people with diabetes and peripheral neuropathy are less active than their peers, however, the group for this study had an average daily step count of 7919 and there were 5 people with diabetes and peripheral neuropathy who were taking more than 10,000 steps per day. The average step counts in this study are consistent with those from Maluf et al¹⁶; however they are significantly higher than those reported by Armstrong et al.¹⁵ Because we were recruiting for an

exercise study, our sample may be biased toward those who have high activity and limited our ability to recruit participants who are less active.

Surprisingly, HbA1C levels were not associated with activity. This is in conflict with other reports in the literature that indicate that exercise and increased physical activity will improve glucose control.³¹ There are several potential explanations for this. First, the participants in this study were not participating in a regular exercise program; the activity measure is a measure of regular daily walking activity. Intensity of exercise is a key factor in maintaining blood glucose control, and perhaps normal daily walking, regardless of total number of steps, does not reach an intensity level that is consistent with regulating blood glucose over the previous 3 to 4 month time period that HbA1c indicates. Also, one limitation of this study is that we do not have any information on diet in these participants and it is possible that dietary habits contributed more to overall HbA1c values than low intensity physical activity in this particular sample. It is also possible that some of the participants in this study were not compliant in taking their daily medication, which could also adversely affect HbA1c values.

While this study provides important information about the role of activity in people with diabetes and peripheral neuropathy, there are limitations that should be considered. First, the participants in this study were all interested in participating in an exercise study, so it is possible that our sample is biased towards those who tend to be more active. It is unclear whether our results are generalizable to people who are taking less than 3000 steps/day. In addition, we excluded people who had a current foot ulcer, peripheral arterial disease, severe foot deformity or amputation, or who weighed greater than 300 pounds. Our results should not be generalized to these populations. There is

evidence that walking capacity and performance decrease in the presence of severe foot deformity or amputation.³² Additional research is needed to clarify the contraindications and safety precautions for a walking program or weight bearing exercise program given various levels of foot deformities and impairments. Also, based on these data, we can only say that step count and IMAT volumes are associated, but we cannot specify causation.

In conclusion, physical activity is associated with IMAT volume and IMAT volume is related to muscle performance and overall physical function. In addition we found that people with diabetes, peripheral neuropathy, and without severe foot deformity appear to be able to take a high number of steps per day. More evidence is needed to determine if an exercise program designed to increase step count will decrease the amount of IMAT volume and enhance muscle performance and physical function.

Acknowledgments

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Table 1. Values are mean (SD) unless otherwise indicated

Step Count (steps/day)	N	Gender (M/F)	Age (yrs)	BMI	HbA1c	Duration of Diabetes (yrs)	Vibration Perception Threshold (Volts)	Average Step Count (steps/day)	IMAT (cm ³)	Muscle Volume (cm ³)	PPT	6MW (feet)	PFPT (ft lbs)
<5000	7	4/3	68(14)	35.5(6.7)	7.0(1.2)	11(6)	42(10)	3995(624)	157(127)*	735(206)	26(5)	1147(300)*	33.3(12.5)
5000-8000	8	6/2	69(10)	30.5(6.1)	6.9(0.4)	11(9)	39(11)	6310(1032)	53(27)	811(253)	30(2)	1564(244)	42.7(10.5)
>8000	7	5/2	55(11)*	33.9(6.2)	7.4(2.2)	11(7)	40(11)	13162(4645)	44(22)	880(66)	29(3)	1471(346)	44.1(7.9)
<i>P</i> values			0.05	0.30	0.78	0.98	0.86	<0.00#	0.02	0.40	0.07	0.04	0.13
Full Range	22	15/7	65(13)	33.2(6.4)	7.1(1.4)	11(7)	40(10)	7754(4678)	84(88)	809(196)	28(4)	1402(336)	40.1(11.1)

P values are from the ANOVA results between the 3 different groups based on activity level.

PPT=modified 9-item physical performance test score; 6MW=six minute walk distance; IMAT=intermuscular adipose tissue volume in the calf; PFPT=plantarflexor peak torque

* indicates difference from other 2 groups based on post hoc t-tests from ANOVA results ($P<0.05$)

indicates all 3 groups are different from each other based on post hoc t-tests from ANOVA results ($P<0.05$)

Table 2—multiple regression analysis results for Step Count as dependent variable.

	R	R Square	R Square Change	Sig. F Change
IMAT	0.44	0.19	0.19	0.04
Age	0.51	0.26	0.07	0.19
BMI	0.53	0.28	0.02	0.51
HbA1c	0.61	0.38	0.10	0.13
DM Duration	0.61	0.38	0.00	0.84
Muscle Performance	0.63	0.39	0.01	0.90
Physical Performance	0.74	0.55	0.16	0.17

IMAT=Intermuscular adipose tissue; Muscle Performance=dorsiflexion and plantarflexion peak torque and power; Physical Performance=6 minute walk and physical performance test scores

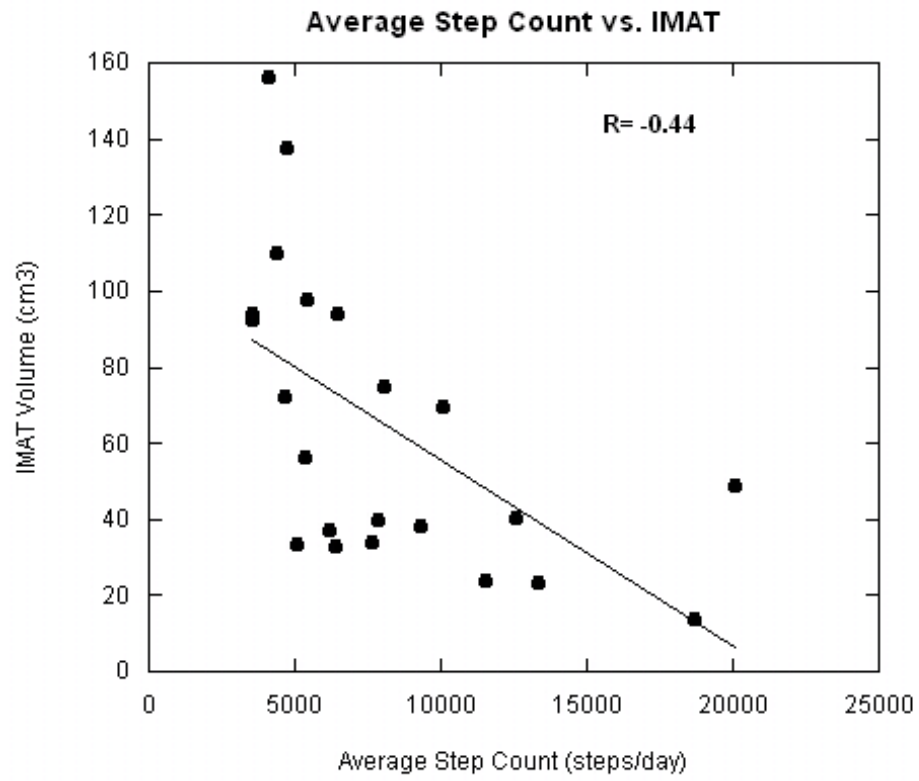
Table 3—univariate correlation matrix

	Steps	MVol	HbA1c	PPT	6MW	IMAT	DFPT	DFPOW	PFPT	PFPOW	DM DUR	VPT
Age	-0.39*	-0.46*	-0.54*	-0.15	-0.22	0.31	-0.20	-0.12	-0.23	-0.21	-0.04	0.36
Steps		0.23	-0.12	0.27	0.10	-0.44*	0.05	-0.02	0.19	0.18	-0.09	-0.03
MVol			0.35	0.09	0.17	-0.45*	0.69*	0.65*	0.36	0.12	-0.02	-0.03
HbA1c				-0.18	0.04	-0.11	0.20	0.15	0.12	0.05	0.22	-0.39*
PPT					0.75*	-0.43*	-0.03	-0.08	0.55*	0.43*	-0.36	-0.08
6MW						-0.48*	0.08	0.03	0.47*	0.48*	-0.27	-0.06
IMAT							-0.06	-0.02	-0.45*	-0.29	-0.05	-0.16
DFPT								0.97*	0.37	0.14	0.03	0.03
DFPOW									0.28	0.02	0.00	0.10
PFPT										0.82*	-0.35	-0.02
PFPOW											-0.43*	-0.08
DM DUR												-0.01

*indicates significance $P < 0.05$

Steps=average daily step count; MVol=muscle volume in the calf; PPT=modified 9-item physical performance test score; 6MW=six minute walk distance; IMAT=intermuscular adipose tissue volume in the calf; DFPT=dorsiflexor peak torque; DFPOW=dorsiflexor power; PFPT=plantarflexor peak torque; PFPOW=plantarflexor power; DM DUR=duration of time with diabetes; VPT=vibration perception threshold (measure of neuropathy)

Figure 1



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Chapter 4

People with Diabetes and Peripheral Neuropathy are able to exercise safely and increase Six Minute Walk Distance

Tuttle LJ, Hastings MK, Mueller MJ. People with Diabetes and Peripheral Neuropathy are able to exercise safely and increase Six Minute Walk Distance.

ABSTRACT

OBJECTIVE— The purpose of this study was to determine the effect of a 12 week progressive walking exercise program on physical performance (Physical Performance Test, 6 minute walk test), calf muscle performance (dorsiflexor and plantarflexor peak torque), and calf muscle structure and composition (intermuscular adipose tissue [IMAT], muscle volume) in people with diabetes and peripheral neuropathy (DM+PN) who complete the 12 weeks of training (EX group) and compare these outcomes to a group of people with DM+PN who do not complete the training (CON group).

RESEARCH DESIGN AND METHODS— Ten subjects in the EX group were compared to 10 subjects in the CON group, all with diabetes and peripheral neuropathy [14 men/6 women; aged 66(12); BMI 36(7)]. Modified 9-item Physical Performance Test, 6 minute walk distance, isokinetic dynamometry of the ankle muscles, and calf IMAT volume (via MRI) were measured.

RESULTS— Following the 12 week exercise intervention, the EX group had a significant increase in the six minute walk distance [1266(263) to 1352(286) feet] compared to the CON group [1409 (293) to 1398 (234) feet] ($P<0.05$). There were no other significant differences between the groups.

CONCLUSIONS—People with diabetes and peripheral neuropathy are able to tolerate a progressive walking and exercise program and increase six minute walk distance. They showed no significant calf muscle changes with this mild exercise intensity.

The benefits of exercise to help control diabetes mellitus (DM) and reduce subsequent complications are well known and accepted (1). In addition to improved DM control and lower mortality rates, walking and increased activity appears to improve complications such as neuropathic pain from peripheral neuropathy (PN) (2). The evidence of the benefits of exercise for people with DM has led the American Diabetes Association (ADA) and the American College of Sports Medicine (ACSM) (1) to recommend that adults with DM participate in at least 30 minutes per day of moderate-intensity physical activity, but the effects of this exercise program is not well known in people with DM+PN.

