Obesity/ Type II Diabetes Promotes Function-limiting Changes in Flexor Tendon Extracellular Matrix Organization that are not Reversed by Restoring Normal Metabolic Function

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Abstract

Type II Diabetes (T2DM) negatively alters baseline functions of flexor tendon range of motion and mechanical properties leading to impaired hand movement. As of yet, the biological mechanism(s) leading to diabetic flexor tendinopathy have not been characterized. Here, we use a murine model of diet-induced obesity (DIO) and T2DM to characterize progressive changes in tendon function and organization and to determine if these changes can be reversed through restoration of normal metabolic function. We show that induction of T2DM/obesity is sufficient to initiate an irreversible cascade of pathological events leading to impaired gliding function and mechanical properties specifically in the flexor tendon. Furthermore, we demonstrate that collagen extracellular matrix (ECM) organization is irreversibly compromised during T2DM/obesity and that restoration of normal metabolism is not sufficient to halt these changes. Finally, western blot analysis demonstrate changes in insulin receptor (IR) signaling in obese/diabetic tendons. Collectively, these results establish and characterize a murine model of diabetic flexor tendinopathy, which will aid in identification of novel targets to treat diabetic flexor tendinopathy.

Introduction

Tendon is a dense connective tissue that is responsible for force transmission between muscle and bone and is required for skeletal locomotion. Composed of a compact collagen extracellular matrix (ECM), tendon is organized in a hierarchical structure, with resident tendon cells (tenocytes) interspersed along the collagen fibers. Tenocytes are responsible for synthesis, deposition, and turnover of the collagen ECM, which serves as the main structural component of the tendon (1, 2)

Type II Diabetes Mellitus (T2DM) is a metabolic disease characterized by hyperglycemia and a decrease in sensitivity to insulin (3), and is strongly correlated with obesity (4). While it is difficult to dissect the systemic effects of Type II Diabetes from obesity, it is clear that induction of both obesity/T2DM leads to severe alterations in metabolic function resulting in a variety of pathological changes throughout the body (5).

While T2DM results in a plethora of systemic pathologies (6), the dramatic impact of T2DM on the musculoskeletal system has only more recently become appreciated. Approximately 83% of patients with

T2DM will experience some form of musculoskeletal system degeneration or inflammation (7). Furthermore, T2DM has been shown to increase the risk of tendinopathy and tendonitis (8), with a variation in sensitivity to T2DM between different tendons (8, 9). The flexor tendons (FTs) of the hand are among the most sensitive to pathological changes of T2DM, with approximately 50% of T2DM patients experiencing limited hand function and mobility (10, 11). FTs facilitate movement of the hand and digits via nearly frictionless gliding of the FTs through the synovial sheath. Diabetic flexor tendinopathy results in severe inflammation and pain in the hand and digits (12), and decreased digit range of motion (ROM) (8). These impairments in tendon function can severely impair use of the hands, and subsequently diminish overall quality of life. At present, the only treatments for diabetic tendinopathy are surgical release of fibrous adhesions, or corticosteroid injections to reduce local inflammation (13, 14). Consistent with disruptions in tendon homeostasis, diabetic tendons are more likely to experience spontaneous rupture requiring surgical repair (13, 14). Furthermore, tendon healing after acute injury is impaired in diabetic patients relative to non-diabetic patients (15, 16). Therefore, there is a strong need for therapeutic interventions to prevent tendon pathology in diabetic patients.

Currently, very little is known about the cellular and molecular mechanisms underlying diabetic tendinopathy, although pathological changes increase as a function of T2DM disease duration (8). To begin to address this gap in knowledge, we have utilized a murine model of diet induced obesity and Type II diabetes (17) to test the hypothesis that flexor tendons from obese/ diabetic mice will undergo progressive, pathological changes in tendon gliding function and mechanical properties. We also examined the effects of restoration normal metabolic function as a potential means to reverse or halt diabetic tendinopathy. Furthermore obese/diabetic mice have altered extracellular matrix organization, impaired mechanical properties and gliding function. We also show that reversal of obesity/diabetes is insufficient to reverse these pathological changes, suggesting that management of the systemic effects of obesity/ type II diabetes is an inadequate approach to treat diabetic tendinopathy.

Methods

Diet Induced Obesity and Type II Diabetes: All animal experiments were approved by the University Committee on Animal Resources (UCAR) at the University of Rochester. Male C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) were housed in groups of five in a 12-hour light/dark cycle. At four weeks of age mice were placed on either a low fat diet (LFD; 10% Kcal, Research Diets #12450D), or high fat diet (HFD; 60% Kcal, Research Diets #D12492) for up to 60 weeks. A third cohort (HFD-LFD) of mice was placed on the HFD for 12 weeks, and then switched to the LFD until sacrifice. Mice were sacrificed at 12, 24, 40, 48 and 60 weeks post-diet initiation, via carbon dioxide inhalation followed by cervical dislocation.

Measurement of Fasting Blood Glucose: At 12, 24, 40 and 48 weeks post-diet-initiation, mice were fasted for five hours (18), weighed, and fasting blood glucose levels were measured using a OneTouch2 blood glucometer (LifeScan Inc. Milpitas, CA).

Glucose Tolerance Test: To assess glucose tolerance at 20 weeks post-diet-initiation LFD, HFD and HFD-LFD mice received a 10 uL/g bolus of 20% glucose in PBS via intraperitoneal injection following a five hour fast. Blood glucose levels were measured at 15, 30, 60 and 120 minutes after the glucose bolus.

Assessment of Body Fat: At 12 and 48 weeks post-diet-initiation the percent body fat was measured using the PIXImus dual-energy X-ray absorptiometer (DXA) (GE Lunar PIXImus, GE Healthcare, WI).

*Assessment of Gliding Function***:** Following sacrifice, hind limbs were harvested for gliding and biomechanical testing at 12, 24, 40, 48 and 60 weeks post diet initiation (n=5-12 per diet per time-point.) The FDL tendon was isolated from the myotendinous junction to the tarsal tunnel, leaving the tendon intact through the digits. The proximal end of the tendon was adhered between two pieces of tape using cyanoacrylate. The tibia was secured using a custom grip and the taped end of the FDL was incrementally loaded from 0-19 g. Upon application of each weight, an image was taken of the Metatarsophalangeal (MTP) joint flexion, and measured using ImageJ

(http://imagej.net). Using these images, MTP flexion angle was calculated as the difference in MTP flexion angle between unloaded (0g) and 19g. The gliding resistance was calculated by fitting the flexion data to a single-phase exponential equation. Non-linear regression was used to determine the gliding resistance (19).

