The Effect of Type II Diabetes on Native Mechanical and Biologic Shoulder Joint Properties in a Rat Model

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Introduction

The incidence of type II diabetes mellitus has substantially increased over the past decade and is linked with many systemic maladies including cardiovascular disease.[1] Research has demonstrated that type II diabetes can result in increased stiffness and increased expression of inflammatory markers of the vascular walls (i.e., IL1-β).[2] Recently, type II diabetes has been linked to rotator cuff disease and adhesive capsulitis,[3] conditions with increased stiffness and inflammation as well. Unfortunately, limited research exists examining how type II diabetes affects the native shoulder (tendon and capsule) properties. A recent type I diabetic rat model study determined that the native mechanical properties of the patellar tendon were decreased after just 19 days.[4] Although this study highlighted the mechanical changes that could occur in the presence of uncontrolled type I diabetes, it was not designed to address the altered tissue properties and biologic mechanisms in the shoulder joint, following the more common type II diabetes. Therefore, the objectives of this study were to compare shoulder joint mechanics, tendon properties (mechanics and protein content), and capsule protein content of healthy control and type II diabetic rats 8 weeks following induction of hyperglycemia with a submaximal dose of streptozotocin (STZ). We hypothesized that there will be an increase in passive shoulder stiffness and a decrease in shoulder range of motion in the diabetic group. In addition, there will be an increase in tendon mechanics along with an increase in protein content (tendon and capsule) for advanced glycated end-products (AGE), IL-1β, and TNF-α

Methods

Eighteen adult male Sprague-Dawley rats were injected with STZ (30mg/kg x 3 doses) to induce diabetes or citrate buffer (control). Type II diabetes was defined as a fasting blood glucose level of ≥ 200mg/dL and was determined with a glucometer. Fasting serum insulin levels were measured by ELISA, and animals were excluded if the values were less than 70% from baseline.

Passive Joint Mechanics

Passive shoulder joint range of motion and stiffness were measured at 8 weeks following induction of diabetes using a custom instrument and methodology.[5] Briefly, under anesthesia, the forearm was placed through a fixture and secured into a rotating clamp at 90° of elbow flexion and 90° of glenohumeral forward flexion. The scapula was manually stabilized in order to isolate glenohumeral motion. The arm was then rotated through the full range of internal and external rotation three times. The range of external and internal motion was determined using data from all three cycles. A bilinear fit utilizing least-squares optimization was applied to calculate joint stiffness in the linear region for both internal and external rotation.

Tendon Mechanical Testing

After sacrifice and dissection, stain lines for local optical strain measurement were placed on the supraspinatus tendon. Cross sectional area was measured using a custom laser device. To determine biomechanical properties, tensile testing was performed as follows: preconditioning, stress relaxation, and ramp to failure.[6]

Histology

Whole shoulders were left completely intact and were processed, sectioned in the sagittal direction (7μm) and stained with hematoxylin-eosin (H&E). Cell density (cells per mm2) and cell shape (aspect ratio; 0-1 with 1 being a circle) were quantified using a bioquantification software system (Bioquant Osteo II). Immunohistochemistry was performed on the whole shoulder sections following established protocols.7 Briefly, following deparaffinization and rehydration, sections were blocked for endogenous peroxidase, washed, and then placed in a protein blocking solution. After draining, slides were incubated with the primary antibody overnight in a humidity chamber. The appropriate secondary antibody was then added followed by DAB, and rinsed. Samples were examined for IL1-β, TNF-α, and AGE. Staining was quantified using a bioquantification software system.

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Statistics

Joint and tendon mechanics and histology (tendon and capsule) were assessed using a one-tailed t-test. Significance was set at $p<0.05$.