People with DM+PN have reduced ankle muscle strength and volume with plantar flexor peak torque values in people with DM+PN being about 50% of that in people without DM+PN (3-5). Calf muscle strength is decreased and declines more rapidly in individuals with DM + PN compared to muscular changes associated with normal aging (5). The lower extremity musculature of individuals with DM+PN also demonstrates substantial intermuscular adipose tissue (IMAT) infiltration and muscle atrophy as measured by magnetic resonance imaging compared to people without DM+PN (6). Intermuscular adipose tissue (IMAT) is defined as the visible extra-cellular adipose tissue that is located beneath the muscle fascia and between and within muscle groups (7). It has been demonstrated that IMAT in the thigh is sensitive to changes in activity and exercise programs in an older population (7) as well as in a group with DM (8), but it is unclear whether an exercise program can impact IMAT or lean muscle volume in the calf of people with DM+PN.

Although people with DM+PN clearly have lower extremity impairments, little evidence exists to determine if these impairments can improve with exercise. The Centers for Disease

Control and Prevention reports adults with DM compared to those without have an odds ratio of 2.0 for having mobility limitations. The incidence of impaired mobility increases with peripheral artery disease and/or peripheral neuropathy; subjects with DM and lower extremity diseases had an almost 3-fold increase in risk of impaired mobility compared to those having neither (9). The most frequently reported mobility limitations were related to patients' inability to walk a quarter mile and to walk up 10 steps without resting (9). The ADA and ACSM have recently reported that individuals with peripheral neuropathy and without acute ulceration may participate in moderate weight-bearing exercise (1) and previous studies (10) have indicated that people with DM+PN were able to participate in a low-intensity walking program without increasing risk of ulceration. It is unclear exactly what "moderate weight-bearing exercise" entails and how that might affect physical function and strength in people with DM+PN. A previous study investigating the effects of exercise in people with DM+PN reported no strength changes with a low-intensity exercise program (11) and a Cochrane review of the literature concluded that there is inadequate evidence to evaluate the effect of strengthening exercise for people with DM+PN (12).

The purpose of this study was to determine the effect of a 12 week progressive walking exercise program on physical performance (Physical Performance Test, 6 minute walk test), calf muscle performance (dorsiflexor and plantarflexor peak torque), and calf muscle structure and composition (IMAT, muscle volume) in people with DM+PN who participated in the 12 weeks of training (EX group) and compare these outcomes to a group of people with DM+PN who do not complete the training (CON group). We hypothesized that 1) the EX group would display improved physical performance compared to the CON group 2) the EX group would display increased dorsiflexor and plantarflexor peak torque after a progressive exercise program compared

to the CON group and 3) the EX group would display decreased IMAT in calf muscles and increased calf muscle volume after a progressive exercise program compared to the CON group.

Research Design and Methods

A total of 20 people with DM + PN (14 men/6 women; aged 66 ± 12 ; BMI 36 ± 7) are reported in this study which was a portion of a larger randomized controlled clinical trial involving exercise for people with DM+PN. The primary reason for studying this subgroup was to use MRI to investigate muscle changes secondary to the progressive exercise program. Participant characteristics by group are listed in Table 1. Participants were excluded if they 1) weighed more than 300 pounds (equipment weight limit), 2) had a history of severe foot deformity or amputation, 3) had any co-morbidity or medications that would interfere with exercise, 4) did not have peripheral neuropathy, 5) were currently exercising regularly, 6) had a step count less than 2,000 steps per day or greater than 10,000 steps per day on average (screened with the StepWatch accelerometer over 7 days), 7) or if they had a current foot ulcer; none of the participants in this study had a history of foot ulcer. Participants provided written informed consent. This study was approved by the Human Research Protection Office at Washington University in St. Louis.

Peripheral Neuropathy

Presence of peripheral neuropathy was determined based on an inability to feel the 5.07 Semmes-Weinstein monofilament on at least one point on the plantar surface of the foot and based on a vibration perception threshold of greater than 25V as measured with a biothesiometer (13,14).

Six Minute Walk Test

All participants performed the six minute walk test (15) as a measure of physical function and walking endurance. The test has been validated in obese adults (16). The participants walked back and forth in a hallway between 2 cones that were placed 100 feet apart. The participants were told that the goal was to walk as far as possible in 6 minutes. Six minute walk distance was recorded as total distance walked in feet.

Physical Performance Test (PPT)

The modified 9-item PPT was used to assess physical function in all participants. This test is designed to mimic activities of daily living and includes items such as stair climbing, and coming sit-to-stand. The 9-item PPT has been shown to correlate well with disability and frailty (17). Each of the items is scored on a scale of 0-4 based on the time it takes to complete the task. Each task is performed twice and the average time is used to determine the 0-4 score. A maximum score is indicated by a score of 36.

Intermuscular Adipose Tissue (IMAT) and Muscle Volumes

Intermuscular adipose tissue and lean muscle volumes were quantified using MRI on the right leg of each participant. The MRI scans were performed with the participant in a supine position with a Siemens CP extremity coil placed over the right calf muscle. The MRI measurements were performed with a 3.0-Tesla superconducting MRI instrument with a pulse sequence of TE=12 milliseconds, TR=1,500 milliseconds, matrix=256x256; both a fat saturated and a non fat saturated image were collected (6). Thirty transverse slices were collected beginning at the joint space of the knee and proceeding distally. The slices were 7mm thick with no interslice gap. Nine consecutive slices were selected to calculate muscle and IMAT volumes. Volumes were quantified using a PC workstation and custom Matlab software. The software uses voxel brightness

to distinguish between muscle and adipose tissues. The subcutaneous adipose tissue was removed from each image by drawing a line along the deep fascial plane surrounding the calf muscle so that only the fat within and between the muscles (IMAT) was remaining. The software uses edge detection algorithms to assist the user in separating the subcutaneous fat from the muscle as well as separating individual muscles and muscle compartments. IMAT and muscle volumes are reported in cm^3 . Based on test-retest reliability of 21 subjects, the error in measuring muscle volume is less than 1% and less than 2% for measuring fat volumes on average in any muscle or compartment.

Ankle Dorsiflexion and Plantarflexion Peak Torque

Concentric isokinetic ankle dorsiflexor and plantarflexor peak torque as measures of muscle performance were assessed using a Biodex Multijoint System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). The tests were performed at angular velocities of $60^\circ/\text{s}$. All participants were given 3 practice trials to ensure they were comfortable with the test. The mean values for peak torque were calculated for 3 trials.

Activity Monitoring

Participants were given an activity monitor (StepWatch by Orthocare) and were instructed to wear the monitor around their ankle during all waking hours to determine baseline activity levels. The participants were given the monitor for at least 9 consecutive days calculating an average of 7 days. The first and last days of wear were deleted because a full 24 hours would not be recorded. For a day to be included, the monitor had to be worn for at least 8 hours, and at least 1 weekend day was included in the 7 day average. The device records strides per day and steps per day were calculated

by multiplying strides by 2 (1 stride equals 2 steps). Results of validity tests performed in our laboratory indicate a mean absolute error of 1.1% (SD, 3.1%) for healthy obese subjects and 1.8% (SD, 2.4%) for subjects with DM+PN (13). Results of the activity monitor were used to screen subjects who were inactive or already too active (i.e. walking less than 2,000 steps per day or greater than 10,000 steps per day) and to prescribe specific walking distance to start their progressive walking program as described below.

Exercise Program

The 20 participants were randomly assigned to CON or EX condition. Randomization methods were successful as there were no differences between the groups on the primary outcome variables before the study ($P>0.05$). Subjects who were assigned to the CON group were given an opportunity to participate in the EX program after their 12 weeks in the CON group. Five subjects participated in both the CON and EX groups. The CON group received foot screenings and education regarding foot care every two weeks over a twelve week period for a total of 6 visits. The EX group participated in a weightbearing exercise program three times a week for twelve weeks. The exercises included in this program were adapted from the “Feet First” intervention (10), and those used in prior interventions that reduced falls in frail older adults and those with peripheral neuropathy (18). Where progression for an activity was not described in the published study, we added an appropriate exercise progression to maximize progressive improvements. Activity performance was supervised by a physical therapist. The exercise sessions began with flexibility and stretching exercises. The balance exercises followed a progressively challenging program in which external support decreased and/or

the perturbation to a balanced individual increased (e.g. more compliant surface, movement of arms/legs). The strengthening exercises were focused on the ankle dorsiflexor and plantarflexor muscles for both groups using body weight resistance (i.e. heel raises, bilateral progressing to unilateral for the plantar flexor muscles; stair-climbing and coming sit-to-stand for hip, knee, and ankle extensor muscles) rather than machines or weights in an effort to make the exercises easier to continue after the end of the study.

The aerobic intervention included walking on a treadmill for a duration individually calculated for the subject based on their own average step count collected over 7 days using a Step Watch Activity Monitor as described above. The goal was for subjects to increase their average step count 10% every 2 weeks by increasing their daily step count 24% on the 3 days that they participated in the exercise program. For example, a subject with a beginning average step count of 5950 step/day had a 2 week goal to walk an average of 6548 step/day. This goal would be achieved by walking at their self-selected pace (40-60% estimated heart rate) an additional 1428 steps (about 14 minutes) on each of the 3 treatment days and with no change in regular activity on the other 4 days. This modest increase in activity (10% increase every 2 weeks) would result in the subject walking approximately 10,700 steps/day at the end of 12 weeks (~80% increase), and would meet the surgeon general's expectation for 10,000 steps per day and the ADA's guideline for 30 min/day of walking on most days. Subjects wore a heart rate monitor to assist the physical therapist in assuring that the exercise intensity was within the mild/moderate range (40-60% of max heart rate). Exercise participation was modified, postponed, or stopped based on guidelines established by the ADA and ACSM

(1). Exercise was postponed if 1) blood glucose >300 mg/dl, 2) resting systolic BP of >200 mm Hg, 3) resting diastolic BP of >110 mm hg. If blood glucose was <80-100mg/dl carbohydrates were ingested.

Precautions to avoid skin injury

Before and after each exercise session, the physical therapist and the participant each performed a visual inspection of the participant's feet and footwear. A digital photograph was taken before and after the exercise session to document the skin condition. Foot skin temperature was recorded using a handheld infrared thermometer (Xilas) before and after each session as described previously (19). The temperature was recorded at 7 sites on the plantar surface of the foot where risk for skin breakdown is greatest: the great toe, the 1st, 3rd, and 5th metatarsal heads, medial and lateral midfoot, and the heel. The temperature measures were used to determine areas that might be at risk for injury and were part of a composite assessment for skin injury that included redness, swelling and pain. If there was an area of redness, swelling, pain and increased temperature, activity was limited and the participant was instructed to closely monitor the area and decrease daily activity.

Data Analysis

Statistical analyses were performed using Systat for windows, version 13.0. A repeated measures ANOVA was performed to determine any group by time interactions. Significance was set at $P < 0.05$.