Tensile Biomechanical Testing: Following gliding function testing, the FDL was released from the tarsal tunnel, and the tibia was removed. The proximal end of the FDL and the digits were held in opposing custom grips in the Instron device (Instron 8841 DynaMight axial servohydraulic testing system, Instron Corporation, Norwood, MA). The tendon was displaced in tension at 30mm/minute until failure. Stiffness and maximum load at failure were calculated from the force displacement curve.

Second Harmonic Generation 2-Photon Confocal Imaging: Whole FDL tendons (n=3 per diet per time-point) were isolated and fixed in 10% neutral buffered formal (NBF). Specimens were scanned with a Titanium:Sapphire laser, tuned to 800nm, under 25x magnification at 0.5μm steps. 3D projections of image stacks were generated using the 3D-Project macro on ImageJ and analyzed for collagen fibril uniformity using the Directionality macro. The Directionality macro utilizes Fourier transform analysis to derive spatial orientation of image stacks. Using a Gaussian fit, directions and angles from Second Harmonic Generation (SHG) image stacks were calculated and clustered based on similarity, resulting in quantification of fibril angle dispersion.

Histology: Collection of human tissue samples was approved by the University Research Subjects Review Board (RSRB) at the University of Rochester. Human flexor tendon samples were obtained from both T2DM (n=3) and non-T2DM patients (n=6) following surgical limb amputation. Murine hind paws were harvested between 12-60 weeks after diet initiation (n=4-8 per diet per time-point). Tissues were fixed for 72hrs in 10% NBF, de-calcified in 14% EDTA (pH 7.2-7.4) for 14 days and processed for paraffin sectioning. Three-micron serial sections were cut through the sagittal plane of the murine hind paw, or through the entire depth of human tendon. Sections were stained with Alcian Blue/Hematoxylin/Orange G (ABHOG).

Transmission Electron Microscopy: FDL tendons were isolated (n=3 per diet per time-point) and fixed in Glutaraldehyde Sodium Cacodylate fixative. One-micron axial sections were cut and stained with Toluidine blue. One-micron sections were then trimmed to 70nm and stained with uranyl acetate and lead citrate. Sections were placed on grids for imaging on a Hitachi 7650 Analytical TEM. Eight to twelve non-overlapping images were taken from mid-substance of each tendon at 15,000x and 40,000x magnification. For measurement of fibril diameter, a region of interest (ROI) of was determined within each image so that a minimum of 80 fibrils could be measured. Diameters were measured along the y-axis. Collagen Fibril density was measured in a 2340 x 1860 pixel area. Collagen fibril density and diameter measurements were made in ImageJ.

Insulin receptor signaling: Whole FTs were isolated from HFD and LFD mice 48 weeks after diet initiation (n=3 per group), and stimulated with vehicle (0.5% BSA in PBS) or 1nM insulin for 15 minutes on ice, followed by protein isolation for western blot analyses. Blots were probed for phospho-AKT Ser473 (1:1000, Cell Signaling, Rabbit mAB #4060), and total AKT (1:1000, Cell Signaling, Rabbit Ab #9272S), developed with SuperSignal West Pico or Femto Chemiluminescent Substrate and imaged on a GelDocXR (BioRad, Hercules, CA).

Statistical Analysis: Body weight, blood glucose levels, biomechanical and gliding data were analyzed using a two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons with significance set as $\alpha = 0.05$. GTT, SHG images, and collagen fibril density and diameter were analyzed using one-way ANOVA with Bonferroni's post-hoc multiple comparisons ($\alpha = 0.05$). All data was analyzed using Prism GraphPad 7.0 statistical software. Data are presented as mean ± SEM.

Results

HFD induces Obesity and Type II Diabetes in Mice

HFD mice had a significant increase in body weight relative to LFD at all time-points, (12 weeks- LFD: 31.13 g ± 4.28 g, HFD: 45.96 g ± 2.08 g, $p<0.0001$) (Figure 1A). Body weights of HFD-LFD mice were not significantly different than LFD from 24-60 weeks. Fasting BG was significantly increased in HFD mice at 12, 40 and 48 weeks, relative to LFD (12 weeks- LFD: 154.6 mg/dL ± 16.1, HFD: 205.2 mg/dL ± 24.7, $p <0.0001$). However this increase was not observed in HFD-LFD compared to LFD between 24-48 weeks (Figure 1B). Glucose tolerance was significantly impaired in HFD, relative to LFD during a glucose tolerance test, with a 37% increase in area under the curve (AUC) in HFD, relative to LFD at 20 weeks post diet initiation ($p<0.0001$) (Figure 1C). In contrast, no change in glucose tolerance was observed between HFD-LFD and LFD at this time (Figure 1C). Significant increases in body fat percentage were observed in HFD, relative to LFD at 12 weeks and 48 weeks (12 weeks: +100%, $p<0.0001$; 48 weeks: +21%, $p=0.004$). Body fat percentage was significantly decreased in HFD-LFD mice, relative to both HFD (-68%, $p=0.0002$), and LFD (-27%, $p=0.03$) at 48 weeks (Figure 1D).

HFD and HFD-LFD alters Flexor mechanical properties

No change in FDL stiffness was observed between groups at 12 weeks. At 24 weeks both HFD and HFD-LFD FTs exhibit a significant increase in stiffness relative to LFD (+49% and +117%, respectively, $p<0.006$), however these increases in stiffness relative to LFD were not sustained through 40 weeks (Figure 2A). FDL max load at failure was not significantly different between groups at 12, 24 and 40 weeks. At 48 weeks max load at failure was significantly decreased in HFD and HFD-LFD FTs, relative to LFD (HFD: -25%, $p<0.01$; HFD-LFD: -21%, $p<0.04$) (Figure 2B). No change in max load at failure was observed between groups at 60 weeks, due to a decrease in max load at failure in LFD tendons from 48-60 weeks (-23.5%) (Figure 2B).

HFD and HFD-LFD Impairs Flexor Tendon Gliding Function

To assess tendon gliding function we quantified metatarsophalangeal (MTP) flexion angle and gliding resistance. No change in MTP Flexion Angle was observed between groups at 12 and 24 weeks. However, at 40 and 48 weeks the MTP Flexion Angle was significantly decreased in HFD mice relative to LFD (48 weeks-

LFD: 59.07° ± 5.0; HFD: 35.4° ± 9.52, p<0.0001). At 48 and 60 weeks, MTP Flexion angle was significantly decreased in HFD-LFD relative to LFD (48 weeks: HFD-LFD: 30.36° ± 9.3, p=0.04) (Figure 3A). Similar to MTP flexion angle between diet groups at earlier time-points, HFD and HFD-LFD at 12 and 24 weeks showed no significant changes in gliding resistance. However at 40, 48 and 60 weeks, gliding resistance was significantly increased in HFD and HFD-LFD, relative to LFD (60 weeks- LFD: 17.47 ± 7.36; HFD: 29.67 ± 10.41; HFD-LFD: 36.68 ± 16.63, p<0.05) (Figure 3B.)