Results

The diabetic group had a significantly higher fasting blood glucose level compared to controls; the fasting serum insulin levels were not significantly different, which confirms our animal model (Figure 1). Passive joint mechanics (Figure 2) demonstrated significantly less external rotation in the diabetic group compared to controls, with no other group differences. Tendon mechanics (stiffness and modulus) were not significantly different between groups at both the insertion site and mid-substance (data not shown). For histology, cell shape was not significantly different between groups at the insertion site and mid-substance of the tendon or the superior capsule. Cell density also was not significantly different between groups at the insertion site or superior capsule; however the diabetic group had a greater cell density at the mid-substance of the tendon (data not shown). Immunohistochemistry staining of the tendon and capsule

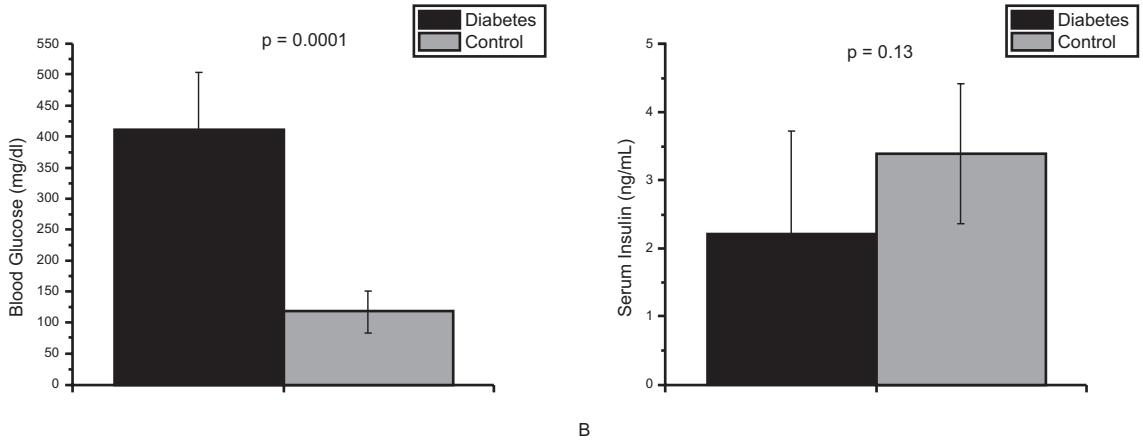

Figure 1. (A) The diabetes group had an increased fasting blood glucose level compared to the control group. (B) There was no difference in fasting serum insulin levels. Data are reported as Mean±SD.

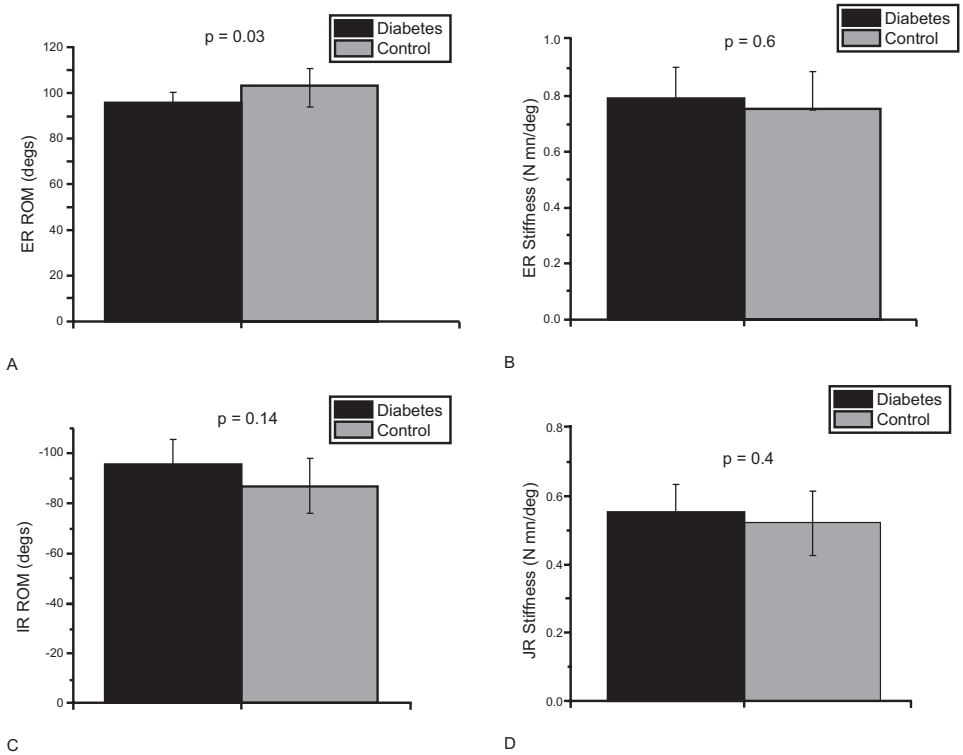

Figure 2. (A) The diabetes group had decreased external rotation (ER) range of motion (ROM) compared to control. (B) There were no differences for IR stiffness. (C) There were no group differences for internal rotation (IR) ROM. (D) There were no group differences for IR stiffness. Data are reported as Mean±SD.