Results

Following the 12 week exercise intervention, the EX group had a significant improvement in the six minute walk distance compared to the CON group. The EX group was able to increase six minute walk distance from 1266 feet to 1352 feet while the CON group changed from 1409 feet to 1398 feet ($P=0.04$). Table 2. There was no difference in PPT scores after a 12 week exercise program (EX 27 to 29 vs. CON 29 to 30) . There was no difference in measures of dorsiflexor peak torque (EX 6.8 to 7.7 vs. CON 5.3 to 3.6 Nm) or plantarflexor peak torque (EX 51.9 to 56.9 vs. CON 54.2 to 57.8 Nm) after a 12 week exercise program. There was no significant difference in IMAT volumes (EX 100 to 103 vs. CON 84 to 93 cm^3) or calf muscle volumes (EX 431 to 425 vs. CON 424 to 420 cm^3) after a 12 week exercise program ($P>0.05$).

Conclusions

In this study, we found that a group of people with DM+PN were able to tolerate a 12 week progressive exercise program and were able to increase their 6 minute walk distance, but showed few other measured changes in calf muscle structure, strength or physical performance. Lord et al (20) and Harada et al (21) demonstrated that 6 minute walk distance appears to provide a measure of overall mobility and physical functioning in an older population rather than a specific measure of cardiovascular fitness which indicates that the EX group improved mobility and physical functioning overall in comparison to the CON group. However, the average 6 minute walk distance for healthy 55-75 year olds has been reported to be 2161 feet (22), so even with this improvement in six minute walk distance, the EX group with DM+PN is still limited in comparison to healthy controls. While the EX group continues to have limitations in comparison to healthy controls, the change in six minute walk distance is consistent with a meaningful

change (>20m or >66 feet) as reported by Perera et al. (23). The change in six minute walk distance that we report is consistent with another recent study involving supervised walking in people with diabetes, but without peripheral neuropathy (24). This study by Negri et al. reports changes in six minute walk distance that are larger than what we report here, but it is important to note that people with diabetes and neuropathy are able to increase six minute walk distance like their peers without neuropathy.

The EX group was able to tolerate the 12 week exercise program and increase their six minute walk distance with a minimum of complications. The EX group attended an average of 85% of the exercise sessions with a total of 4 foot-related complications. One person had an injury from trimming his own toenails, one person had a small abrasion on the second metatarsal head from walking barefoot at home that resolved within 3 days, and one person had a full-thickness wound on the 5th metatarsal head that appeared after callus was removed during a visit to the podiatrist. One person suffered a calf strain during exercise, but was able to continue to exercise with a lower intensity (shorter time on treadmill, fewer heel raises) and the strain resolved within one week. The CON group had two foot-related complications. One person had a cut on the great toe due to walking barefoot at home, and one person had an open lesion between the 4th and 5th toe. Both lesions resolved within 2 weeks. The annual population based incidence of diabetic full-thickness foot ulcers ranges from 1.0-4.1% (25). Due to a relatively low incidence rate of ulceration, we are unable to truly detect differences in ulceration rates in this small sample. We believe the daily pre-post exercise visual inspection to the feet helped to minimize skin injury. In addition, it has been reported (26) that high variability in activity may contribute to plantar ulceration in people with DM+PN. It is possible that

consistently attending the exercise sessions and monitoring overall activity helped to maintain a consistent activity level and reduced the risk of ulceration in this study population.

While six minute walk distance improved in the EX group as expected, we had several unexpected findings from this study. The 12 week progressive exercise program did not have a measureable impact on calf muscle volume, IMAT volume, dorsiflexor or plantarflexor peak torque. Physical performance as measured by PPT score was also unchanged after exercise, which is different from what we had expected. Other investigators have reported significant reduction in IMAT, increased muscle mass in the thigh and increased muscle strength after an exercise program for people with diabetes who do not have peripheral neuropathy (8). This study involved the use of eccentric exercise and higher resistance than in our study. In our study, we did not find changes in IMAT, muscle, or muscle strength in the calf after an exercise program. It is possible that the intensity of our strengthening exercises was not sufficient to cause changes like those that Marcus et al. report. Our study population was specifically people with diabetes and peripheral neuropathy rather than those with diabetes alone, so it is possible that the presence of neuropathy minimized the ability of the calf muscles to respond to the exercise program in the manner that we anticipated.

Goodpaster et al (7) has reported that older adults who participate in an exercise program that uses walking as a main intervention were able to maintain muscle strength and minimize IMAT infiltration in comparison to a group who did not exercise. In that study, the comparison group lost strength and gained IMAT, whereas our CON group remained unchanged in these measures. In that particular study, the exercise intervention

was for one year rather than 12 weeks, so it is possible that with a longer intervention or longer follow up, we might have noticed similar results (i.e. greater decline in CON group and maintenance of muscle strength in EX group). As in the Marcus study, Goodpaster et al measured IMAT and muscle in the thigh rather than the calf. Other investigators (5) have noted that people with diabetes and peripheral neuropathy have more severe involvement distally compared to proximally. It is possible that our intervention could have had an effect on the thigh, but we did not measure thigh changes in this particular study.

In the “Feet First” randomized controlled trial (11), a population with DM + PN participated in an exercise program and was followed for one year. These investigators also did not see a change in muscle strength, but the intensity of the walking program and the strengthening exercises were less than in our study. In addition, part of their study was done by the participants at home and the authors report that compliance with the exercise was a problem, which could have limited the potential to observe meaningful changes in this population. We had a compliance rate of 85% in this study, so we believe that the lack of changes that we report is not due to poor compliance.

This study provides important information about exercise in people with DM + PN, but there are limitations that should be considered. First, this study has a small sample size and was not powered to detect subtle changes in outcome measures. Furthermore, we included only people already walking 2000-9000 steps per day and it is unknown whether these results apply to people with more severe involvement or DM complications. Also, there were 5 people who were in the CON group who then completed the exercise intervention, so these groups are not truly independent of each

other. In addition, we did not measure the thigh muscle or thigh muscle performance, so it is possible that the exercise program had an effect on the lower extremities that we are not capturing. Our measures of IMAT and muscle volume are gross morphological measures, and we do not have a measure of changes that might be occurring on a smaller scale such as mitochondrial function in the muscle. Finally, we do not have a follow up on these subjects beyond the end of the exercise program, so we are unable to comment on the longer term effects of the exercise program compared to those who do not exercise.

Despite these limitations, this study provides evidence that people with DM + PN are able to participate in a progressive walking and strengthening program and demonstrate improved overall mobility and function based on six minute walk distance. These findings are consistent with the recently updated ADA guidelines indicating that individuals with DM+PN and without acute ulceration may participate in moderate weight-bearing exercise (1). Future studies that include a larger number of subjects receiving a higher intensity of resistance exercise than used in this study, and evaluating all lower extremity muscles, are needed to determine the appropriate safe dosage and intensity of activity to optimize changes in muscle morphology, strength, and physical mobility measures in this population.

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Table 1. Subject Characteristics. Values are means(SD).

Group	N	Gender (M/F)	Age (yrs)	Weight (lbs)	BMI (kg/m ²)	HbA1c (%)	DM Duration (years)	Vibration Perception Threshold (V)
EX	10	8/2	68(9)	246(41)	35.5(6)	6.8(0.9)	11.6(7.7)	40(11)
CON	10	6/4	66(14)	242(57)	36.5(8)	7.7(2.2)	10.3(5.5)	37(11)

Table 2. Group Data Pre and Post Exercise. Values are Mean (SD)

	Pre	Post
6MW (feet)*		
EX Group	1266 (263)	1352 (286)
CON Group	1409 (293)	1398 (234)
PPT		
EX Group	27 (5)	29 (5)
CON Group	29 (3)	30 (3)
Muscle Volume (cm ³)		
EX Group	431 (89)	425 (108)
CON Group	424 (128)	420 (116)
IMAT (cm ³)		
EX Group	100 (51)	103 (50)
CON Group	84 (50)	93 (50)
DF PT (Nm)		
EX Group	6.8 (13.3)	7.7 (9.1)
CON Group	5.3 (6.8)	3.6 (3.2)
PF PT (Nm)		
EX Group	51.9 (20.2)	56.9 (28.7)
CON Group	54.2 (16.7)	57.8 (26.4)

*Indicates significant Group x Time interaction based on repeated measures ANOVA and significant post hoc difference between pre and post measures (P<0.05)

The groups were not different from each other on any pre test measures (P>0.05)

6MW=6 minute walk distance

PPT=modified physical performance test score

Muscle volume=calf muscle volume measured via MRI

IMAT=intermuscular adipose tissue volume measured via MRI

DF PT=dorsiflexor peak torque

PF PT= plantarflexor peak torque

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Chapter 5

A weight-bearing exercise program for people with diabetes and peripheral neuropathy: A case report

This chapter is in review:

Tuttle LJ, Hastings MK, Mueller MJ. A weight-bearing exercise program for people with diabetes and peripheral neuropathy: A case report. *Phys Ther.*

ABSTRACT

Background and Purpose

The exercise guidelines for people with diabetes mellitus and peripheral neuropathy (DM+PN) have recently changed to allow moderate intensity weight-bearing exercise, but there are few reports in the literature describing appropriate weight-bearing exercise for those with DM+PN. This case report describes a successful and safe progressive exercise program for an individual with DM+PN.

Case Description

The subject was a 76 year old man with a 30 year history of DM + PN. He participated in a 12 week progressive exercise program (3 times per week) involving walking on a treadmill, balance exercises, and strengthening exercises for the lower extremities using body weight resistance.

Outcomes

Measures were taken before and after the 12 weeks of exercise. Six minute walk distance increased from 1200 to 1470 feet. Physical performance test score changed from 29 to 30. The Foot and Ankle Ability Measure questionnaire improved from 89-98. Dorsiflexor and plantarflexor peak torque increased (right dorsiflexor peak torque: 4.5 to 4.6 Nm, left dorsiflexor peak torque: 2.8 to 3.8 Nm; right plantarflexor peak torque: 44.7 to 62.4 Nm, left plantarflexor peak torque: 40.8 to 56.0 Nm), as did his average daily step count (6176 to 8273 ave steps/day). Calf intermuscular adipose tissue changed from 48 to 56 cm³ and calf muscle volume changed from 426 to 390 cm³.

Discussion and Conclusions

Evidence from this case report provides clinicians with a potential exercise program for people with DM+PN that may be able to increase muscle strength, physical function and activity.

People with diabetes and peripheral neuropathy (DM+PN) clearly have lower extremity impairments including reduced ankle muscle strength and volume.¹⁻³ Evidence suggests that the lower extremity muscles in people with DM+PN have substantial intermuscular adipose tissue (the visible extra-cellular adipose tissue that is located beneath the muscle fascia and between and within muscle groups⁴ [IMAT]) and muscle atrophy compared to their peers.⁵

These muscle changes likely contribute to their limitations in physical mobility. Adults with DM have an odds ratio of 2.0 for having mobility limitations compared to those without DM.⁶ The incidence of impaired mobility increases with peripheral artery disease and/or PN; subjects with DM and lower extremity diseases had an almost 3-fold increase in risk of impaired mobility compared to those having neither.⁶ An inability to walk a quarter mile and to walk up 10 steps without resting are the most frequently reported mobility limitations.⁶ Little evidence exists to determine if these impairments in muscle and mobility can improve with exercise.