Increased Disorganization of the Collagen Extracellular Matrix is observed in T2DM Patients

Dramatic differences were observed between representative non-T2DM patients and T2DM patients. Flexor tendons from non-diabetic patients exhibit characteristic collagen ECM organization with tightly packed and fairly linear collagen fibers. In contrast, flexor tendons from obese/diabetic patients have some variation in pathological changes; however, there is a consistent decrease in collagen ECM organization (Figure 4A). Histologically, no apparent changes in the tendon ultrastructure were observed between groups in our murine model. FTs from LFD, HFD and HFD-LFD appeared compact and organized through 48 weeks (Figure 4B).

Flexor Tendons from HFD and HFD-LFD Mice have Decreased Collagen Fiber Alignment

To further examine the impact of T2DM on collagen fibril organization we utilized Second Harmonic Generation Imaging (SHG). The Three-Dimensional projections of SHG imaging revealed a loss of collagen fiber compactness in HFD tendons at 24 weeks (white arrows, Figure 5A) that was not observed in LFD. Quantification of fiber alignment using Fourier analysis demonstrated an increase in collagen fibril dispersion in HFD tendons at 24 weeks relative to LFD (LFD: 7.73°±0.324; HFD: 14.01°± 2.84, p<0.04). No difference in fibril dispersion was observed between LFD and HFD-LFD (10.16° ± 0.77) at 24 weeks (Figure 5B). At 40 weeks collagen fibril dispersion was significantly increased in HFD (10.28 ± 0.59, p<0.0001), and HFD-LFD (8.71 ± 0.57, p<0.01), relative to LFD (6.28 ± 0.42)(Figure 5C & D).

HFD and HFD-LFD Leads to Loss of Collagen Fibril Density

To further elucidate the impact of T2DM on collagen fibril density and compactness we utilized Transmission Electron Microscopy (TEM) at 40 weeks post-diet initiation. At 3500x magnification, there were no observed changes in collagen packing and organization between LFD, HFD and HFD-LFD. However, lipid deposits were observed in the mid-substance of HFD tendons (black arrows, Figure 6A). At 40,000x magnification there were significant changes to collagen fibril density and diameter in both the HFD and HFD-LFD relative to LFD (Figure 6A-D). Collagen fibril diameter was significantly increased in HFD and HFD-LFD, relative to LFD at 40 weeks ($p<0.0001$). The median fibril diameter increased from 150.2nm in LFD to 180nm in HFD ($p<0.0001$), and 180.2nm in HFD-LFD ($p<0.0001$) (Figure 6B & C). Collagen fibril density was decreased in HFD and HFD-LFD, relative to LFD, at 40 weeks (-23% and -19% respectively, $p<0.01$) (Figure 6D).

Insulin Receptor Signaling is attenuated in HFD tendons

Based on changes in insulin sensitivity in other diabetic tissues (20), we examined changes in activation of Insulin Receptor (IR) signaling in obese/ T2DM and non-diabetic murine tendons. Upon insulin stimulation tendons from LFD mice demonstrate a robust induction of p-Akt expression, indicating activation of IR signaling. In contrast, induction of p-Akt was blunted in HFD tendons (Figure 7).

Discussion

Both Type I and Type II diabetic patients are at an increased risk of developing tendinopathy (11). While several tendons can be affected by diabetic tendinopathy, including the Achilles and the supraspinatus tendons, the flexor tendons are most commonly impacted by T2DM (10, 11). Patients with diabetic tendinopathy will experience decreased tendon strength with an increased likelihood of tendon rupture, requiring surgical repair (13). Currently, diabetic tendinopathy treatment options are limited to corticosteroid injections, or surgical intervention (13, 14). Therefore it is imperative to understand the biological mechanism driving this pathology in order to develop novel therapeutic approaches to improve tendon function. To address the gap in knowledge surrounding the progression of diabetic tendinopathy, we have characterized a murine model of diet induced obesity and Type II diabetes and assessed its impact on tendon homeostasis. To our

knowledge, this is the first study to investigate the mechanistic, cellular, and molecular changes that occur during long-term diabetic tendinopathy in a murine model. We have demonstrated that induction of T2DM/obesity recapitulates the phenotypes observed clinically in diabetic flexor tendinopathy, including decreased mechanical properties and tendon range of motion. Furthermore, profound changes in collagen organization in both HFD and HFD-LFD tendons support a strong link between altered ECM organization and impaired mechanical properties in diabetic tendinopathy. Perhaps most interestingly, we demonstrate that resolution of metabolic dysfunction from obesity/T2DM is insufficient to reverse diabetic tendinopathy, highlighting the need for tendon-specific treatment options, rather than simply managing or reversing T2DM.

Clinically, one of the pathological hallmarks of diabetic tendinopathy is a decrease in tendon strength, rendering the tendon more susceptible to rupture. Several studies have demonstrated that hyperglycemia is associated with inferior mechanical properties. Maffulli *et al*., demonstrated that mechanical properties are reduced in the Achilles and supraspinatus tendons in a hyperglycemic environment (21), while impairments in restoration of tendon mechanical properties after injury is delayed in the Achilles tendon of hyperglycemic rats (22). Consistent with this, we observe a significant decrease in tensile strength of the FT at 48 weeks in both the HFD and HFD-LFD tendons relative to LFD, although it is not yet clear to what extent these phenotypes are driven by elevated blood glucose levels, relative to other components of metabolic dysfunction that occur in obese/ diabetic mice. Interestingly, max load at failure was not significantly different between HFD and HFD-LFD compared to LFD at 60 weeks. We believe this is due a dramatic decrease in max load of the LFD tendons from 48 to 60 weeks. We have recently demonstrated that aging does not alter baseline mechanical properties of the FDL (23), suggesting this is not simply a reflection of advanced age in these mice. Indeed, low calorie diets have been demonstrated to negatively impact cortical and trabecular bone architecture and volume, which can increase risk of fracture (24). Therefore, calorie restriction may have a similar impact on tendon architecture in the LFD mice, potentially accounting for this finding.

Tendon mechanical properties and ROM are dependent on organization of the collagen ECM (2). Given the decrements in mechanical properties of FDL tendons from HFD and HFD-LFD mice, we have focused extensively on changes in collagen organization and alignment. While no changes in tissue level collagen

organization were observed histologically, SHG and TEM demonstrated substantial changes in microscale and nanoscale collagen ECM organization. Increased collagen fibril angle dispersion was observed in HFD tendons at 24 weeks, and in HFD and HFD-LFD tendons at 40 weeks, relative to LFD, indicating a decrease in collagen fiber alignment and loss of ECM organization. Moreover, when we examined collagen packing using TEM, we observed a decrease in collagen fibril density, as well as loss of compactness in HFD and HFD-LFD tendons, compared to LFD. The observed changes in fibril organization are consistent with impaired gliding function, which implicates impairments in collagen ECM as a mechanism for loss of flexor tendon function in diabetic tendinopathy. Consistent with this, an increase in tendon bulk is observed clinically in type II diabetic patients (25), suggesting that loss of collagen compactness may impede normal gliding function leading to decreased tendon functionality and range of motion.

