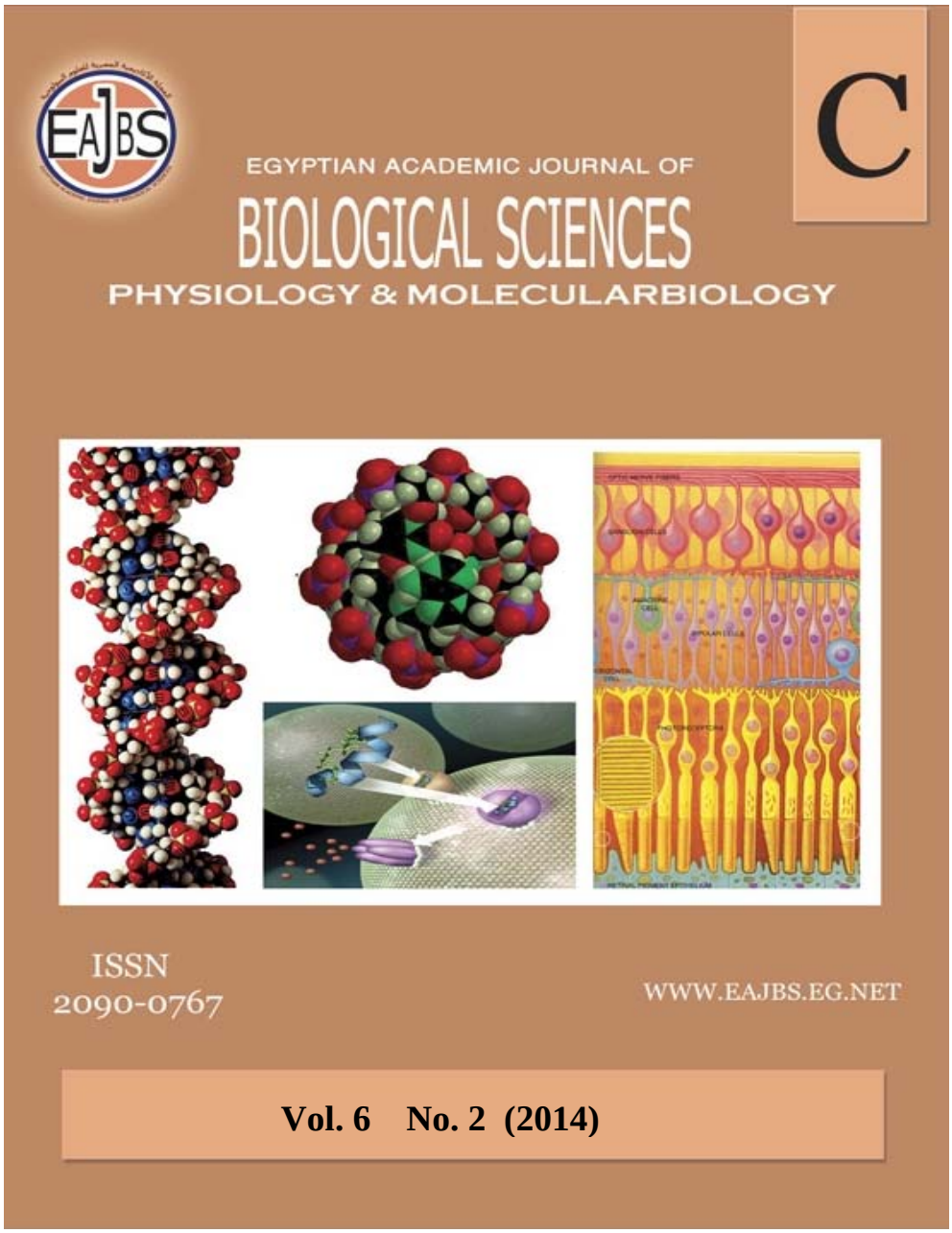

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Exercise ameliorates diabetes-induced osteoarthritis in rats

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ABSTRACT

Introduction: Osteoarthritis secondary to diabetes is one of the common public health problems arising from diabetic complications in humans. Exercise was reported to alleviate cardiovascular diabetic complications. We sought to determine whether exercise can also ameliorate diabetes-induced osteoarthritis condition. **Material and methods:** Using basic histological staining, I studied the morphological changes in the articular cartilage of diabetic rats with and without swim exercise and compare it with the control, untreated rats. In addition, interleukin-6 (IL-6), a biomarker that is known to be elevated in osteoarthritis was assayed in the blood of the three rat groups by enzyme-linked immunosorbent assay (ELISA).

Results: Compared to the control group, induction of type I diabetes mellitus (T1DM) in Wistar rats caused a profound damage to the knee joint cartilage as demonstrated by disrupted lacunae, condensing of the matrix and enlargement of the chondrocytes and its nucleus with the disappearance of both, the euchromatin staining and the discrete cytoplasmic vacuoles. Furthermore, there was a four-fold increase in IL-6 in the T1DM group that was significantly ($p<0.01$) reduced in the diabetic group with exercise. More interestingly, exercise resolved diabetes-induced cartilage damage by restoring the chondrocytes spherical intact nucleus and vaculated cytoplasms with regular lacunae.

Conclusion: In this report, we have demonstrated a model of diabetes-induced osteoarthritis in rats where swim exercise was able to ameliorate both articular cartilage damage and IL-6 inflammatory biomarker in T1DM rats.