In addition to these documented limitations in physical mobility, individuals with DM+PN lack the critical level of sensory feedback to protect their feet from injury. Historically, their inability to monitor their own skin condition led the ADA and ACSM to recommend exercise that was restricted to non-weight bearing activities (2006 guidelines). The exercise guidelines have recently changed, however, to allow individuals with DM+PN and without acute ulceration to participate in moderate weight-bearing exercise.⁷ This change was prompted, in part, by the recent “Feet First”

randomized controlled clinical trial⁸ that reported that people with DM+PN were able to participate in a low-intensity walking program and show a slight improvement in daily walking activity without increasing their rate of ulceration. Despite the benefits of this study, this same “Feet First” study reported no lower extremity strength changes with their low-intensity, community based exercise program⁹ and a Cochrane review of the literature concluded that there is inadequate evidence to evaluate the effect of strengthening exercise for people with DM+PN.¹⁰ Because the intervention intensity was quite low in the previous reports, it is unclear if people with DM+PN are able to increase lower extremity strength or reduce mobility impairments with an appropriately dosed exercise program.

The purpose of this case report is to describe the effects of a 12 week progressive walking and resistance exercise program on physical performance (6 minute walk test, Physical Performance Test, Foot and Ankle Ability Measure), calf muscle performance (dorsiflexor and plantarflexor peak torque), and calf muscle structure and composition (IMAT, muscle volume) in a single individual with DM+PN.

Case Description

Background to the case report

The case reported here is from an individual who participated in a randomized controlled trial comparing weight-bearing exercise, non weight-bearing exercise, and a non-exercising condition for people who have DM+PN. The overall goal of the trial is to determine whether people with DM+PN are able to perform weight-bearing exercise to increase activity level and physical mobility without increasing the incidence of foot ulcers or skin breakdown. The subject provided written informed consent and the study

was approved by the Human Research Protection Office at Washington University in St. Louis.

Patient

The subject was a 76 y.o. man, with a BMI of 27.1, with a 30 year history of Type 2 DM. His comorbid conditions included a history of myocardial infarction, history of coronary artery bypass graft, and hypertension that was controlled with medication. His blood sugar was controlled through oral medication.

Tests and Measures

Peripheral Neuropathy (PN)

The subject had severe PN based on his inability to feel any Semmes-Weinstein monofilament on the plantar surface of the foot and based on his vibration perception threshold of 50V as measured with a biothesiometer (threshold for neuropathy is 25V, 50 V is maximum value of machine).^{11,12}

Six Minute Walk Test

The participant performed the six minute walk test¹³ as a measure of physical function and walking endurance. The test has been validated in obese adults.¹⁴ The participant walked back and forth in a hallway between 2 cones that were placed 100 feet apart. The participant was told that the goal was to walk as far as possible in 6 minutes. Six minute walk distance was recorded as total distance walked in feet.

Physical Performance Test (PPT)

The modified 9-item PPT was used to assess physical function. This test is designed to mimic activities of daily living and includes items such as stair climbing, and

coming sit-to-stand. The 9-item PPT has been shown to correlate well with disability and frailty.¹⁵ Each of the items is scored on a scale of 0-4 based on the time it takes to complete the task. Each task is performed twice and the average time is used to determine the 0-4 score. A maximum score is indicated by a score of 36.

Foot and Ankle Ability Measure (FAAM)

The FAAM is a reliable, responsive, and valid self-report measure of physical function for individuals with a broad range of musculoskeletal disorders of the lower leg, foot, and ankle.¹⁶ The questions investigate the subjects perception of 26 activities of daily living (i.e. walking on even ground, walking up hills, stepping up and down curbs, home responsibilities, recreational activities). The FAAM was found to be more responsive to changes in functional status compared to the SF-36 and has a high test-retest reliability of 0.89.¹⁶ A higher score on the FAAM indicates better physical function.

Ankle Dorsiflexion and Plantarflexion Peak Torque

Concentric isokinetic ankle dorsiflexor and plantarflexor peak torque as measures of muscle performance were assessed using a Biodex Multijoint System 3 Pro isokinetic dynamometer.^{††} The tests were performed at angular velocities of 60°/s. He was given 3 practice trials to ensure he was comfortable with the test. The mean values for peak torque were calculated for 3 trials.

Intermuscular Adipose Tissue (IMAT) and Muscle Volumes

Intermuscular adipose tissue and lean muscle volumes were quantified using MRI on the right leg. The MRI scans were performed with the participant in a supine position

^{††} Biodex Medical Systems, 20 Ramsay Road, Shirley, NY 11967.

with a Siemens CP extremity coil placed over the right calf muscle. The MRI measurements were performed with a 3.0-Tesla magnet^{††} with a pulse sequence of TE=12 milliseconds, TR=1,500 milliseconds, matrix=256x256; both a fat saturated and a non fat saturated image were collected.⁵ Thirty transverse slices were collected beginning at the joint space of the knee and proceeding distally. The slices were 7mm thick with no interslice gap. Nine consecutive slices were selected to calculate muscle and IMAT volumes. Volumes were quantified using a PC workstation and custom Matlab software. The software uses voxel brightness to distinguish between muscle and adipose tissues. The subcutaneous adipose tissue was removed from each image by drawing a line along the deep fascial plane surrounding the calf muscle so that only the fat within and between the muscles (IMAT) was remaining. The software uses edge detection algorithms to assist the user in separating the subcutaneous fat from the muscle as well as separating individual muscles and muscle compartments. IMAT and muscle volumes are reported in cm³. Based on test-retest reliability of 21 subjects, the error in measuring muscle volume is less than 1% and less than 2% for measuring fat volumes on average in any muscle or compartment.

Activity Monitoring

The participant was given a StepWatch activity monitor^{§§} and was instructed to wear the monitor around his ankle during all waking hours to determine baseline activity levels. The participant was given the monitor for at least 9 consecutive days calculating an average of 7 days. The first and last days of wear were deleted because a full 24 hours

^{††} Siemens Corp, Citicorp Center, 153 E. 53rd St., New York, NY 10022

^{§§} Orthocare Innovations, 840 Research Parkway, Suite 200, Oklahoma City, OK 73104

would not be recorded. For a day to be included, the monitor had to be worn for at least 8 hours, and at least 1 weekend day was included in the 7 day average. The device records strides per day and steps per day were calculated by multiplying strides by 2 (1 stride equals 2 steps). Results of validity tests performed in our laboratory indicate a mean absolute error of 1.1% (SD, 3.1%) for healthy obese subjects and 1.8% (SD, 2.4%) for subjects with DM+PN.¹¹ Results of the activity monitor were used to prescribe his specific walking distance to start his progressive walking program as described below.

The subject was determined to be appropriate for participation in this exercise program due to the presence of DM+PN and lack of any severe foot deformities or comorbidities that might limit his ability to tolerate 60 minutes of exercise.

Exercise Program

The subject participated in a weightbearing exercise program three times a week for twelve weeks. The exercises included in this program were adapted from the “Feet First” intervention,⁸ and those used in prior interventions that reduced falls in frail older adults and those with PN.¹⁷ Where progression for an activity was not described in the published study, we added an appropriate exercise progression to maximize progressive improvements. Activity performance was supervised by a physical therapist. The exercise sessions began with flexibility and stretching exercises. The stretches included: toe stretches aimed at stretching the toe extensor muscles, a supine hamstring stretch, prone knee flexion, rocking on hands and knees, and a standing calf stretch. Each stretch was performed twice with a 30 second hold. The balance exercises followed a progressively challenging program in which external support decreased and/or the perturbation to a balanced individual increased (e.g. more compliant surface, movement

of arms/legs). The strengthening exercises were focused on the ankle dorsiflexor and plantarflexor muscles using body weight resistance (i.e. heel raises, bilateral progressing to unilateral for the plantar flexor muscles; stair-climbing and coming sit-to-stand for hip, knee, and ankle extensor muscles) rather than machines or weights in an effort to make the exercises easier to continue after the end of the study (Table 1).

The aerobic intervention included walking on a treadmill for a duration individually calculated for the subject based on his own average step count collected over 7 days using a Step Watch Activity Monitor as described above. The goal was for the subject to increase his average step count 10% every 2 weeks by increasing his daily step count 24% on the 3 days that he participated in the exercise program. In this case, he began with a step count of 6176 steps/day and the goal was for him to reach 10,712 steps/day (See Figure 1 for step count weekly goals). The subject wore a heart rate monitor to assist the physical therapist in ensuring that the exercise intensity was within the mild/moderate range (40-60% of max heart rate). Exercise participation was modified, postponed, or stopped based on guidelines established by the ADA and ACSM.⁷ Exercise was postponed if 1) blood glucose >300 mg/dl, 2) resting systolic BP of >200 mm Hg, 3) resting diastolic BP of >110 mm hg. If blood glucose was <80-100mg/dl carbohydrates were ingested.

Precautions to avoid skin injury

Before and after each exercise session, the physical therapist and the participant each performed a visual inspection of the participant's feet and footwear. A digital photograph was taken before and after the exercise session to document the skin condition. Foot skin temperature was recorded using a handheld infrared thermometer

(Diabetica Temptouch^{***}) before and after each session as described previously.¹⁸ The temperature was recorded at 7 sites on the plantar surface of the foot where risk for skin breakdown is greatest: the great toe, the 1st, 3rd, and 5th metatarsal heads, medial and lateral midfoot, and the heel. The temperature measures were used to determine areas that might be at risk for injury and were part of a composite assessment for skin injury that included redness, swelling and pain. If there was an area of redness, swelling, pain and increased temperature, activity was limited and the participant was instructed to closely monitor the area and decrease daily activity.

Adherence to Exercise

All exercise sessions were monitored by a physical therapist. In order to assist with transportation costs, the subject received ten dollars for each exercise visit attended. The subject attended 86% of the scheduled exercise classes. The subject missed 5 exercise sessions due to illness and conflict with previously scheduled doctor's appointments.

Outcomes

Data for outcome measures were obtained before and after the 12 week exercise program (Table 2). He was able to progress on all of the prescribed exercises and was able to increase his time on the treadmill at his self-selected pace of 2.0 mph from 14min to 24min. The subject improved his six minute walk distance from 1200 to 1470 feet. His PPT score improved from 29 to 30 and his FAAM score improved from 89-98%. The participants average daily step count increased from 6176 to 8273 steps/day. Glucose control improved as indicated by a decrease in HbA1c from 6.9 to 6.4%.

^{***} Diabetic solutions Inc. 12665 Silicon Drive, San Antonio, TX 78201

Dorsiflexor and plantarflexor peak torque increased (right dorsiflexor peak torque: 4.5 to 4.6 Nm, left dorsiflexor peak torque: 2.8 to 3.8 Nm; right plantarflexor peak torque: 44.7 to 62.4 Nm, left plantarflexor peak torque: 40.8 to 56.0 Nm) (Figure 1). IMAT volume increased (48 to 56 cm³) and calf muscle volume decreased (426 to 390 cm³). He also lost 13 pounds over the 12 week intervention.

This subject was able to tolerate a 12 week exercise program without complications or skin breakdown. His only injury came from trimming his own toe nails and this injury resolved in less than one week.

