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Adipose tissue content, muscle performance and physical function in obese adults with type 2 diabetes mellitus and peripheral neuropathy

Daniel C. Bittel, PT, DPT^{a,*}, Adam J. Bittel, PT, DPT^a, Lori J. Tuttle, PT, PhD^b, Mary K. Hastings, PT, DPT, MSCI^a, Paul K. Commean, BEE^a, Michael J. Mueller, PT, PhD, FAPTA^a, W. Todd Cade, PT, PhD^a, and David R. Sinacore, PT, PhD, FAPTA^a

^aProgram in Physical Therapy, Washington University School of Medicine, 4444 Forest Park Ave, St Louis, MO, 63110

^bDoctor of Physical Therapy Program, School of Exercise and Nutritional Sciences, San Diego State University, 5500 Campanile Dr, San Diego, CA, 92182

Abstract

Aims—To determine leg intermuscular (IMAT) and subcutaneous (SQAT) adipose tissue and their relationships with muscle performance and function in obese adults with and without type 2 diabetes and peripheral neuropathy (T2DMPN).

Methods—Seventy-nine age-matched obese adults were studied, 13 T2DM, 54 T2DMPN, and 24 obese controls. Leg fat (%IMAT, %SQAT) volumes were quantified using MRI. Ankle plantar flexion (PF) torque and power were assessed with isokinetic dynamometry. Physical function was assessed with 9-item Physical Performance Test (PPT), 6-minute walk distance, single-limb balance, and time to ascend 10 stairs. One-way ANOVAs determined group differences, and multiple regression predicted PPT score from disease status, % IMAT, and PF power.

Results—T2DMPN participants had 37% greater IMAT volumes and 15% lower SQAT volumes than controls ($p=.01$). T2DMPN and T2DM showed reduced PF torque and power compared to controls. T2DMPN participants had lower PPT score, 6' walk, single-limb balance, and stair climbing than controls (all $p<.05$). %IMAT volume correlated inversely, and %SQAT correlated directly, with PPT. Leg %IMAT and disease status predicted 49% of PPT score.

Conclusions—T2DMPN may represent a shift in adipose tissue accumulation from SQAT to IMAT depots, which is inversely associated with muscle performance and physical function.

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*Corresponding author: Daniel C. Bittel, 1(314)-747-0648, bitteld@wusm.wustl.edu, Program in Physical Therapy at Washington University School of Medicine, Campus Box 8502, 4444 Forest Park Ave., St Louis MO, 63110.

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Keywords

Diabetes; Adipose Tissue; Muscle; Physical Function; Neuropathy

1. Introduction

The excessive accumulation of intermuscular adipose tissue (IMAT), an ectopic fat deposit developing between skeletal muscle fibers and beneath the deep fascia of muscle, is a morphological maladaptation present in a wide range of clinical conditions, including disuse atrophy, spinal cord injury, human immunodeficiency virus (HIV) infection, chronic obstructive pulmonary diseases (COPD), and diabetes mellitus (DM) (Addison et al. 2014; Mjtahedi et al. 2008; Nardo et al. 2014).

The IMAT infiltration in large muscle groups of the lower extremity is a particularly important etiological factor in the onset and progression of type 2 diabetes (T2DM) and its complications due to its hormonal and structural influence on skeletal muscle – the tissue responsible for 90% of peripheral glucose uptake (DeFronzo and Tripathy, 2009). While it remains uncertain what precipitates the accrual of excessive IMAT accumulation, previous studies have implicated an increased rate of free fatty acid (FFA) release from obese, diabetic adipose tissue stores, leading to inhibition of skeletal muscle glucose uptake (via glucose-fatty acid cycle or Randle cycle competition), amplified gluconeogenesis, beta cell lipotoxicity, and the development of hypertriglyceridemia (DeFronzo, 2004; Jensen, 2008; Randle et al. 1963). The preferential uptake of FFA in skeletal muscle, due to high availability and competitive inhibition of glucose transport, may initiate the accretion of excessive intermuscular lipids. Over time, with increasing resistance to insulin's action, excessive IMAT accumulation develops and proliferates, particularly in lower extremity musculature, due to concomitant, progressive mitochondrial dysfunction and declining oxidative capacity (Schrauwen and Hesselink, 2004). Proper glycemic control is of paramount importance for the maintenance of neuronal structural integrity and function, with chronic hyperglycemia implicated in peripheral nerve demyelination, decline in nerve conduction velocity, axonal atrophy and degeneration, glial insult, and lipid peroxidation (Obrosova, 2009; Yu et al. 2008); processes linked to the development of diabetic symmetric peripheral neuropathy.