At the molecular level, the mechanisms driving diabetic tendinopathy are unclear. However, several studies have suggested that Advanced Glycation Endproducts (AGEs), which are dramatically increased in T2DM tissue (26), may promote loss of collagen organization due to altered cross-linking resulting from AGE-collagen binding (27). However, Fessel *et al*., have recently demonstrated that AGEs are unable to induce tissue level impairments in tendon mechanical properties (28), suggesting other mechanisms may be driving this phenotype. Alternatively, T2DM has been shown to decrease serum levels of Matrix Metalloproteinases (Mmps) (29), which mediate collagen ECM remodeling, while insulin receptor (IR) signaling can promote Mmp expression and activity (30, 31). Consistent with this, we observed a substantial decrease in insulin sensitivity in HFD tendons at 48 weeks, suggesting that loss of IR signaling in HFD tendons may contribute to ECM disorganization via loss of Mmp activity and remodeling. Further studies will be needed to precisely understand how changes in IR-Mmp signaling in obese/T2DM may promote tendinopathy.

While we clearly demonstrate that a murine model of diet induced obesity and type II Diabetes recapitulates many of the clinical findings of diabetic tendinopathy, there are several limitations that must be considered. We have confined these studies to male mice, as male C57Bl/6J mice are susceptible to diet induced obesity and T2DM, while females become obese but not diabetic (32, 33). Future studies will delineate the relative contributions of obesity and diabetes to the phenotypes presented here, although a similar incidence of

tendinopathy is observed between obese/ T2DM individuals and non-obese Type I diabetics (10, 11), suggesting that impairments in IR signaling may be driving these phenotypes to a greater degree than obesity. We have also initiated the development of diet induced obesity and T2DM in juvenile mice rather than adult mice, which may impact the severity or development of the phenotypes observed in our model. However, given that the incidence of obesity/T2DM is rapidly rising in the pediatric population (34), modeling this phenomenon is scientifically justified and understanding how age of onset affects diabetic tendinopathy progression is an important focus for future studies.

In summary, obesity/ T2DM is sufficient to induce an irreversible cascade of pathologic changes in the flexor tendon including altered ECM organization and alignment, ultimately leading to diminished mechanical properties and tendon range of motion. Resolution of metabolic dysfunction was not sufficient to prevent disease progression in the tendon, highlighting the need for tendon-based treatment options.

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Figure Legends

Figure 1. A High Fat Diet induces obesity and Type II Diabetes Mellitus. A) Body weight was measured in LFD, HFD mice between 12 and 60 weeks after diet initiation. HFD-LFD mice were initiated on a HFD, then switched to a LFD 12 weeks after initiation, as such body weights from these mice were measured only between 24-60 weeks (12-48 weeks after switching to the LFD). B) Changes in fasting blood glucose were measured after a 5hr fast between 12-48 weeks after diet initiation. C) At 20 weeks after diet initiation a significant impairment in glucose tolerance was observed in HFD, compared to LFD and HFD-LFD. A sustained increase in glucose levels post glucose bolus is indicative of impaired glucose tolerance. D) Body fat percentages of LFD, HFD, and HFD-LFD mice at 12 and 48 weeks post diet initiation. (*) Indicates $p<0.05$

Figure 2. T2DM/obesity leads to a decrease in flexor tendon tensile strength in HFD and HFD-LFD. A) Stiffness, and B) Maximum load at failure of the FDL between 12-60 weeks after diet initiation. (*) Indicates $p<0.05$.

Figure 3. T2DM/obesity impairs tendon ROM and gliding function. A) Measurement of metatarsophalangeal (MTP) joint flexion angle and B) Gliding Resistance in LFD, HFD and HFD-LFD tendons between12-60 weeks after diet initiation. (*) Indicates $p<0.05$

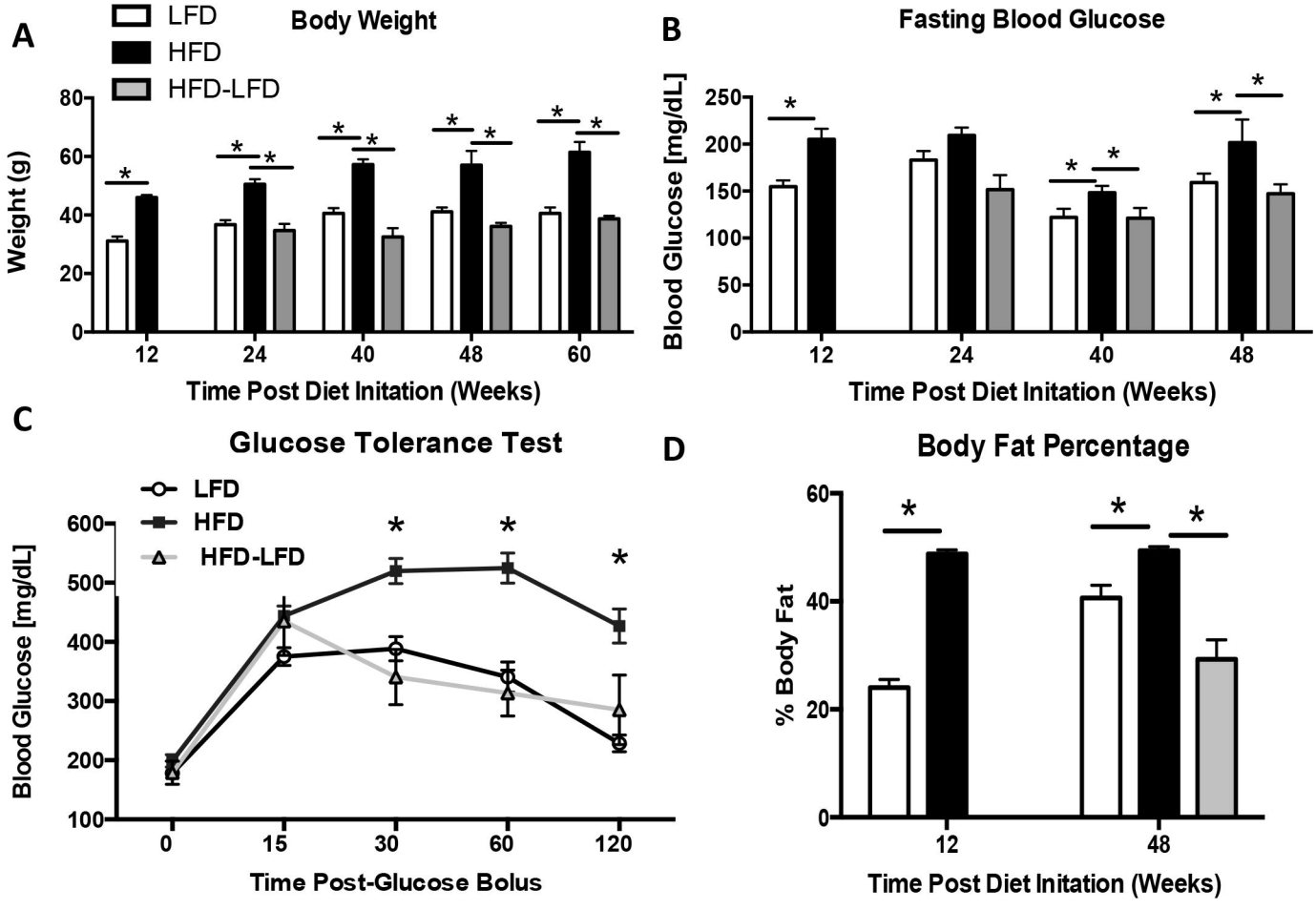
Figure 4. Decreased collagen ECM organization is observed in tendons from diabetic patients. Alcian Blue/ Hematoxylin/ Orange G (ABHOG) staining of tendon samples from A) non-diabetic and T2DM human flexor tendons, and B) FDL tendons from HFD, LFD, and HFD-LFD mice 24 weeks after diet initiation. A loss of collagen alignment is observed in T2DM human tendons, compared to non-diabetic, while no obvious tissue level changes in collagen organization are observed in murine tendons. Scale bars represent 50 microns.