Discussion

The guidelines from the ADA and ACSM have recently changed to indicate that people with DM+PN may participate in moderate weight bearing exercise. Prior to this change in guidelines, it was recommended that people with DM+PN limit weight bearing activity due to concerns for increased risk for skin injury. This case report provides an example of a moderate intensity, weight-bearing exercise program that was successful in safely increasing six minute walk distance, average daily step count, and ankle strength in a person with DM+PN.

The subject presented in this case report was able to increase his six minute walk distance from 1200 to 1470 feet after the 12 week exercise program. The six minute walk test has been shown to be a measure of overall mobility and physical function^{19,20} and a change of 270 feet is consistent with a substantial change as reported by Perera et al.²¹ It seems reasonable that the six minute walk distance improved as the subject was part of a duration based walking program. Even though he did not increase his velocity during treadmill training, he was able to increase his velocity during the six minute walk

test. Essentially, he was training to walk and he was successful in improving his ability to walk.

In the case of this subject, PPT scores did not improve substantially (29-30). Based on an initial score of 29 out of 36 possible points, this particular subject did not demonstrate frailty or severe limitations in function, so it is possible that he had little room to improve on the PPT measure. However, his FAAM score did improve by 9 points, which is beyond the minimal detectable change (5.7 points) and minimal clinically important change (8 points) of the test.¹⁶ The subject reported improvements on the FAAM in: walking uphill, climbing stairs, going up and down curbs, squatting, doing heavy work (pushing/pulling, climbing, carrying), and in recreational activities. The FAAM is a measure of physical function that is specific to the foot, ankle and lower extremity activities whereas the PPT incorporates upper extremity activities as well. The exercise program reported here is focused entirely on the lower extremity, so it is possible that the FAAM is a more sensitive measure of improvement than the PPT in this case.

Dorsiflexor and plantarflexor strength measures (peak torque) responded differently from each other in this intervention. The dorsiflexor peak torque values did not change as much as the plantarflexor peak torque, but the plantarflexor peak torque increased by 37-40%. There are several reasons that could account for this difference in response. First, more of the exercises in this program were focused on increasing strength in the plantarflexors. The primary exercise for the dorsiflexors was heel stands, while the plantarflexors had toe raises (heels up), stair climbing, and sit to stand. It has been reported previously that dorsiflexion is impaired in people with DM+PN.³ It is

possible that due to the process of DM and DM+PN, the dorsiflexors may be harder to strengthen or less able to adapt than the plantaflexors.

The “Feet First” study⁹ described a low-intensity intervention for people with DM+PN aimed at increasing walking activity and improving strength, but they report no strength changes in their exercise program. The intervention that we describe here is more intensive than the “Feet First” study based on our goal of increasing step count by 10% every 2 weeks. This subject was able to tolerate this more aggressive program without injury or complications and was able to increase his average daily step count from 6176 to 8273 steps per day (34% increase) (Figure 1). However, this subject did not actually meet the target goal of increasing step count by 10% every 2 weeks (goal of 10,712 steps/day). Because he was walking at his self selected pace and we based the walking program on a predetermined length of walking time, his actual number of steps taken during the treadmill walk was less than what was required for the 10% increase to occur.

While this subject was able to increase his average daily activity, ankle muscle strength and six minute walk distance, he lost weight overall and actually lost muscle volume in the calf and gained IMAT in the calf. Previous studies of a walking program in older adults²² indicate that an exercise program can attenuate strength loss and IMAT gain in the thigh. It is unknown whether people with DM+PN are able to respond in a similar manner and it is unknown whether neuropathic muscle is able to adapt or what the intensity of the stimulus should be to elicit change in muscle structure. Based on this case study, it appears that change in calf muscle volume and calf IMAT volume are not directly related to change in muscle performance or physical function, but this is in

conflict with other reports in the literature.⁵ Future studies should focus on teasing out the relationship between IMAT and muscle function and its response to exercise.

Other investigators⁸ have indicated that muscle quality (peak torque/muscle volume) may be a key component in muscle function. In this case study, strength increased despite a decrease in muscle volume which would indicate an improvement in muscle quality as defined by peak torque/muscle volume. It is possible that the underlying contractile properties of the muscle were changed which could account for the increase in peak torque independent of a change in muscle volume. It is also possible that the improvement in muscle strength is due to a change in the nervous system rather than a change in the muscle structure. We do not have measures that answer these questions, but they should be investigated in future studies.

Of note, this subject's HbA1c dropped by 0.5% from 6.9% to 6.4% indicating an improvement in glucose control over the 12 weeks of exercise. Other exercise studies have reported HbA1c reductions of 0.6-1.6% with combined aerobic and resistance exercise programs of 8weeks-24months.²³⁻²⁷ In all of these studies, the exercise programs were of higher intensity than the one we report here, and further study is needed to determine if a higher intensity exercise program is safe and can result in larger drops in HbA1c in people with DM+PN. We can only speculate that perhaps even with this moderate intensity exercise program, the muscles improved insulin sensitivity which could be responsible for the improvement in HbA1c. More research is needed to determine if this speculation is accurate.

While this case study provides important information for clinicians, there are limitations to be considered. First, this is a case study of a single subject and it is unclear

whether these results are generalizable to the larger population of people with DM+PN. This person did not have severe foot deformity or a history of plantar ulcer and more studies are needed to determine the safety of this type of exercise for people with these diabetic complications. We believe that our precautions of a detailed foot exam before and after exercise are important components in people with DM+PN exercising safely. We also used temperature measures from the plantar surface of the foot as a means of identifying areas that might be at risk for skin breakdown.¹⁸ However, there were multiple false positives (cases where the temperature difference was >4 degrees between the right and left foot without other signs or symptoms indicating tissue damage). For this subject, we concluded that the detailed visual foot examination before and after exercise was adequate to prevent injury. Additional research is needed to investigate the usefulness of temperature readings in populations at risk for skin injury.

This case report provides an example of a weightbearing exercise program that was successfully implemented for a person with DM+PN. He was able to complete the program without injury and increase six minute walk distance, increase plantarflexor peak torque, and increase his average daily step count. More work is needed to determine the appropriate intensity of exercise to elicit changes in muscle structure and to determine the safety of high intensity resistance and/or aerobic exercise in people with DM+PN.

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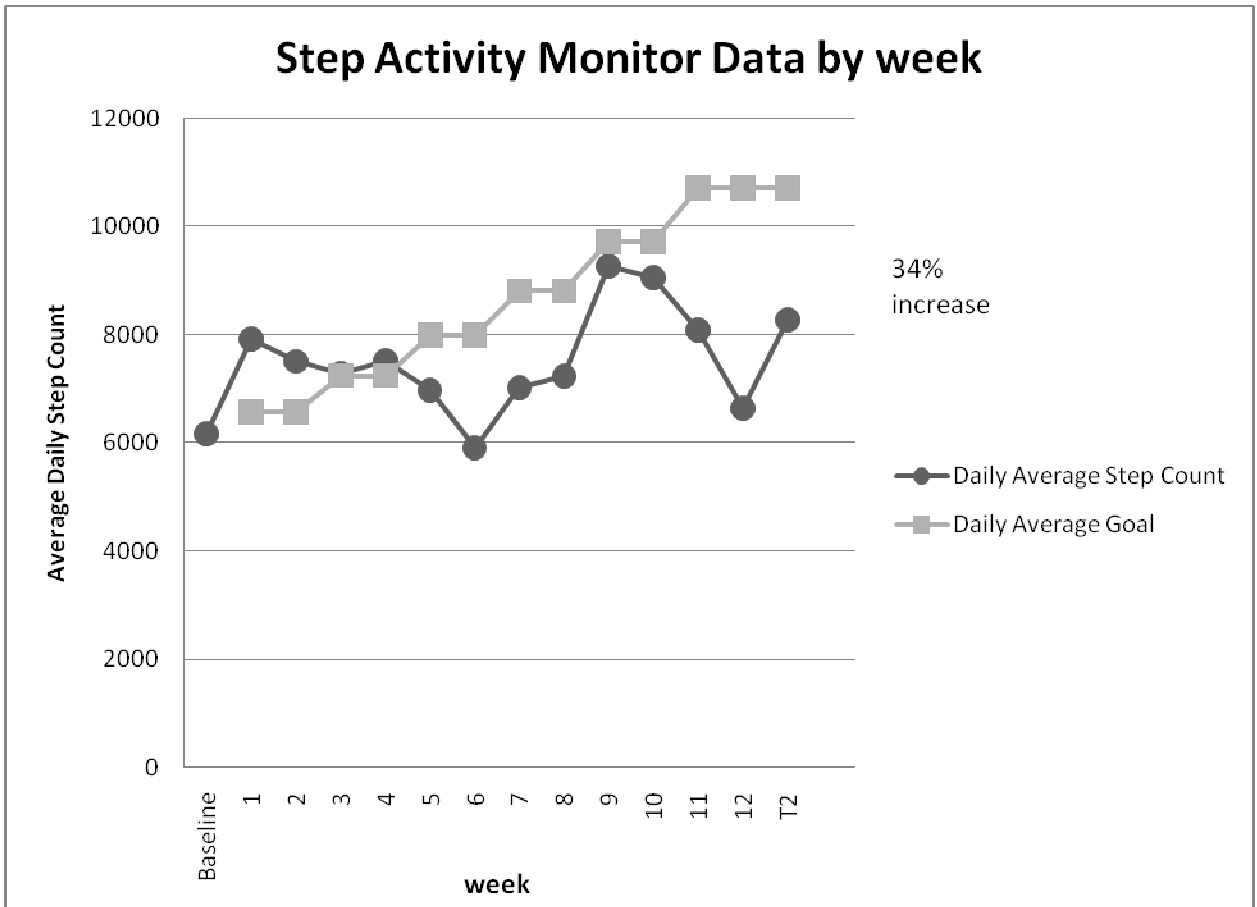
Table 1. Exercises performed during week 1 and week 12. Progression was determined at each exercise session based on the subject's subjective report of an exercise becoming easy and his tolerance for increasing his repetitions on an exercise.

Week 1	Week 12
Toe crunches, 10 reps 2 times	Toe crunches, 35 reps 2 times
Single leg stand with one hand support, 30 seconds 2 times	Single leg stand with no hand support, 30 seconds 2 times
Step sideways and step backwards, 10 steps each 2 times	Step over 5 cones, 3 times
2 leg heel stands (toes up), 10 reps 2 times	2 leg heel stands (toes up) 20 reps 2 times
2 leg toe raises (heels up), 10 reps 2 times	Single leg toe raises (heels up) 40 reps 2 times
Sit to stand, 5 reps 2 times	Sit to stand 20 reps 2 times
Step Ups with one hand support, 10 reps 2 times	Climb 2 flights of stairs, 2 times
14 min walk on treadmill, self-selected pace	24 min walk on treadmill, self-selected pace

Table 2. Outcome measures.

Measure	Pre Exercise	Post Exercise
Six Minute Walk Distance (feet)	1200	1470
PPT Score	29	30
FAAM Score (%)	89	98
Self-Report FAAM Score (%)	98	100
Dorsiflexor Peak Torque (Nm) Right/Left	4.5/2.8	4.6/3.8
Plantarflexor Peak Torque (Nm) Right/Left	44.7/40.8	62.4/56.0
Average Daily Step Count	6176	8273
HbA1c (%)	6.9	6.4
IMAT volume (cm ³)	48	56
Muscle volume (cm ³)	426	390
Weight (lbs)	178	165

Figure 1: Step Activity Monitor Data by Week.