Since IMAT accumulation has been shown to negatively correlate with insulin sensitivity, it has been hypothesized that IMAT levels in the skeletal muscles of individuals with T2DM and peripheral neuropathy (T2DMPN) would be higher than those with T2DM alone, or age- and BMI-matched controls (Boettcher et al. 2009). However, Tuttle et al. (2012), in a limited sample size, found no differences in total IMAT volume in the calf across these groups.

In addition to the volume of IMAT accumulation, the distribution of adipose tissue deposition may also change with the severity and progression of T2DM complications. The expansion of visceral adipose tissue depots (including IMAT), as opposed to subcutaneous depots (SQAT), has been associated with peripheral insulin resistance, hyperglycemia, and cardiovascular disease in obese populations (Lebovitz and Banerji, 2005). In contrast, higher

amounts of SQAT are associated with enhanced glucose metabolism and a reduced risk of dyslipidemia due to its lower lipolytic activity, preferential absorption of plasma FFA, and enhanced secretion of adiponectin (an adipokine associated with increased insulin sensitivity) (Porter et al. 2009; Wronska and Kmiec, 2012).

While many of the previous reports have detailed the protective or deleterious roles of subcutaneous compared to visceral adipose tissue accumulation, the focus has been on the abdomen and abdominal viscera (Klein et al. 2004), with recent evidence suggesting this concept can be extended to lower extremity adipose tissue accumulation. Goodpaster et al. (2000), demonstrated that a loss of thigh intramuscular adipose tissue and visceral adipose tissue, but not SQAT, was correlated with higher insulin sensitivity. Goodpaster et al. (2000) did not assess IMAT or SQAT volumes in the leg (calf) and did not examine the association between adipose tissue deposition and physical and functional outcomes. Therefore, the inter-relationships between leg IMAT infiltration, SQAT volume, muscle performance, and physical function across a spectrum of T2DM severity with increasing complications (e.g., the presence of peripheral neuropathy) require further study. Changes in tissue composition in the leg may be particularly important because of its propensity for excess IMAT accumulation (most especially in the gastrocnemius muscle) (Tuttle et al. 2012), and because impairment of calf muscle function may lead to the development of impairments such as foot deformities, ulceration, and impaired gait mobility.

Therefore, the purposes of this study were to: 1) determine leg SQAT, IMAT, and muscle volume, as well as compartmental (anterior, lateral, deep, soleus muscle, gastrocnemius muscle) IMAT, and muscle volumes among obese T2DM, T2DMPN, and non-diabetic obese control groups; 2) determine differences in muscle performance and physical function in groups with different disease and complication severity; and 3) determine the relationship between leg adipose tissue volume and measures of both muscle performance, and physical function.

2. Methods

Seventy-nine BMI- and age-matched participants were studied (22 obese controls, 10 T2DM participants, and 47 T2DMPN participants). All participants had physical and demographic data and completed tests for body composition (leg muscle and adipose tissue volumes), and muscle performance. Thirty eight of these participants (10 controls, 10 T2DM, 18 T2DMPN) also completed a series of physical performance tests to determine function in simulated activities of daily living (detailed in sections 2.4.1–2.4.4). Inclusion criteria included ambulatory individuals; with or without a diagnosis of T2DM and with and without evidence of PN as determined by diminished or absent plantar sensation to light touch or pressure or vibration perception (detailed in section 2.2).

Individuals were excluded from the study if they weighed more than 300 pounds (equipment weight limit), presented with any recent history of illness or hospitalization (within previous 6 months), any active infection or ulceration of either foot, previous botulinum toxin injection, ABI < .45, the presence of non-MRI compatible implants and women who may be pregnant, a history of severe foot deformity or amputation, and the presence of any co-

morbidity or medications that would interfere with activity testing (rheumatic disease, peripheral arterial disease, dialysis, current cancer treatment). Each participant read and signed an IRB approved protocol and informed consent that was approved by the Human Research Protection Office at Washington University in St. Louis, MO.