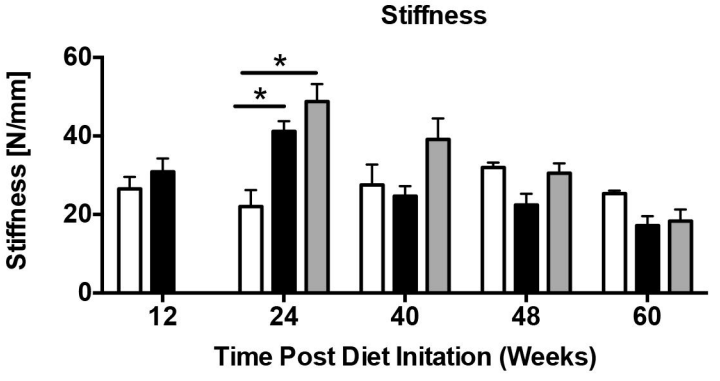
Figure 5. T2DM/obesity leads to increased collagen fibril dispersion in HFD and HFD-LFD tendons. A) Second harmonic generation imaging 3D projection sagittal images of the FDL tendon from LFD, HFD, HFD-

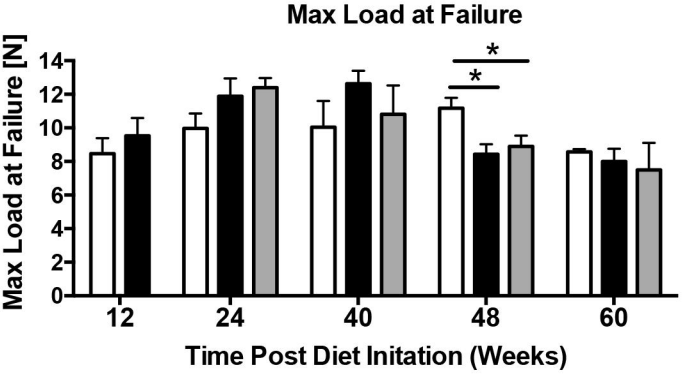
LFD at 24 weeks post diet initiation. White arrows identify areas of disrupted collagen fiber compactness. B) Average angular collagen fibril dispersion at 24 weeks. An increase in dispersion is associated with decreased alignment of collagen fibers. C) Second harmonic generation imaging 3D projection sagittal images of the FDL tendon from LFD, HFD, HFD-LFD at 40 weeks post diet initiation. D) Average angular dispersion of collagen fibrils at 40 weeks post diet-initiation. (*) Indicates $p<0.05$

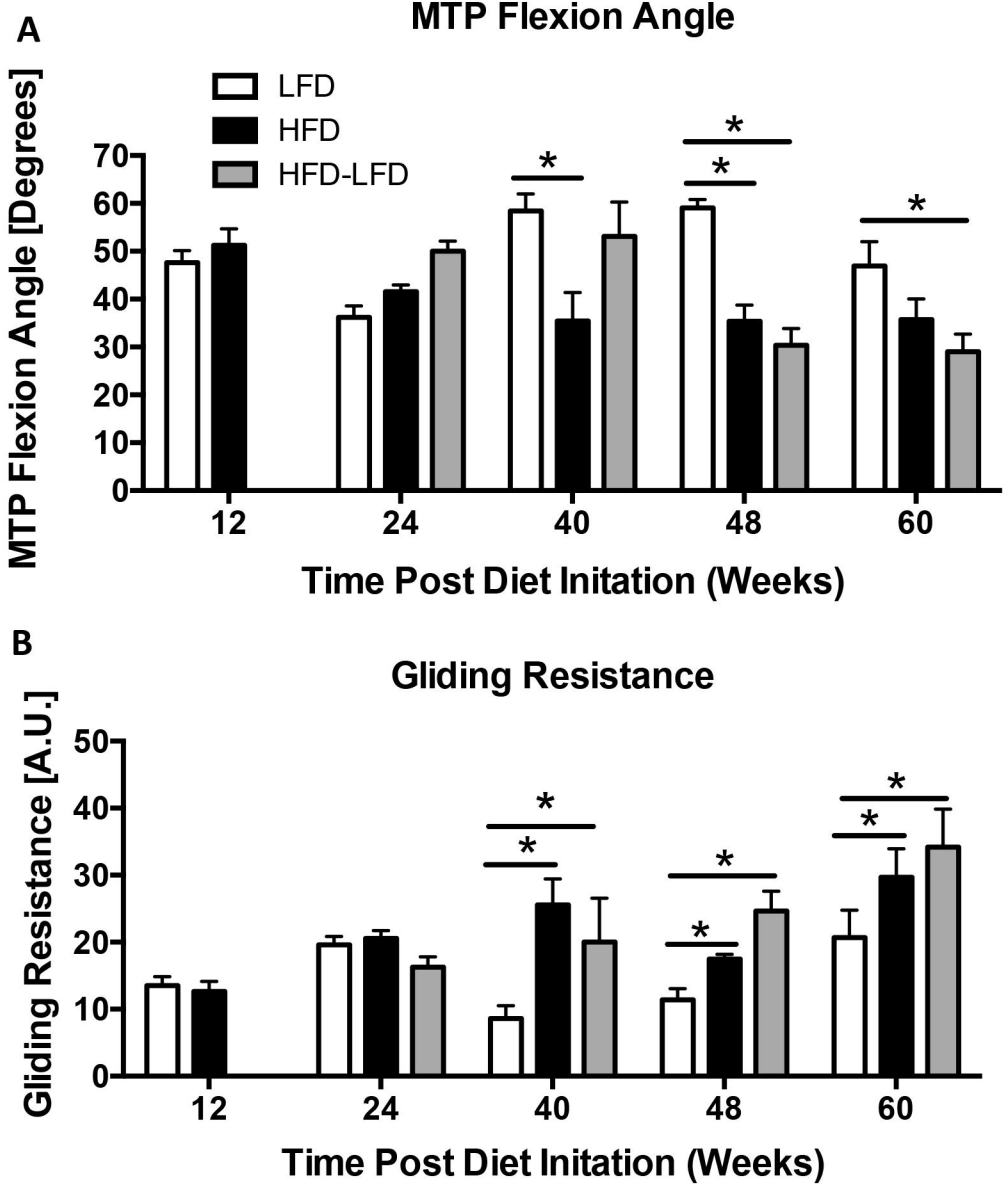
Figure 6: T2DM/obesity leads to loss of collagen fibril density and increased fibril diameter. A) TEM axial images of the FDL tendon from LFD, HFD and HFD-LFD mice at 40 weeks post diet initiation. Lipid deposits in HFD tendons are noted by black arrows. Scale bars represent 5 microns in 3500X magnification images, and 0.5microns in the 40,000X magnification images. B) Collagen fibril diameter histograms demonstrating an increase in median fibril diameter in HFD and HFD-LFD tendons compared to LFD. C) Collagen fibril diameter distributions with boxplot whiskers spanning data between the 5^{th} and 95^{th} percentiles. Data outside this range are plotted as individual points. D) Average collagen fibril density. A decrease in density is indicative of a decrease in collagen compactness. (**) Indicates $p<0.01$, while (****) indicates $p<0.0001$.