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Chapter 6
Summary of Major Findings

Summary of Key Findings

The objectives of this research were to describe skeletal muscle structure in people with diabetes and peripheral neuropathy, to determine whether neuropathic muscle structure and composition is associated with movement impairments and functional limitations, and to determine whether neuropathic muscle can be modulated by activity level and/or an exercise intervention.

Chapter 2 asked the questions: does the presence of diabetes mellitus (DM) and peripheral neuropathy (PN) have an additive affect on IMAT in the calf beyond the presence of obesity? And how does IMAT impact function in a group with DM, a group with DM+PN and a group with NoDMPN? Our results suggest that the presence of DM and DM+PN do not have an additive affect on calf IMAT as there were no differences in calf IMAT between the 3 groups. IMAT was associated with worse physical performance, however, and both the DM and DM+PN group had lower measures of physical performance in comparison to the NoDMPN group. In addition, it appears that calf muscles may be affected by IMAT differently. Specifically, the gastrocnemius muscle had the highest ratio of IMAT/Muscle Volume of any other calf muscle.

These results add to our knowledge of IMAT in that it continues to be related to physical performance, but DM and PN do not seem to increase IMAT accumulation independently of obesity. However, the group with DM and DM+PN had lower measures of physical performance, indicating that the presence of pathology such as DM and DM+PN impact physical performance beyond what is fully explained by IMAT in

the calf. This is also the first study to report muscle specific differences in IMAT accumulation, which will warrant future study.

Chapter 3 asked the question: how active are people with DM+PN and is activity (average daily step count) related to calf IMAT in people with DM+PN? Our results show that people with DM+PN are able to maintain a higher level of activity on average than what we originally expected. Activity was inversely associated with IMAT, but was not associated with measures of performance or glucose control. Interestingly, IMAT was associated with measures of muscle performance and physical function, indicating that activity level may be a modifiable factor with the potential to impact IMAT and possibly impact physical function in people with DM+PN.

Chapters 4 and 5 asked the question: can neuropathic muscles adapt after an exercise program and can people with DM+PN improve physical function after an exercise program? Our results show that in response to a duration based walking program, overall, people with DM+PN are able to increase 6 minute walk distance. Changes in muscle strength as a group were variable, but some individuals were able to increase average activity, increase muscle strength and improve physical performance. Interestingly, these changes occurred without a consistent change in muscle volume or IMAT.

In summary, our data suggests that IMAT is associated with physical performance, but the presence of DM+PN are key factors that influence performance overall. In addition, activity level is also related to IMAT, but more study is needed to determine the exact exercise prescription that is safe and appropriate to cause changes in

muscle volume and IMAT in people with DM+PN. It is possible to have improvements in overall performance that are not reflected in changes in gross muscle morphology in the calf muscles.

Limitations

There are several limitations to these studies; 1) small sample sizes, 2) potential for a selection bias due to recruitment for an exercise study, 3) generalizability to those with ulceration and foot deformity, 4) lack of activity measures for all participants, and 5) lack of histology measures. First, the sample sizes for these studies are relatively small. It is possible that with larger sample sizes, we would have been able to better characterize variables that contribute to IMAT. Secondly, the people with DM+PN who participated in these studies were all interested in an ongoing study involving exercise for people with DM+PN. It is possible that this led us to recruit the most active and highest functioning people with DM+PN and we are not fully capturing a broad spectrum of the whole population with DM+PN. Third, one of the requirements for participation in these studies was a lack of foot deformity and we had very few participants with a history of ulcer. These results should not be generalized to people who have these complications. Fourth, we do not have activity measures for the groups with DM only or NoDMPN, so it is possible that these groups are different from the DM+PN group in activity, which could be a confounding variable in our results. Fifth, we do not have histology measures or measures of changes that may be occurring on a smaller level in the muscle. We do not know whether we would see group differences in histology between people with DM, DM+PN and NoDMPN, or whether the exercise program was perhaps altering muscle

composition in a manner that we are not able to observe due to the gross MRI measures that we are using.

Clinical Implications

Based on these results, people with DM+PN display lower levels of physical function than their peers, although it is unclear whether IMAT is the key predictor of this decline in physical function and whether or not interventions aimed at decreasing IMAT will result in improved physical function. People with DM+PN are able to tolerate high levels of physical activity without suffering from skin breakdown. Health care workers should continue to educate people with DM+PN on the importance of increased physical activity and the necessary precaution of a detailed foot examination daily to prevent skin injury. While the 12 week duration-based intervention did not successfully change muscle or adipose tissue morphology in the calf, the participants were successful in increasing six minute walk distance. This speaks to the importance of specificity of training—these participants were training using walking as the primary exercise and they were indeed able to walk further.

Future Studies

Future studies are needed with larger sample sizes and with imaging data collected on the entire lower extremity rather than just the calf or just the thigh to determine whether IMAT accumulation is muscle specific throughout the lower extremity. Our data suggest that the gastrocnemius is affected by IMAT accumulation more so than other muscles and it is unknown whether other muscles in the lower extremity are preferentially affected, but there is evidence in the literature that there are higher amounts of IMAT in the foot muscles compared to more proximal muscle in the

lower extremity in people with DM+PN. Future studies should attempt targeted interventions for the gastrocnemius muscle in an effort to determine if IMAT can be changed. Also, studies with more longitudinal data and longer follow ups that what is presented here are needed. The natural history of the disease of DM and DM+PN is one of decline in strength and function, so a longer follow up would provide more insight on the effects of higher levels of activity or of an intervention.

The ideal magnitude, duration and type of intervention to cause safe changes in the morphology of neuropathic muscle remain unclear. The American Diabetes Association and the American College of Sports Medicine have only recently changed their guidelines to indicate that people with DM+PN may participate in moderate intensity exercise. Future studies should examine a higher intensity exercise program—both aerobic and resistance based—to determine the impact this has on muscle structure and physical performance and the safety of high intensity exercise in people with DM+PN. Based on our data, it is unclear whether neuropathic muscle has the potential to adapt muscle morphology in response to exercise, but a higher intensity strengthening program would provide clinicians with more useful information in this regard. In addition, measures of nerve function such as nerve conduction velocity testing would provide information about adaptation in this population. It is possible that the exercise program altered the nervous system in some way and that accounts for the changes in 6 minute walk distance and would explain why we did not see changes in muscle morphology.

Overall, our results suggest that people with DM+PN have lower levels of physical performance than their peers and increased IMAT is associated with poor

physical performance and may be muscle specific. Increased activity levels are associated with decreased IMAT volume, and people with DM+PN are able to safely increase their walking distance following an exercise intervention, but we did not see a change in muscle composition. Additional research is needed to determine the specific roles of IMAT in skeletal muscle and function.

Appendix A

Magnetic Resonance Spectroscopy

Originally, I proposed to use magnetic resonance spectroscopy to investigate intramyocellular and extramyocellular lipid content in the calf muscles of people with diabetes and peripheral neuropathy. I was able to collect and analyze these data, however, because of difficulty in finding appropriate voxel placement in muscles with high amounts of visible adipose tissue and difficulty in interpreting the results from these scans, the information has been included in this appendix rather than in a primary chapter of the dissertation.

Magnetic resonance spectroscopy (MRS) is a non-invasive imaging technique that allows investigators to examine metabolites within the muscle. ^1H -MRS makes it possible to observe intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) within the muscle. Intramyocellular lipids are stored in the form of lipid droplets in the cytoplasm in close contact with the mitochondria in the muscle cell (1). The ability to observe IMCL via ^1H -MRS gained popularity when it became clear that IMCL can be recruited for use during strenuous exercise (2). The observation of increased IMCL in diabetic and insulin-resistant individuals supported the hypothesis that increased IMCL within the muscle could be a part of skeletal muscle insulin resistance (3-6).

Recently, measures of IMCL have been used to measure outcomes in a cohort of elderly obese individuals participating in either a diet only intervention or a combined diet and exercise intervention (7). The preliminary results of this study show an increase in IMCL in the soleus muscle in the diet and exercise group and no change in the IMCL content of the medial gastrocnemius. It is possible that increased IMCL in the soleus muscle may have been a positive adaptation in the diet and exercise group as the result of endurance activity such as the IMCL noted in highly trained athletes (8). Any

information on how neuropathic muscle responds or fails to respond to an intervention will provide health care providers with important information about how diabetic and neuropathic muscle adapts.

The goal was to determine if there was a difference in IMCL and EMCL in the gastrocnemius and soleus muscles in a group with diabetes (DM), a group with diabetes and peripheral neuropathy (DM+PN) and an age and BMI matched group without diabetes and peripheral neuropathy (NoDMPN). A secondary goal was to determine if IMCL and EMCL in the gastrocnemius and soleus muscle changes in response to an exercise program for people with DM+PN.

Methods

IMCL and EMCL values were quantified using proton spectroscopy on the right calf of each participant with a 3.0-T superconducting MRI instrument using a point resolved spectroscopy (PRESS) single-voxel technique (9). The participants were positioned in supine with an extremity coil over the calf. Spectra were collected at echo times of 30 and 60 ms to approximate and correct for the T_2 decay of the signals (9). An initial scout scan was performed in order to localize the calf musculature. A 1cm³ voxel region of interest was chosen, avoiding vessels and apparent adipose depots in the medial gastrocnemius muscle (2 voxels) and the soleus muscle (3 voxels). Fig 1. The data was processed and analyzed using jMRUI 3.0 with an AMARES fitting algorithm and using prior knowledge of the water and IMCL frequencies (10-13). Fig 2. An average of the IMCL from the voxels in each muscle was used for analysis and are reported as a percentage of the water peak in arbitrary units.

A one-way ANOVA was used to determine group differences (DM vs. DM+PN vs. NoDMPN) and a repeated measures ANOVA was used to determine differences in groupxtime. A power analysis was conducted based on the means and standard deviations listed in Table 2 using GPower 3.0.10 software to determine the sample size necessary to detect differences using repeated measures ANOVA and the results of this analysis are listed in Table 2.

Results

Table 1: Group differences in IMCL and EMCL. Values are mean (SD). P-values are from one way ANOVA.

	DM	DM+PN	NoDMPN	<i>P</i> -Values
Soleus IMCL(AU)	2.8(1.9) N=9	3.1(2.5) N=18	3.7(5.6) N=8	0.83
Soleus EMCL(AU)	3.1 (1.8) N=9	5.6 (5.4) N=18	5.6 (6.5) N=8	0.46
Gastrocnemius IMCL(AU)	2.1(1.7) N=8	3.5(4.7) N=10	1.6(1.4) N=8	0.42
Gastrocnemius EMCL(AU)	4.5 (3.4) N=8	11.4 (19.6) N=10	5.7(5.3) N=8	0.48

Table 2: Pre and post exercise IMCL and EMCL values. Values are mean (SD).