2.1 Participant Demographics

Participant's age, duration of T2DM, weight, height, and BMI were collected through participant interview, weight balance, and stadiometer, respectively at the initial visit, prior to any imaging, dynamometry or physical performance testing.

2.2 Lower Extremity Sensation

Biothesiometry and Semmes Weinstein Monofilament testing were used to determine the presence of peripheral neuropathy -- defined clinically as the inability to feel the 5.07 (10 gram) monofilament on at least one non-callused site on the plantar aspect of either foot or the inability to perceive vibration < 25 volts on the biothesiometer (Biomedical Instrument, Newbury, OH, USA) applied to the hallux (Maluf and Mueller, 2003).

2.3 Muscle Performance Assessment

Maximal isokinetic torque, power, and work were measured at 60 deg/sec and 120 deg/sec for ankle plantarflexion and dorsiflexion using a Biodex System 3 Isokinetic Dynamometer (Shirley, NY, USA). Each isokinetic movement was repeated three times, with the average of the three trials used in the final analysis. Additionally, isometric ankle peak plantar flexion and peak dorsiflexion torque were measured, and averaged from three repetitions. Plantarflexion and dorsiflexion torque per unit lean tissue volume (torque (Nm)/muscle volume (cm³)) were also calculated for each participant.

2.4 Physical Function

2.4.1 9-Item modified Physical Performance test—Functional physical performance was assessed using the 9-item modified Physical Performance Test (PPT). The PPT is a timed observational assessment of performance on nine daily physical activities including: 5 consecutive sit to stand transfers without arm assistance, climbing a flight of stairs with 10 steps, a 50 foot walk test, turning 360 degrees, picking up a penny from the floor, donning/doffing a jacket, lifting a book onto an overhead shelf, a Romberg standing balance assessment with eyes open, and ability to ascend 4 flights of stairs (Reuben and Siu, 1990; Villareal et al. 2011). Each item is scored from 0 to 4 based on the time to taken to complete each task, with a maximum score of 36 (higher score indicates better performance).

2.4.2 6 Minute Walk Distance—The distance walked in six minutes was recorded. The test was administered as outlined by the American Thoracic Society (2002).

2.4.3 Stair Vertical Power—Stair vertical power was calculated from the stair component of the PPT using the equation as previously described (Tuttle et al. 2012):

$$Power = \frac{mass \times (-9.8 \text{ m/s}^2) \times Height(10 \text{ Steps})}{Time \text{ to Climb Stairs}(sec)}$$

2.4.4 Single Limb Balance—Participants were asked to stand on their right lower limb, and then their left lower limb with their eyes open. They were allowed to move their arms to maintain their center of gravity. The timer was started when they lifted their non-stance foot from the floor and stopped when they lost balance (indicated by a return of the non-stance foot to the floor). Participants performed the test twice, with the average of the two trials recorded for analysis.

2.5 Leg Composition

Calf IMAT, SQAT, and muscle volumes were quantified using T1-weighted magnetic resonance imaging (MRI). MRI measurement was performed with a 3.0 Tesla superconducting magnet with pulse sequence TE = 12 msec, TR = 1500 msec, matrix 256×256, with both fat-saturated and non-fat saturated images collected (Hilton et al. 2008). We scanned 30 consecutive, 7 mm thick, transverse slices spanning from the tibial plateau and progressing distally. We analyzed 9 consecutive slices beginning from the 11th slice distal to the tibial plateau to assess tissue volumes using customized MatLab software. This software differentiates both muscle and fat volumes based on voxel brightness, and reports calf tissue volumes in cm³. Volumetric assessments were made as described by Tuttle et al. (2012), and Commean et al. (2011) for the anterior, lateral, and deep compartments, as well as the gastrocnemius and soleus muscles. Additional tissue volume metrics were calculated from the MRI analysis, including percent IMAT volume (%IMAT), calculated as the ratio of IMAT volume to total fat volume (total fat volume = SQAT volume + IMAT volume), and percent subcutaneous fat volume (%SQAT) as the ratio of SQAT volume to total fat volume. With this method of tissue volume quantification, Tuttle et al. (2012) reported <1% measurement error in muscle volume quantification, and <2% error in adipose tissue volume quantification regardless of the individual muscle or compartment analyzed.