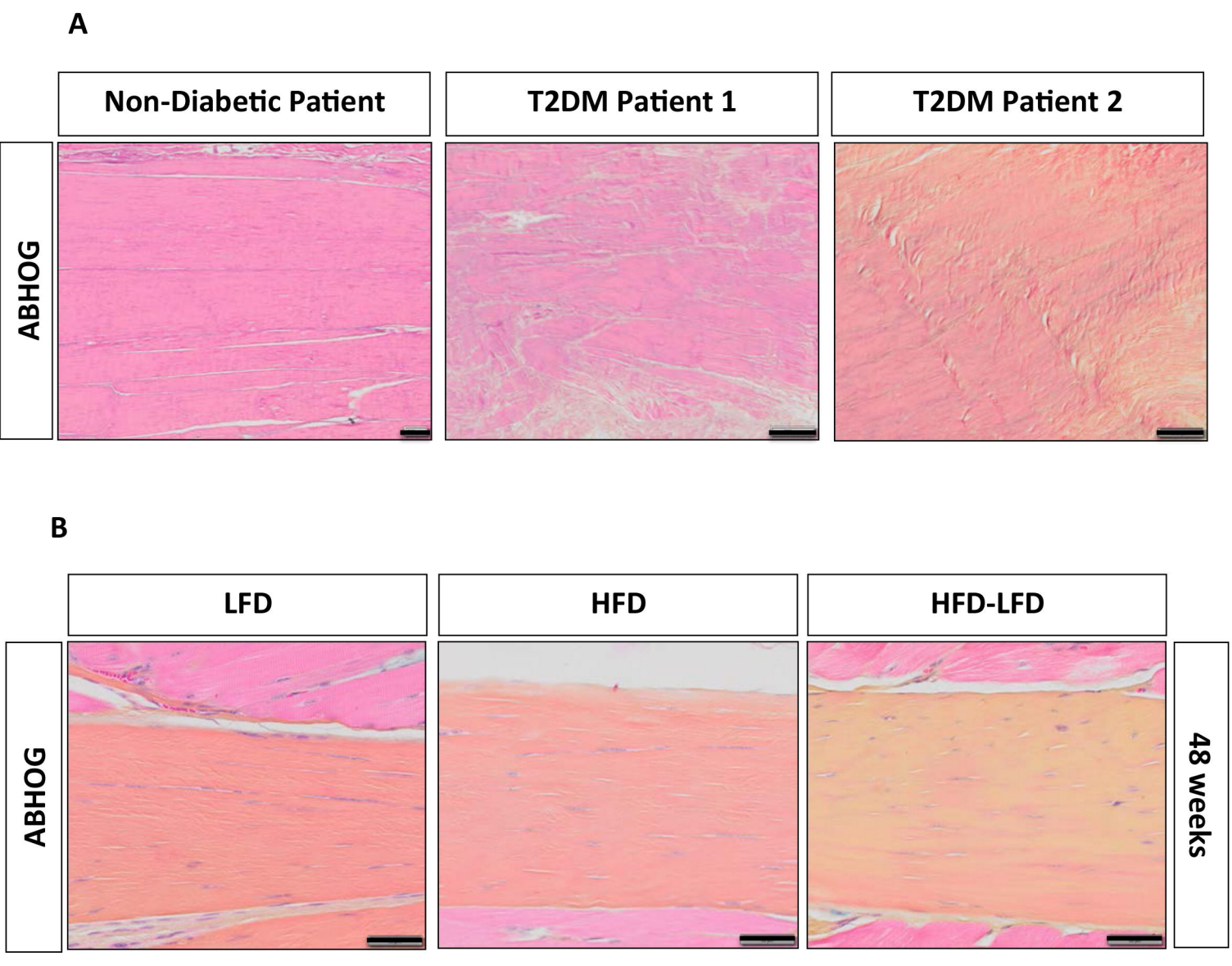
Figure 7: Activation of insulin receptor signaling is impaired in obese/ T2DM tendons. Tendons from HFD and LFD mice were stimulated with insulin or vehicle. Activation of IR signaling was observed in LFD tendons based on the increased expression of p-Akt. In contrast, no increase in p-Akt expression was observed in insulin stimulated HFD tendons compared to vehicle treatment indicating blunted sensitivity to insulin in HFD tendons.