	Pre	Post	Power Analysis
SOL IMCL			1962
EX Group N=9	4.9 (7.2)	4.9 (5.0)	
CON Group N=7	3.9 (3.7)	5.9 (8.1)	
SOL EMCL			830
EX Group N=9	6.3 (6.7)	7.3 (5.9)	
CON Group N=8	7.5 (7.5)	6.4 (7.2)	
GAST IMCL			150
EX Group N=6	6.6 (8.1)	2.8 (4.4)	
CON Group N=3	4.2 (3.2)	3.0 (3.6)	
GAST EMCL			26
EX Group N=6	13.2 (17.5)	6.5 (8.1)	
CON Group N=3	2.3 (3.2)	3.8 (3.2)	

No groupxtime differences (P>0.05)

Power Analysis: total number of subjects needed to determine group differences using a repeated measures ANOVA.

Discussion

There were no differences in IMCL and EMCL values between the DM, DM+PN and NoDMPN groups (Table 1). There was also no change in IMCL and EMCL in the EX group or the CON group from pre to post exercise testing (Table 2). There are several limitations to be considered. First, the IMCL and EMCL values in the gastrocnemius and soleus muscles are highly variable—notice that the standard deviations often exceed the mean value. This high variance makes detecting group differences or changes over time difficult, particularly in a small sample. Also, as noted in the methods, when choosing a voxel, the goal is to avoid vessels and frank adipose tissue. Appropriate voxel placement becomes impossible in cases where the gastrocnemius or soleus has large amounts of intermuscular adipose tissue (Fig 3) and makes the results either invalid or difficult to interpret. The N is variable in the groups because of the invalid data. The average ratio of IMAT/Muscle volume in the gastrocnemius muscle of the subjects with invalid data (N=19) across the DM, DM+PN and NoDMPN groups was 0.43 with a standard deviation of 0.53 and a range of 0.11 to 1.848. More data is needed to determine a true threshold of IMAT infiltration that makes MRS data difficult to interpret due to the difficulty in voxel placement. It is unclear whether the results would be improved with the use of larger magnets or smaller voxel, but this should be considered for future studies. While MRS provides data regarding IMCL and EMCL in individual muscles and allows investigators to measure fat within a muscle cell, it seems that this technique is not feasible as a measure in populations with large amounts of IMAT such as people who are obese, and have DM and PN due to difficulty in voxel placement as well as interpretation of large fat peaks using current analysis methods.

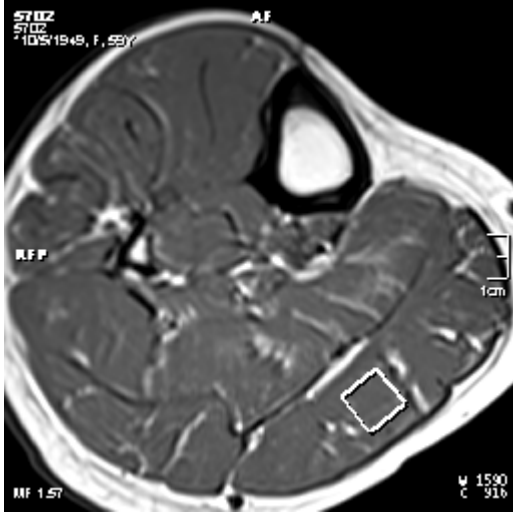


Figure 1. Example of voxel placement in the medial gastrocnemius muscle of a subject with low intermuscular adipose tissue.

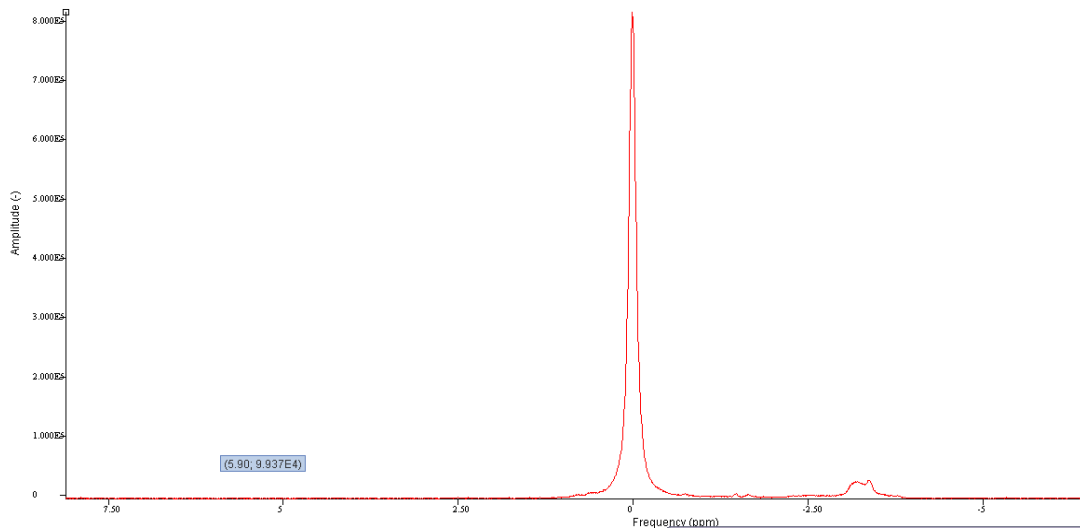


Figure 2. Example of a spectrum collected using MRS protocol. Notice the large water peak and the much smaller IMCL and EMCL peaks to the right of the water peak.

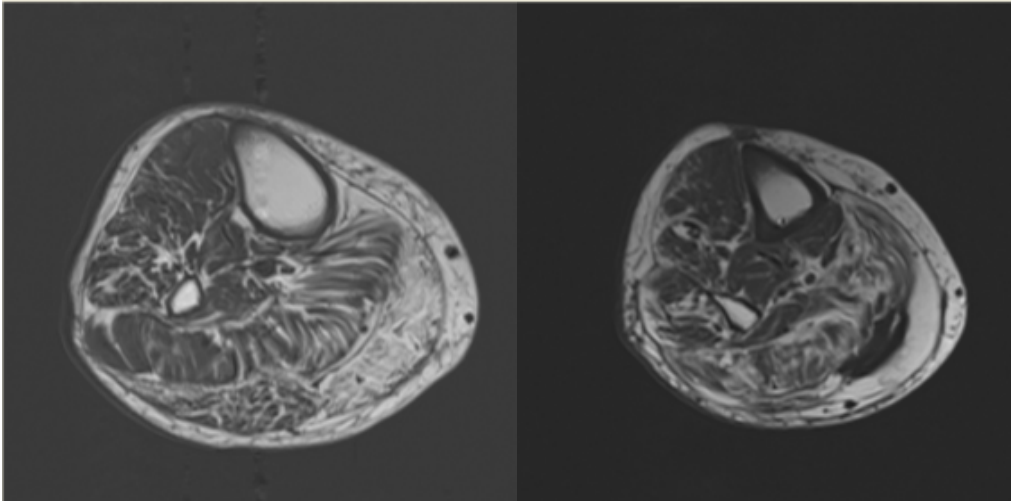


Figure 3. Examples of subjects with large amounts of IMAT in the gastrocnemius and soleus muscles making voxel placement in areas without adipose tissue impossible.

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Appendix B

DXA vs. MRI for IMAT analysis

There are multiple ways to determine body composition including amount of fat and muscle in a particular area of the body. Dual-energy x-ray absorptiometry (DXA) can be used to distinguish soft tissue from bone and to distinguish fat from lean (muscle) tissue. DXA is reliable and valid¹ and allows the user to look at regional areas of fat and lean muscle, such as the leg. DXA has the benefit of being non-invasive, as well as a very fast imaging tool (6 minutes for a whole body scan), but has the drawback of using radiation and has limited resolution.

MRI can also be used to determine fat and lean tissue in a region of interest due to inherent tissue contrast because of different amounts of water in different tissues. MRI is also a non-invasive imaging tool, but the MR scans typically will take longer and will image a smaller area of interest. For example, the MR protocol that is used to scan the calf takes 4.5 minutes to scan 21cm of the calf compared to 6 minutes for a whole body DXA scan. Unlike DXA, MRI does not use radiation and has superior resolution to look at body structures, however the cost for MRI is significantly higher than DXA.

One might be interested in trying to parse out the fat between and within an individual muscle compartment (Intermuscular adipose tissue or IMAT), but it is unclear whether or not DXA would provide this capability. It is also unclear whether the fat reported in DXA measures correspond entirely with subcutaneous fat or if it is able to capture IMAT as well.

Using the data set of the 45 participants from chapter 2—11 people with type 2 diabetes [5men/6 women; aged 56(9); BMI 35.5(6.4)], 24 people with diabetes and peripheral neuropathy [15 men/9 women; aged 64(13); BMI 32.6(6.3)], and 10 people

without diabetes and without peripheral neuropathy [4 men/6 women; aged 64(9); BMI 32.9(4.6)], I compared DXA measures and MRI measures using Pearson correlations.

There was a high correlation between calf total fat in cm^3 via MRI (subcutaneous fat and IMAT) and the leg fat in grams measured via DXA ($r=0.78$) (Figure 1). There was a low correlation between calf IMAT in cm^3 measured via MRI and the leg fat in grams measured via DXA ($r=0.20$) (Figure 2). There was also a significant correlation between BMI and body fat percentage as measured using DXA ($r=0.60$) (Figure 3), but there was a fair amount of variability around the trend line.

Based on this analysis, it is necessary to use MRI to measure IMAT, but DXA provides an alternate tool for measures of whole body and regional body composition. In addition, DXA may provide another way to characterize obesity or regional adiposity that is more specific than BMI and may be more powerful when looking at specific regions of interest between groups.

Figure 1.

MRI vs. DXA Leg Fat Measures

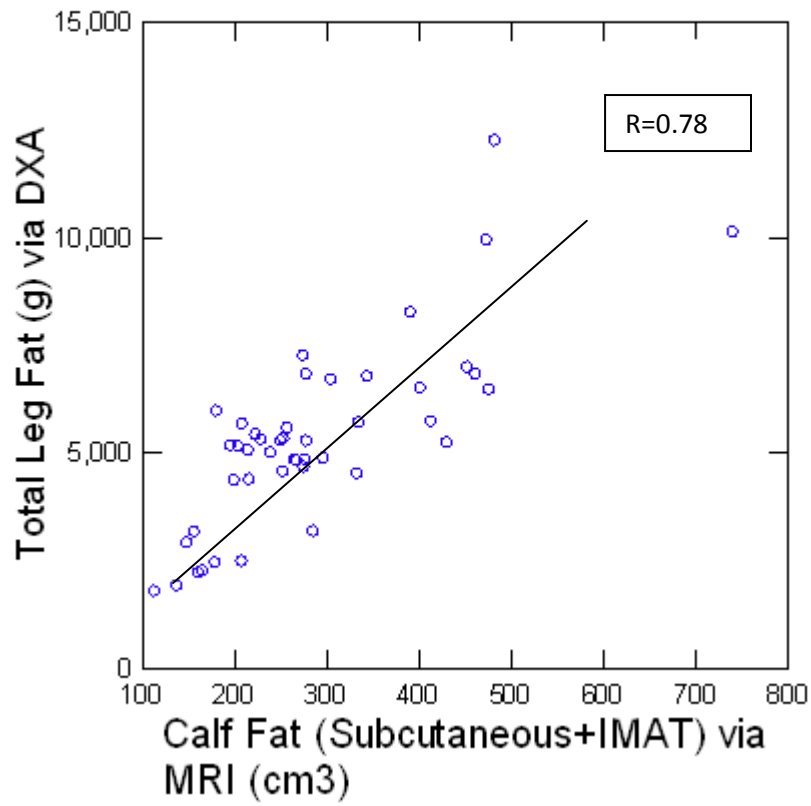


Figure 2.