2.6 Statistical Analysis

Chi square test for equality of proportions was used to determine the homogeneity of sex distribution of the three groups for disease status (T2DM only, T2DMPN, and Controls). One-way analyses of variance (ANOVAs) were performed to determine group differences for age, weight, height, BMI, and duration of DM and to identify group differences across measures of body composition, muscle function, and physical performance. Tukey's HSD was used during post hoc testing to determine specific group differences. Based on *a priori* hypotheses regarding muscle performance, post-hoc testing for muscle performance was completed with independent t-tests. Bivariate Pearson product correlation coefficients were calculated to determine inter-relationships between measures. All analyses were evaluated at an alpha level set at 0.05.

Finally, a forced entry multiple regression was used to predict 9-item PPT score using the following predictors: group status (Control as reference, T2DM only, or T2DMPN), normalized plantar flexion power ((PF power at 60 deg/sec)/(gastroc-soleus muscle

volume)), and % IMAT volume. Overall model fit was assessed using the Hosmer-Lemeshow Goodness of Fit Test and the potency of the model was determined using Nagelkerke's Pseudo R^2 . All statistical analyses were performed in IBM SPSS Version 21 (Armonk, NY: IBM Corp.).

3. Results

3.1 Group Demographics

As shown in Table 1, there were no group differences in sex, age, BMI, weight, height or years of diabetes. Vibration perception threshold (VPT) and monofilament testing confirmed that participants in the T2DMPN group had PN while the other groups did not have PN.

3.2 Leg Composition

As shown in Figure 1, there were no differences between groups on total muscle (Fig. 1-A) or total fat volumes in the calf (Fig. 1-B). Lean compartmental muscle volumes were not different between groups. Participants in the T2DMPN group had 42% greater %IMAT volumes (cm^3) in the deep compartment than the T2DM and 33% greater than control group as well as 72% greater %IMAT volumes in the lateral compartment than the T2DM group (Fig. 1-D). No differences were found between groups for anterior muscle compartment volume (Fig. 1-C). Most importantly, the T2DMPN group demonstrated 37% greater %IMAT volumes than controls, 15% lower %SQAT volumes than controls (Fig. 1-F), and 5% lower gastrocnemius-soleus muscle volume relative to total leg muscle volume ($[\text{gastrocnemius} + \text{soleus lean volume}] / \text{total lean volume}$) than controls (Fig. 1-E, Figure 2).

3.3 Muscle Performance

As shown in Table 2, both the T2DM and T2DMPN groups had lower plantarflexion torque than control participants at 60 deg/sec (33% and 24% lower respectively). Similarly, both the T2DM and T2DMPN groups demonstrated 38% lower plantarflexion peak power at 120 deg/sec than controls. The T2DMPN group produced lower absolute and normalized (to leg muscle volume) dorsiflexion peak torques at 60 deg/sec compared to the T2DM (95% lower absolute torque, 84% lower normalized torque) and control groups (82% lower absolute torque, 67% lower normalized torque). The T2DMPN group produced 94% lower dorsiflexion power at 60 deg/sec than controls.

3.4 Physical Function

Table 2 shows that T2DMPN participants had lower 9-Item PPT scores than controls. On single limb balance, the T2DMPN group had shorter balance times than the T2DM group. On the 6-minute walk test, the T2DMPN group walked shorter distances than controls. No group differences were found for vertical stair power. Additionally, group performance on 4 of the tasks of the 9-Item PPT is reported in Table 2 (coin pickup, 50 foot walk, time to climb one flight of stairs, and chair rise), representing the functional domains that account for group differences. T2DMPN participants had slower times and lower scores on the coin pickup test and 50 foot walk test than controls. While the omnibus F-test did not reach the $p < .05$ level of significance, for the 50 foot walk test the probability was close, making the authors confident in reporting a significant difference between groups, given post-hoc

results. The T2DMPN group took a longer time to climb a single flight of stairs than controls, and longer time to complete the chair rise task. No differences were found for the other 5 subtests of the PPT.

3.5 Multiple Linear Regression

Based on the results of the linear regression, the model using group status, normalized plantar flexion power at 60 deg/sec, and %IMAT accounted for 50% of the variance in 9-item score (Table 3). After controlling for normalized PF power, group status and % IMAT volume were both significant predictors of 9-item PPT score. %IMAT volume accounted for 17% of the variance in 9-Item score, while T2DMPN status accounted for 12% (normalized PF power accounted for 1.4%, and T2DM status accounted for 4%).