Table 1. Immunohistochemistry data.

Tissue	Group	Region	IL1-β Staining Density (%)	p-value	AGE Staining Density (%)	p-value	TNF-α Staining Density (%)	p-value
Supraspinatus tendon	Diabetes	Insertion	1.78±1.29	0.03*	0.57±0.41	0.02*	2.7±1.44	0.5
	Control		0.51±0.40		0.06±0.05		2.74±1.88	
	Diabetes	Mid-substance	3.34±1.54	0.005*	2.37±0.95	0.01*	1.81±1.05	0.3
	Control		0.61± 0.34		0.62±0.51		1.31±1.1	
Superior capsule	Diabetes	Mid-substance	4.41±1.6	0.4	1.18±0.83	0.4	6.69±3.65	0.02*
	Control		4.17±1.89		1.01±0.65		2.51±1.0	

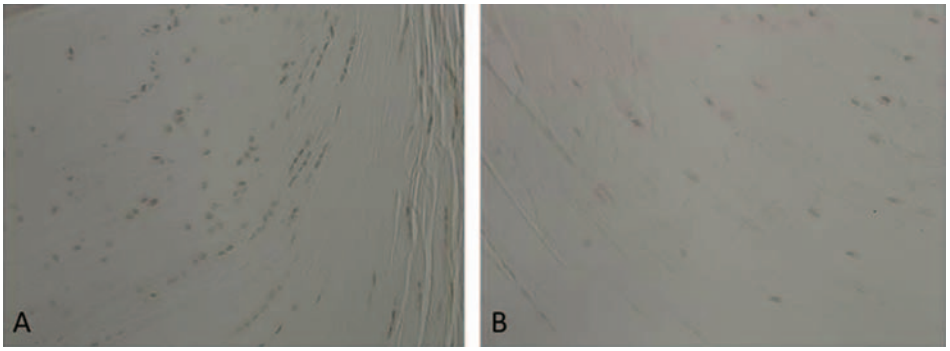

Figure 3. Representative images of the immunohistochemistry stain for IL1-β. (A) Diabetes group demonstrating increased staining at the insertion site of the supraspinatus tendon. (B) Control group demonstrating decreased staining at the insertion site of the supraspinatus tendon.

(Table 1) demonstrated significantly increased, IL1-β and AGE staining localized to the insertion and mid-substance of the tendon but not the capsule (Figure 3). In addition, TNF-α staining was significantly increased in the superior capsule but not the supraspinatus tendon.

Discussion

While the relation of type II diabetes to cardiovascular disease has been well studied, there is limited information with respect to rotator cuff disease and adhesive capsulitis. Results demonstrate that 8 weeks of type II diabetes, defined as hyperglycemia and partial insulin deficiency, leads to a decrease in external rotation range of motion but no other mechanical changes in the joint or tendon. However, there was a large biologic response with elevated levels of inflammatory markers and AGE. The decrease in external rotation and the increase in TNF-α in the superior region of the capsule are similar to the findings in patients with adhesive capsulitis[8] and should be further investigated. The lack of tendon mechanical property differences was surprising, although unlike some animal models of diabetes, this animal model had minimal reductions in insulin, which could allow maintenance of the mechanical properties. The increased biologic response (cell density and immunohistochemistry staining) agrees with findings from cardiovascular research,[3] which identify a chronic underlying inflammatory response to type II diabetes. This underlying elevation of inflammatory markers did not affect the mechanical properties of the supraspinatus tendon at 8 weeks. However, the elevated presence of inflammation in native tissue may have detrimental effects to tissue healing. Future studies will examine additional inflammatory markers and begin to investigate the effect on tendon healing.

Significance

This study demonstrates that type II diabetes does not diminish shoulder mechanical properties but does induce a chronic inflammatory response.

Acknowledgments

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