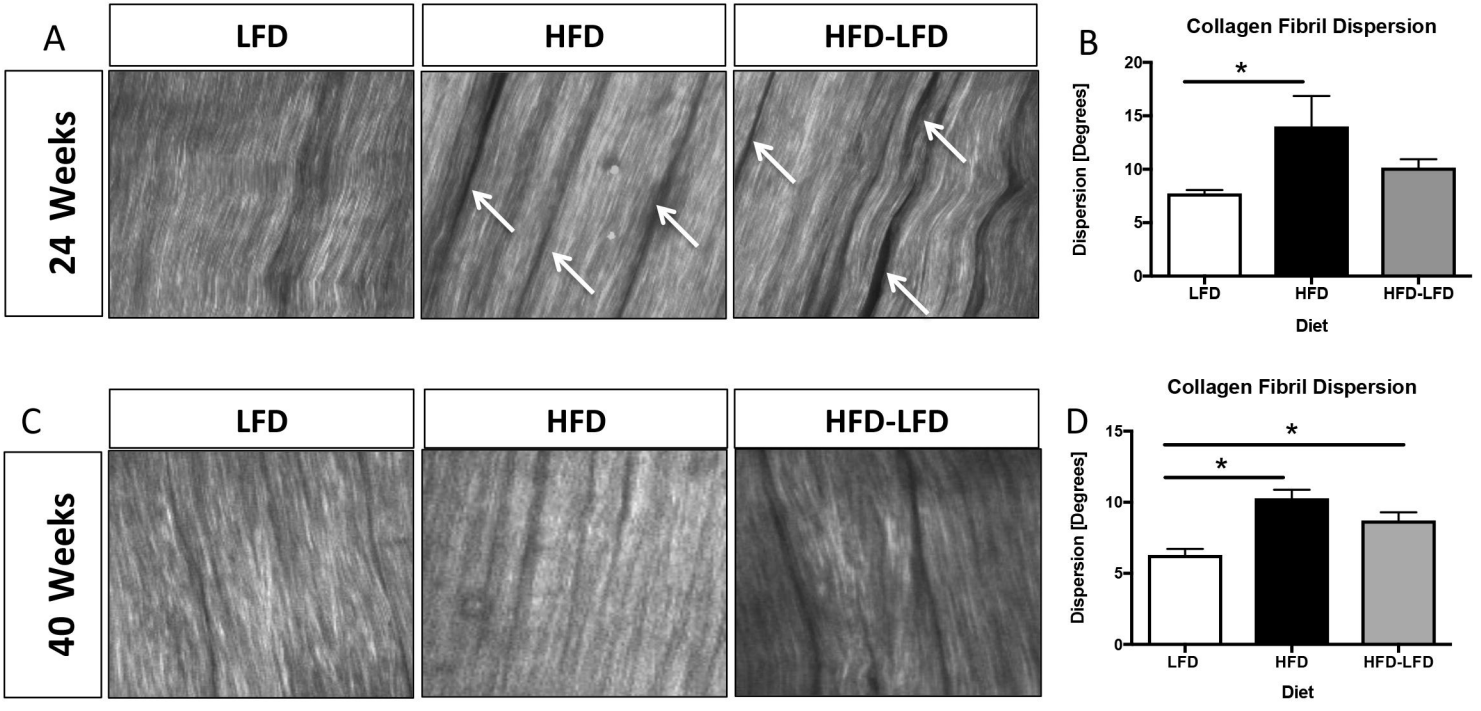

A
LFD
HFD
HFD-LFD
Body Weight
Weight (g)
80
60
40
20
0
12
24
40
48
60
Time Post Diet Initation (Weeks)
B
Fasting Blood Glucose
Blood Glucose [mg/dL]
250
200
150
100
50
0
12
24
40
48
Time Post Diet Initation (Weeks)
C
Glucose Tolerance Test
LFD
HFD
HFD-LFD
Blood Glucose [mg/dL]
600
500
400
300
200
100
0
15
30
60
120
Time Post-Glucose Bolus
D
Body Fat Percentage
% Body Fat
60
40
20
0
12
48
Time Post Diet Initation (Weeks)

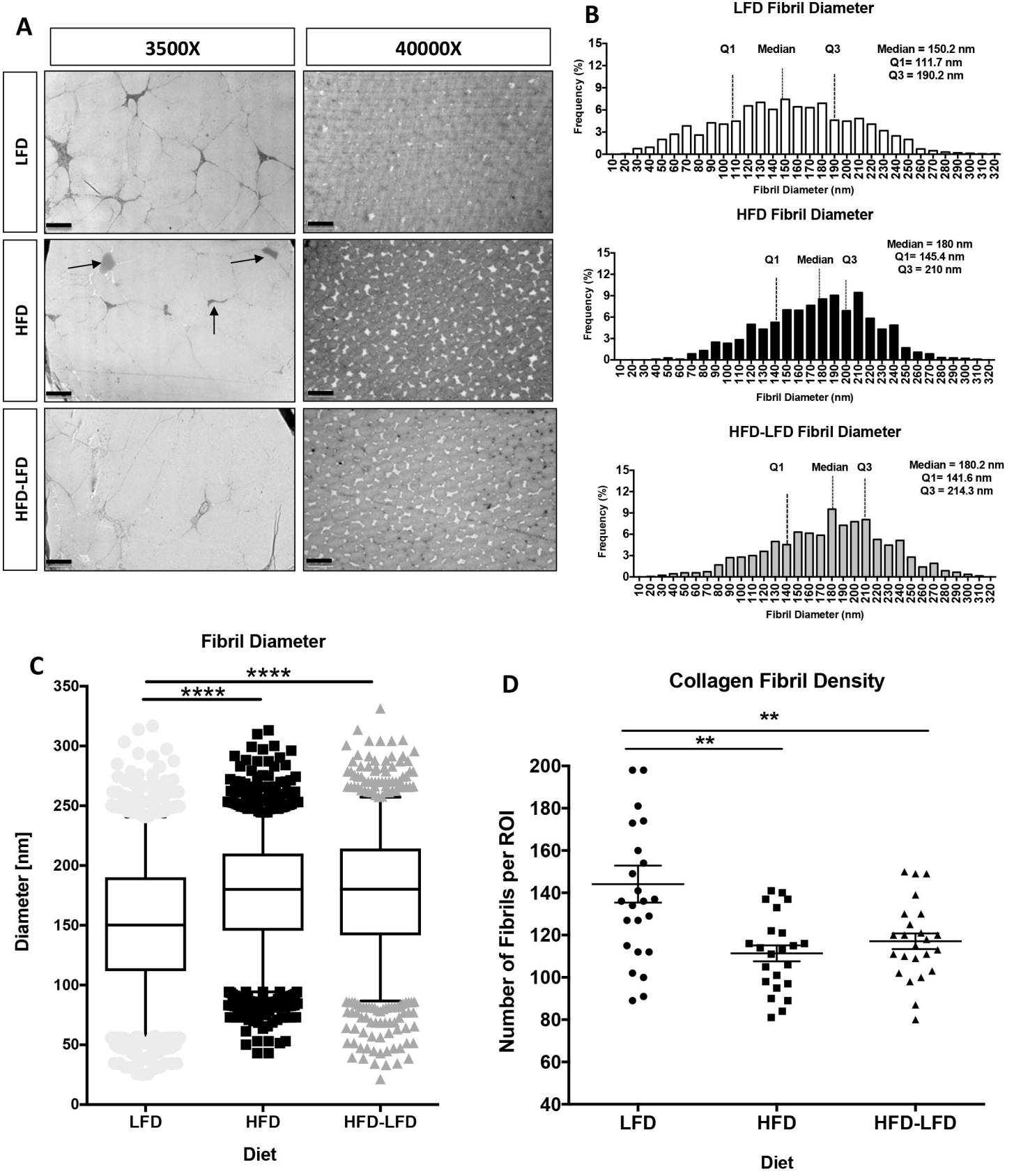

Stiffness
Stiffness [N/mm]
60
40
20
0
*
*
12
24
40
48
60
Time Post Diet Initation (Weeks)