3.6 Bivariate Correlations

The %IMAT volume was inversely correlated with 9-Item PPT score ($r = -.59$, $p = .008$), 6-minute walk distance ($r = -.36$, $p = .016$), PF power at 120 deg/sec ($r = -.39$, $p = .031$), vertical stair power ($r = -.41$, $p = .013$), coin pickup score ($r = -.38$, $p = .02$), and gastrocnemius-soleus percent muscle volume ($r = -.32$, $p = .005$). In contrast, %SQAT was directly correlated with 9-item PPT ($r = .59$, $p < .001$), 6 minute walk distance ($r = .36$, $p = .016$), PF power at 120 deg/sec ($r = .43$, $p = .018$), stair power ($r = .43$, $p = .012$), coin pickup ($r = .41$, $p = .013$), gastrocnemius-soleus percent muscle volume ($r = .32$, $p = .005$), and inversely correlated with vibration perception threshold (indicating better vibration perception, $r = -.39$, $p = .027$). Additionally, gastrocnemius-soleus percent muscle volume was directly correlated with 9-Item PPT, coin pickup, vertical stair power, and PF power at 120 deg/sec correlated with 9-Item PPT score (all $p < .05$).

4. Discussion

4.1 Leg Composition

The results of this study demonstrated that after matching participants for age, weight, height, BMI, and years of diabetes, those with T2DMPN, a more severe complication of diabetes, had higher levels of leg IMAT. Similarly, concurrent with increasing IMAT, participants with T2DMPN had less SQAT volume than T2DM, and obese control participants without diabetes (Figure 2). In the lower extremity, Snijder et al. (2005) found that larger subcutaneous thigh fat is associated with better glucose and lipid control in a healthy population. Subcutaneous adipose tissue may be more apt to take up non-esterified fatty acids (NEFA) than IMAT, thereby protecting skeletal muscle from exposure to high NEFA concentrations and subsequent ectopic triglyceride accumulation (Frayn, 2002). Indeed, Miljkovic-Gacic et al. (2008) found that with aging, IMAT accumulation in the leg indicated partitioning of lipids away from the protective subcutaneous depots of the thigh and calf. Our study demonstrated, for the first time, that protective qualities of lower extremity subcutaneous adipose depots might extend beyond the thigh to the calf among participants with obesity, and T2DM.

This altered pattern of fat storage suggests that the progression from obesity to T2DM to T2DMPN may involve an escalating shift from subcutaneous to intermuscular fat

deposition. This is exemplified in Figure 2. Moreover, long-term intermuscular fat storage may be associated with enhanced lipid peroxidation and the release of damaging, catabolic cytokines such as TNF- α and IL-6 (Skundric and Lisak, 2003; Stenho-Bittel, 2008). Together, these impair Schwann cell-axonal homeostasis, and induce nerve fiber degeneration and peripheral neuropathy (Skundric and Lisak, 2003; Stenho-Bittel, 2008). The elevated IMAT volume in the T2DMPN group in our study lends support to this hypothesis-- IMAT accumulation is a detrimental complication of T2DM that, too, is exacerbated by disease progression. Similarly the previous high correlation between insulin resistance and IMAT (Boulton, 2005), may explain the relationship between increasing disease severity (demarcated by the complication of peripheral neuropathy) and elevated IMAT accumulation. While this interpretation is plausible, so too is the theory of genotype determination of fat accumulation in this population – which suggests altered genotypic expression of genes involved in the leptin-signaling cascade, PPAR system of lipid metabolism and genes involved in preadipocyte differentiation, among others (Joffe and Zimmet, 1998). This genetic theory requires further investigation.

Our findings that the T2DMPN group had significantly more %IMAT in the deep compartment of the calf may have important consequences as the deep compartment muscles, consisting of the flexor hallucis longus, flexor digitorum longus, and tibialis posterior muscles maintain the biomechanical stability and function of the ankle and foot (Rattanaprasert et al. 1999). The accumulation of IMAT in the deep compartment of the leg may be a precipitating factor in the development of common disease-related impairments, including acquired neuropathic hind foot and medial column mid foot deformities, which results in elevated medial forefoot pressures during gait from impaired muscle function (Caselli et al. 2002; Rogers et al. 2011).