MRI vs. DXA Leg Fat Measures

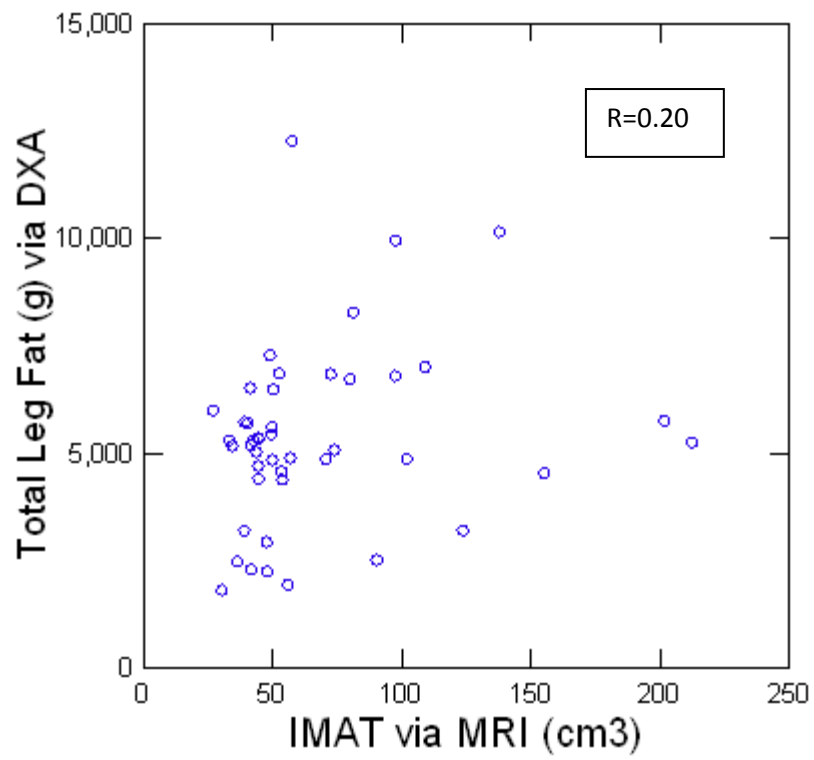
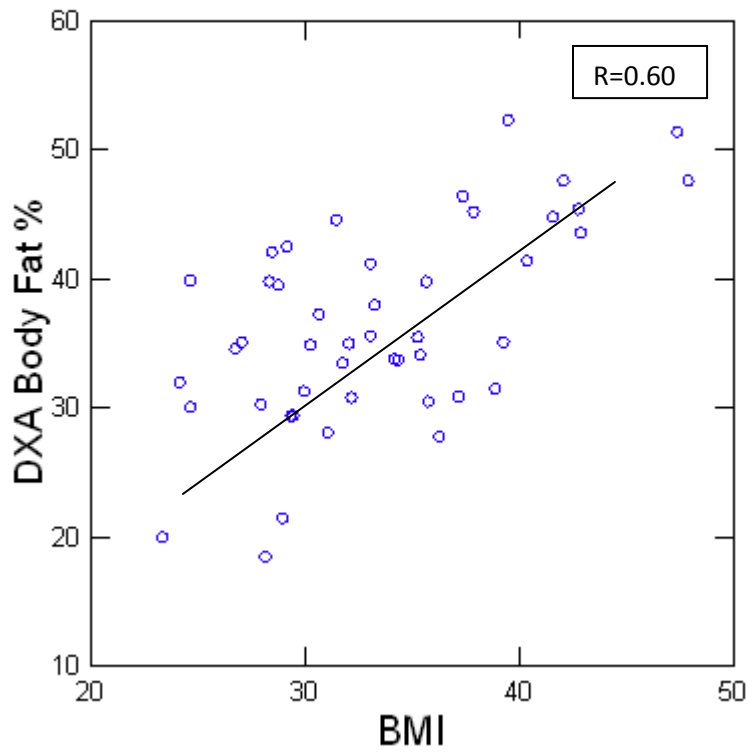


Figure 3.

BMI vs. DXA Body Fat Percentage



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Appendix C

Exercise Intervention Daily Checklist, Stretching and Weight-Bearing Exercises

**Exercise Study for People with Diabetes and Peripheral Neuropathy
Program in Physical Therapy, Washington University School of Medicine**

Daily Stat and Check Sheet -To be completed before and after every intervention session

Date: _____

Subject ID: _____

DOB: _____

Weight: _____

Participant comments before exercise: _____

Screening: Footwear: Adequate Not adequate (See footwear checklist for guidelines)

Digital Photograph	Pulse	Blood Pressure	Glucose Level
Before Yes <input type="checkbox"/>	_____	_____/_____	_____
After Yes <input type="checkbox"/>	_____	_____/_____	_____

Plantar Skin Temperature					Med	Lateral	
	Great Toe	Met 1	Met 3	Met 5	Midft	Midft	Heel
Before							
Left	_____	_____	_____	_____	_____	_____	_____
Right	_____	_____	_____	_____	_____	_____	_____
Difference	_____	_____	_____	_____	_____	_____	_____

(4 degree F (2.2 degree C) difference side to side indicates participant should monitor skin carefully and reduce activity as per study coordinator)

After							
Left	_____	_____	_____	_____	_____	_____	_____
Right	_____	_____	_____	_____	_____	_____	_____
Difference	_____	_____	_____	_____	_____	_____	_____

(4 degree F (2.2 degree C) difference side to side indicates participant should monitor skin carefully and reduce activity as per study coordinator)

Barefoot Visual Inspection Describe any blisters, redness, cracking, rash, lesion, nail color, and describe any area with a temperature change of 4 degrees F or greater--Complete wound documentation form if any new lesion exists.

Before _____

After _____

Intervention Target heart rate range (220-age): _____

HR monitored during exercise [] _____

20 minutes of flexibility, balance, and strengthening exercises

Provide group and level specific instructions WB or NWB Level_____.

Blood Glucose Monitored: Pre exercise:_____ Post exercise_____.

Minutes of walking / stationary bike: Target _____ Actual_____

Step count during intervention: Pedometer _____ SAM_____

RPM (bike)_____

Exercise Precautions for all participants: Exercise postponed because

_____1) Blood glucose >300 mg/dl,

_____2) Resting systolic BP of >200 mm Hg,

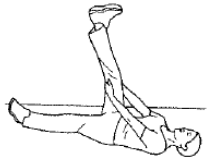
- _____3) Resting diastolic BP of >110 mm hg.
- _____4) Blood glucose is <80-100mg/dl (ACSM guidelines) carbohydrates were ingested.
- _____5) Target heart rate is intended to be 40-60% of age-predicted maximum, and activity will be adjusted to stay within those limits using a heart rate monitor.

Participant comments, complaints, or observations from Physical Therapist or study coordinator.

Toe Stretch

- *Sitting, cross right foot up onto thigh
- *Grasp toes of right foot with your hand and curl the toes down
- *Then point ankle and foot down (in the direction you push on the gas pedal)
- *Hold 30 seconds
- *Repeat with left leg
- *Repeat entire sequence 2 times

Hamstring Stretch



- *Lie on your back
- *Clasp your right thigh and pull it toward you
- *Extend your knee, keeping your back and thigh still, until you feel a gentle stretch in the back of your right thigh
- *Hold stretch 30 seconds, return to start position
- *Repeat with left leg
- *Repeat entire sequence 2 times

Knee Flexion, Face lying



- *Lie face down with your legs straight and relatively close together
- *Bend your right knee
- *Don't let your back move as you bend your knee
- *Hold for 30 seconds then return your leg to starting position
- *Repeat with left leg
- *Repeat entire sequence 2 times

Hands and Knees Rocking Back



- *Get on your hands and knees
- *Rock back toward your heels, keeping your back straight
- *Return to start position
- *Repeat 2 times

Standing Calf Stretch



- *Stand facing the wall
- *Lean to the wall and place right foot forward
- *Make sure your foot is facing straight forward, not turned out to the side
- *Keeping your back heel on the ground, lean forward till gentle stretch is felt in your calf
- *Hold stretch 30 seconds, return to start position
- *Repeat with left leg
- *Repeat entire sequence 2 times

Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
1. Toe Crunches, on mat *10 reps	1. Toe Crunches, on mat *15 reps	1. Toe Crunches, on mat *20 reps	1. Toe Crunches, on mat *25 reps	1. Toe Crunches, on mat *30 reps	1. Toe Crunches, on mat *35 reps
2. One-leg stand with bilateral hand support *30 seconds, 2 times	2. One-leg stand with one hand support *30 seconds, 2 times	2. One-leg stand no hand support *30 seconds, 2 times	2. Stand with 2 feet on balance disc, bilateral hand support *30 seconds, 2 times	2. Stand with 2 feet on balance disc, no hand support *30 seconds, 2 times	2. One-leg stand on balance disc, with bilateral hand support *30 seconds, 2 times
3. Step sideways and then step backwards with one hand support *10 steps (across room), 2 times each	3. Step sideways and then step backwards with no hand support *10 steps (across room), 2 times each	3. Step sideways on exercise mat, and then backwards on exercise mat with one hand support *10 steps, (across room), 2 times each	3. Step sideways on exercise mat, and then backwards on exercise mat with one hand support *10 steps, (across room), 2 times each	3. Step over objects, no hand support *5 objects, 3 times (Ok to alternate with level 5 content but increase to 3 times each)	3. Step over objects, no hand support *10 objects, 3 times (Ok to alternate with level 5 content but increase to 3-4 times each)
4. 2 leg heel stand (toes up) , back against wall *5 reps	4. 2 leg heel stand (toes up), back against wall *10 reps	4. 2 leg heel stand (toes up), back against wall *15 reps	4. 2 leg heel stand (toes up), back against wall *20 reps	4. Single Leg heel stand (toes up), back against wall *10 reps	4. Single Leg Heel Stand (toes up), back against wall *15 reps
5. 2 leg toe raises (heels up) *5 reps	5. 2 leg toe raises *10 reps	5. 2 leg toe raises *15 reps	5. 2 leg toe raises *20 reps	5. Single leg toe raises *5 reps each side	5. Single leg toe raises *10 reps each side
6. Sit to stand *3 reps	6. Sit to stand *5 reps	6. Sit to stand *7 reps	6. Sit to stand *10 reps	6. Sit to stand * 12 reps	6. Sit to stand *15 reps
7. step ups, with two hand support *10 reps	7. step ups, with one hand support *10 reps	7. step ups, with no hand support *10 reps	7. stair climbing, with one hand support *up and down 5 stairs	7. stair climbing, with one hand support *up and down 1 flight	7. stair climbing, hand support as needed *up and down up to 4 flights