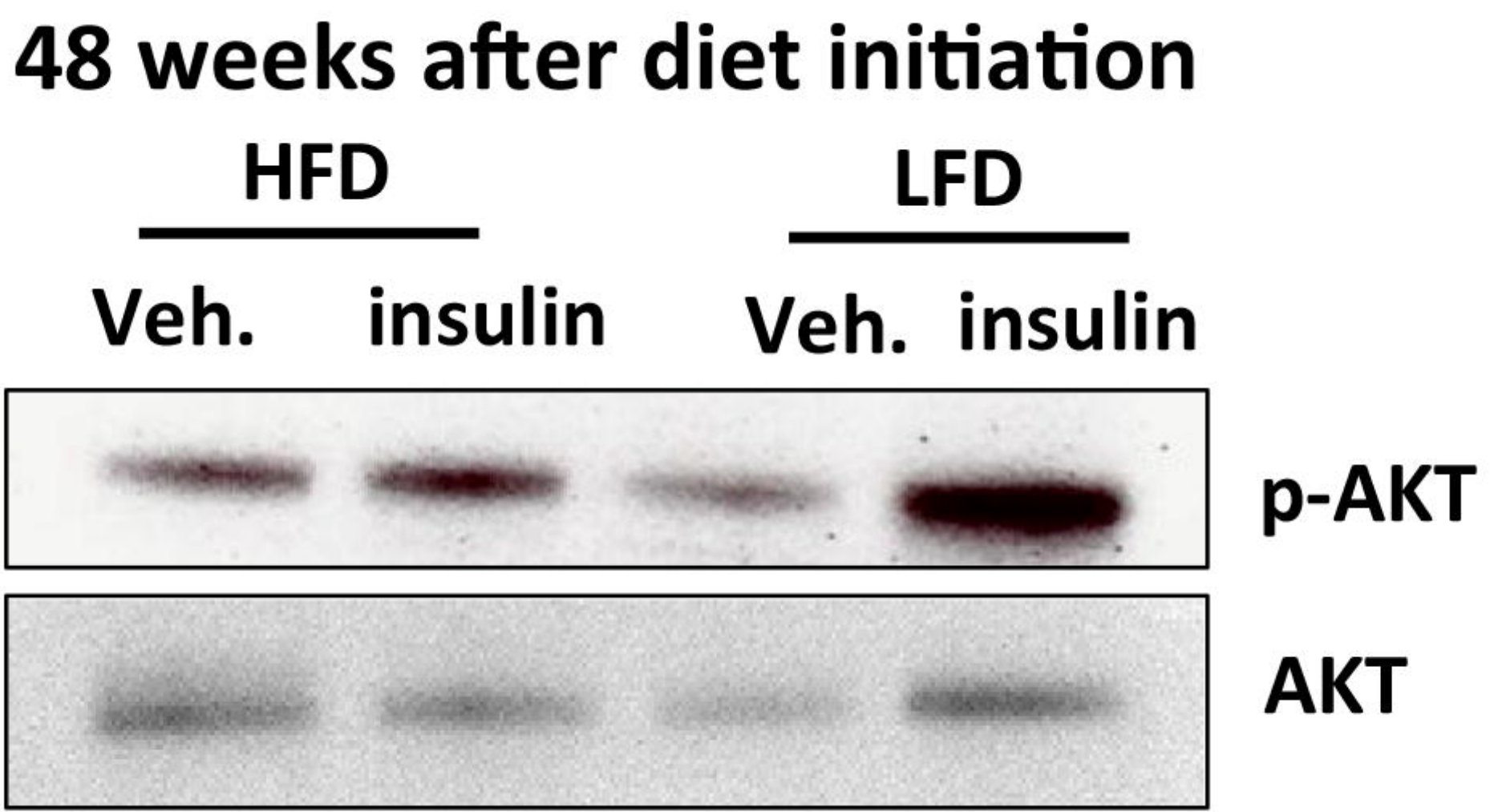

Max Load at Failure
Max Load at Failure [N]
14
12
10
8
6
4
2
0
*
*
12
24
40
48
60
Time Post Diet Initation (Weeks)


A
MTP Flexion Angle
LFD
HFD
HFD-LFD
MTP Flexion Angle [Degrees]
70
60
50
40
30
20
10
0
12
24
40
48
60
Time Post Diet Initation (Weeks)
B
Gliding Resistance
Gliding Resistance [A.U.]
50
40
30
20
10
0
12
24
40
48
60
Time Post Diet Initation (Weeks)


A
Non-Diabetic Patient
T2DM Patient 1
T2DM Patient 2
ABHOG
B
LFD
HFD
HFD-LFD
ABHOG
48 weeks


A
LFD
HFD
HFD-LFD
24 Weeks
B
Collagen Fibril Dispersion
Dispersion [Degrees]
20
15
10
5
0
*
LFD
HFD
HFD-LFD
Diet
C
LFD
HFD
HFD-LFD
40 Weeks
D
Collagen Fibril Dispersion
Dispersion [Degrees]
15
10
5
0
*
*
LFD
HFD
HFD-LFD
Diet


A
3500X
40000X
LFD
HFD
HFD-LFD
B
LFD Fibril Diameter
Q1
Median
Q3
Median = 150.2 nm
Q1= 111.7 nm
Q3 = 190.2 nm
Frequency (%)
Fibril Diameter (nm)
HFD Fibril Diameter
Median = 180 nm
Q1= 145.4 nm
Q3 = 210 nm
HFD-LFD Fibril Diameter
Median = 180.2 nm
Q1= 141.6 nm
Q3 = 214.3 nm
C
Fibril Diameter
****
****
Diameter [nm]
LFD
HFD
HFD-LFD
Diet
D
Collagen Fibril Density
**
**
Number of Fibrils per ROI
LFD
HFD
HFD-LFD
Diet


48 weeks after diet initiation
HFD
LFD
Veh.
insulin
Veh.
insulin
p-AKT
AKT