4.2 Muscle Performance

Our previous research addressing the effect of excess IMAT infiltration on muscle performance of the calf has shown negative associations between excess lipid accumulation and measures of ankle torque and power (Hilton et al. 2008; Tuttle et al. 2012). In the present study, participants with T2DMPN also demonstrated significantly higher % IMAT volume in the calf and reduced plantar flexion and dorsiflexion strength and power. IMAT may impair extrinsic muscle power by interfering with muscle fascicle arrangement, pennation angle, excursion during contraction, and perimysial function (Gillies et al. 2011; Wakeling et al. 2011). Additionally, IMAT infiltration has been associated with a slowing of contractile ability -- indicated by a decline in the rate of torque development and prolonged contraction duration exacerbated by a preferential loss of type II (fast-twitch) motor units and the development of sensorimotor neuropathy in those with T2DMPN (Allen et al. 2014). Indeed, Allen et al. (2014) noted that participants with T2DMPN displayed reduced maximal voluntary contraction, slower rates of torque development, weaker and slower twitch potentials, and greater twitch contraction duration, which contributed to the loss of dorsiflexion torque and power. Furthermore, reductions in gastrocnemius-soleus muscle volume suggest that volume previously occupied by lean muscle tissue is infiltrated by IMAT. Further research is needed to investigate the effect of IMAT on muscle ultra-structural arrangement and contractile mechanics of individual leg muscles.

4.3 Physical Function

Our results demonstrated that individuals with T2DMPN, a group with higher levels of IMAT infiltration, had reduced physical functioning on a wide range of activities. Those with T2DMPN scored an average of 5 points lower than obese-matched controls on the 9-item PPT, and 3 points lower than those with T2DM only. Previous research has shown that a change of 3.7 points on the PPT differentiated those who were living independently and those who were living in a nursing home or had died among older adults (Reuben et al. 1992). Additionally, the clinically meaningful change in PPT scores is 2.4 points (King et al. 2000). Thus, the decline in physical performance and function observed between groups in this study suggests that disease progression is paralleled by meaningful declines in functional capacity.

The T2DMPN group had shorter 6 minute-walk distances and slower walking speeds over 50 feet, which coincides with the findings of previous investigations (Nardo et al. 2014; Tuttle et al. 2011; Visser et al. 2002). However, this study demonstrates additional functional constructs affected by excess calf IMAT accumulation, including balance dysfunctions (single-limb stance time, coin pickup time), and dynamic strength and power (e.g. longer time required to climb stairs, and lower chair rise scores). While balance deficits could result from neuropathy-related impairment of sensory responses, IMAT infiltration may also exacerbate this deficit by superimposing a reduced level of central drive, muscle activation, and contractile mechanics in response to postural sway and perturbation (Yoshida et al. 2012).

4.4 Multiple Regression

The results of the linear regression highlight the functional decrements associated with excess leg IMAT accumulation. Because PF power is an independent predictor of functional capacity (Suzuki et al. 2002), we wanted to control for this variable to isolate the unique contributions of group status and IMAT volume on physical function. With all other variables held constant, having T2DMPN immediately results in a 3.5-point drop in 9-item PPT score (relative to the control group), while having T2DM results in an immediate 2-point drop. Additionally, after controlling for PF power and group status, for every 1-point increase in %IMAT volume, there is a 0.1-point reduction in PPT score. This model raises an intriguing question: if excess increases in IMAT volume impair physical performance, can interventions designed to reduce excess IMAT accumulation in the calf improve functional outcomes? Preliminary research has produced inconclusive results. In the thigh, Jacobs et al. (2014), recently showed that three-months of eccentric resistance training or traditional resistance training failed to reduce IMAT volume in older adults. In contrast, Marcus et al. (2008) showed that thigh IMAT was significantly reduced after 12 weeks of combined eccentric and aerobic exercise in T2DM participants without PN. Further investigation is needed to determine if IMAT is amenable to reduction through exercise interventions.

4.5 Relationships Between Adipose Tissue Deposition and Muscle Performance and Physical Function

Observed correlations between adipose deposition in the leg, strength and power production, and physical performance, underscore the relationships between muscle structure and function. The positive correlations between SQAT volume and 9-item PPT, 6 minute walk distances, ankle plantar flexion muscle power, power ascending stairs, vibration perception (VPT), balance during dynamic reaching tasks (picking up a coin), and normalized gastrocnemius-soleus contractile tissue, associates subcutaneous adipose deposition with enhanced muscle composition, muscle function, and physical performance. Conversely, IMAT volume was negatively correlated with all performance measures. The transition from subcutaneous to intermuscular fat deposition may, therefore, play a role in the functional declines observed during the progression from obesity to T2DM to T2DMPN.

4.6 Limitations

This study has limitations. Only 38 participants (10 obese controls, 10 T2DM, 18 T2DMPN) had physical performance data, which may alter some point estimates in some of the outcome variables. Interpretation of muscle morphology, beyond volumes of fat and lean tissue, cannot be made, as neither biopsies nor biochemical analyses were used in this study. Similarly, while muscle impairment may suggest alterations to its ultrastructure, this information was not collected from included participants and can only be speculated. Finally the correlational relationships observed in this study indicate association, not causality.

4.7 Power Analysis

Based on post-hoc power analysis, the effect size (Cohen's f) for the difference in %IMAT volume between groups was .36, a medium effect size (Cohen, 1988). With an effect size of .36, at an alpha level of $\alpha = .05$, and a sample size of 79 with three groups, the calculated power was 81%. The same power was achieved for detecting group differences in leg %SQAT volume (Cohen's $f = .36$, power=81%).

5. Conclusion

This study shows group differences in adipose distribution of the leg between obese, T2DM and T2DMPN participants. These groups' differences suggest that a transition from SQAT to IMAT deposition is a complication that characterizes the progression from obesity, to T2DM, to T2DMPN. IMAT negatively affects muscle performance, which, in turn precipitate reduced physical function – creating a cyclical exacerbation of disease progression that dramatically impairs mobility. %IMAT volume is a strong predictor of physical function and interventions designed to reduce excess leg IMAT accumulation need to be investigated.

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Figure 1.

Leg Composition of participant groups was compared with 1-way ANOVAs, with post-hoc Tukey's HSD for pairwise comparisons. Bar heights represent group mean and error bars represent $\pm$ SEM. The following symbols represent post-hoc test pairwise differences : ^ (between T2DM and T2DMPN), + (T2DMPN and Controls). **A.** Total muscle volume of the leg across groups, ($p=.17$, NS), **C.** Anterior compartment muscle volume expressed as a % of total leg muscle volume for each group, ($p=.45$, NS), **E.** Gastroc-soleus muscle volume expressed as % of total leg muscle volume for each group. +, T2DMPN and Controls ($p=.01$). **B.** Total fat volume of the leg across groups, ($p=.10$, NS), **D.** Fat volume in deep and lateral compartments between groups. Deep muscle compartment, +, T2DMPN and Controls ($p=.03$), and ^, T2DM and T2DMPN ($p=.02$). In the lateral muscle compartment, ^, T2DM and T2DMPN ($p=.03$). **F.** The % of total fat volume attributed to IMAT and SQAT. +, %IMAT Volume between T2DMPN and Controls ($p=.02$); and (+, % SQAT $p=.02$).

Figure 2.

T1 cross-sectional images of the legs of a control, T2DM, and T2DMPN participant. These images exemplify the progression of obesity (control) to T2DMPN and the accompanying loss of SQAT, accumulation of IMAT, loss of muscle volume (gastroc-soleus% vol.), and reduced physical function (PPT).

Table 1

Physical and demographic characteristics of participants.

	Controls (N=22)	DM (N=10)	DMPN (N=47)	Statistical Analysis	Post-hoc
Gender	N=10 female, 12 male	N=5 female, 5 male	N=18 female, 29 male	p=.72	
Weight (kg)	102.8±25.7	103.5±16.3	98.4±26.6	p=.71	
BMI	34.2±6.3	34.7±6.2	34.3±7.4	p=.97	
Age	61±12	55±10	61±12	p=.27	
Years of Diabetes	NA	8±7	12±9	p=.16	
Biothesiometry VPT (volts)	L Hallux=19±9	L Hallux=19±13	L Hallux=38±11	p<.001 *	^ (p=.001), + (p=.002)
	L MT Head=18±11	L MT Head=15±14	L MT Head=36±15	p<.001 *	^ (p<.001), + (p=.001)
Semmes Weinstein Monofilament (Avg. Score)	L=1±0	L=2.1±.7	L=3.3±.7	p<.001 * ▽	^ (p=.002), + (p<.001), # (p=.003)

Participant demographics were compared using 1-way ANOVA, and chi-square was used for sex distribution between groups.

* denotes significant F-test indicating difference between groups,

▽ denotes Games-Howell post-hoc for violation of homogeneity of variance.

The following symbols represent post-hoc pairwise comparisons :

^ (between T2DM and T2DMPN),

+ (between T2DMPN and Controls),

(between T2DM and controls).

Not shown: Biothesiometry and Semmes Weinstein Monofilament testing of the right foot. Monofilament testing was identical between feet and VPT differed by less than 3 volts for all locations. ANOVA and post-hoc results identical for both feet.

Table 2

Muscle performance and functional performance measures.

Biodex Muscle Performance Measures					
Variable	Controls (N=22)	DM (N=10)	DMPN (N=47)	F-statistic P-value	Post-Hoc
PF peak torque 60/sec (Nm)	61±27	43±9	47±16	p=.02*	+ (p=.01) # (p=.02)
PF power 60/sec (Watts)	48±23	35±8	39±16	p=.06	
PF work 60/sec (Nm)	37±16	30±16	26±12	p=.21	
PF peak torque 120/sec	56±22	46±11	41±14	p=.07	
PF torque per unit muscle volume (Nm/cm ³)	.24±.11	.17±.06	.23±.09	p=.18	
PF power 120/sec	94±30	64±19	64±24	p=.02*	+ (p=.01) # (p=.02)
DF peak torque 60/sec	12±19	14±8	5±7	p=.01*	+ (p=.02) ^ (p=.02)
DF power 60/sec	8±15	9±7	3±5	p=.04*	+ (p=.03)
DF torque per unit muscle volume	.18±.24	.22±.16	.09±.11	p=.02*	+ (p=.04) ^ (p=.01)

Functional Performance Measures**					
Variable	Controls (N=10)	DM (N=10)	DMPN (N=18)	F-statistic P-value	Post-Hoc
9-item PPT (total score)	34±1	32±2	29±4	p=.003*	+ (p=.002)
Chair Rise Time (seconds)	12±2	14±2	18±6	p=.004* ∇	+ (p=.002)
Single Leg Balance (seconds)	14±12	22±12	10±9	p=.035*	^ (p=.02)
6 minute walk (meters)	1681±160	1467±274	1396±323	p=.03*	+ (p=.03)
Coin Pickup Time (seconds)	1.9±.4	2.5±.9	3.2±1	p=.01* ∇	+ (p=.002)
Stair Power (Watts)	809±327	660±176	633±219	p=.18	
Stair Climb Time (1-flight seconds)	4±1	5±2	6±2	p=.05* ∇	+ (p=.01)
50 ft walk time (seconds)	10±1	11±2	12±2	p=.03*	+ (p=.02)

Participant demographics were compared across groups using 1-way ANOVA.

* denotes significant F-test indicating difference between groups,
∇ denotes Games-Howell post-hoc for violation of homogeneity of variance.
The following symbols represent post-hoc pairwise comparisons:
^ (between T2DM and T2DMPN),
+ (between T2DMPN and Controls),
(between T2DM and controls).
** (These analysis performed on a subgroup of 38 participants who completed these measures- see section 2 and 2.4.1–2.4.4).

Table 3

Multiple Regression Analyses.

	Unstandardized Coefficients		Standardized Coefficients		t	Sig.	β 95% CI
	β	Std. Error	β				
Constant	34.977	2.098			16.68	<.001	(30.706, 39.289)
T2DMPN	-3.468	1.318	-.446		-2.632	.01	(-6.163, -.773)
T2DM	-2.132	1.439	-.252		-1.481	.14	(-5.074, .811)
%IMAT	-.096	.030	-.461		-3.159	.004	(-.158, -.034)
Normalized PF Power 60 deg/sec	8.876	9.917	.127		.895	.37	(-11.406, 29.158)
Model Sig.: F = 7.121, df=4, p<.001 Nagelkerke R ² = .496 Unique Variance (PPT) accounted for: %IMAT= 17.4%, T2DMPN status= 12%, PF Power= 1.4%, T2DM status= 3.8%							

Linear regression model to determine 9-item PPT score from PF power, muscle quality and composition (%IMAT), and disease status. Beta coefficients for each predictor represent change in PPT score due to disease status or 1 unit change in %IMAT or PF power. Model significance, amount of variance in 9-item PPT score explained by the model (R²), and amount of variance in PPT explained by each predictor are shown in the bottom panel.