INTRODUCTION

Diabetes is a global health care problem with an estimated 347 million people around the world has diabetes (Danaei *et al*. Lancet 2011) that clams the life of about 3.4 million every year (Global health risk, World Health Organization 2009). Diabetes has been found to be associated with increased risk of hip fracture as well as fracture of other sites (Schwartz 2003). People with type 1 (juvenile) diabetes (T1DM) tend to have mild osteopenia as adults, with bone marrow density (BMD) values around 10% lower than normal (Bouillon 1991).

This modestly low BMD caused an increase in hip fracture risk of about 2-fold (Hofbauer *et al.* 2007), yet prospective studies indicate that postmenopausal women with T1DM have about 10 times greater risk of hip fracture compared with age-matched non- diabetics (Forsen *et al.* 1999). The clinical evidence indicates that fracture risk in diabetic patients is disproportionately high relative to BMD, which suggests reduced material strength of diabetic bone (Schwartz 2003).

Osteoarthritis (OA) is a degenerative joint disease that involves degradation of joints including articular cartilage leading to pain, swelling and reduce joints movement (Manen *et al.* 2012; Pottie *et al.* 2006). It affects millions of people worldwide and regarded as one of the most prevalent condition leading to disability particularly in elderly population as a consequence of the knee OA and/or hip OA (Grazio and Balen 2009). Knee OA is more important not only for its high prevalence rate compared with other types of OA but also for its presentation at earlier age groups particularly in younger age groups of obese women (Bliddal and Christensen 2009; Hayami 2008).

A recent longitudinal cohort study predicted the development of severe OA in diabetic patients with type 2 diabetes that were followed over twenty years (Schett *et al.* 2013). Animal models have supported claims of diminished whole bone strength in diabetes, although studies have not adequately addressed material properties (Schwartz 2003). Streptozotocin-induced T1DM in young rats leads to low bone formation (Shires *et al.* 1981), with diminished bone mass after 8–12 wk (Suzuki *et al.* 2003).

Exercise is recommended to treat or manage both diabetes and OA (Colberg *et al.* 2010; Ivy 1997; Messier *et al.* 2000). However, little is known about the effect of exercise on diabetes-induced OA in animal models. Therefore, the aim of the present work is to test the hypothesis that swim exercise may reduce the deleterious effects of diabetes on knee joint using T1DM-induced OA animal model.

MATERIALS AND METHODS

Animals

The experiments were performed on healthy 30 male Wistar rats of 10 weeks old and weighting 150-200 g. The rats were fed with standard laboratory diets, given water ad libitum and maintained under laboratory conditions of temperature (22±3°C), with 12 h light and 12 h dark cycle. All experimental procedures involving the handling and treatment of animals were approved by the Ethical Committee of King Khalid University Medical School (Abha, KSA) and were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Experimental Design

After one week adaptations, the animal were classified and randomly allocated into 3 groups (n= 10) as follows: group1: Non-treated (C): rats were injected intraperitoneally (i.p.) once with citrate buffer only (0.1 M, pH 4.5), group 2: Diabetic (T1DM) group: DM was induced in rats by a single i.p. injection of streptozotocin in a dose of 65mg/kg (DM type 1), (Haidara *et al.* 2004) , group 3: Diabetic + Exercise group : Diabetic rats were subjected to exercise by letting them swim for 30 min, 3 times per week (Rocha *et al.* 2013).

At the end of the 8th weeks of the experiment, 5 mL retroorbital blood samples was obtained under anesthesia using 40 mg Kg-1 sodium thiopentone, i.p., after an overnight fast 3 mL was collected into 3.8% Na citrate anticoagulant for plasma separation. While 2 mL was collected into plain tubes, then allowed to clot for 20 min then centrifuged at 14000 rpm for 10 min for serum separation. Then sera were stored at-80°C, for subsequent measurements of biochemical parameters. After withdrawal of the blood samples, the knee joints were

opened, dissected and fixed in formalin and kept for histological examination.

Biochemical Parmeters

Rat ELISA kit for IL-6 (Ray Biotech Inc., Norcross, GA, USA) was used as recommended by the manufacturer.

Histological Examination:

At the end of the experiment, 8 weeks after induction of diabetes, blood was withdrawn for biochemical examination, then the knee joints were opened, dissected and fixed in formalin and kept for histological examination. Samples then examined using Masson's Trichrome counter-stained with Haematoxylin and Euosin (H&E). (X1000).

Statistical Analysis

Values are measured as mean ± SD. Comparison of data was performed by using ANOVA test (analysis of variance test) using graph pad prism analysis software, version 5. Probability (P) values of <0.05 were considered to be significant.

RESULTS

Development of T1DM-induced OA animal model

As previously reported by our group (Haidara *et al.* 2004), Wistar rats were injected with a single dose of streptozotocin (65 mg/kg) and T1DM was induced with a blood sugar level reached 220 mg/dl compared to 80 mg/dl in the control group injected with citrate buffer. Tissue preparations for histological analysis from the articular cartilage of the knee joint of the sacrificed control rats (Figure 1A) and T1DM rats (Figure 1B) revealed OA development in the T1DM group. T1DM H&E stained tissues showed disrupted lacunae, condensing of the matrix and enlargement of the chondrocytes and its nucleus with the disappearance of both, the euchromatin staining and the discrete cytoplasmic vacuoles (Figure 1B). Whereas, H&E stained control tissues revealed a normal lacunae and chondrocytes (Figure 1A).

Exercise inhibits T1DM-induced OA

Exposing of T1DM group to swim exercise (Figure 1C) reversed OA histological picture of the articular cartilage (Figure 1C) to become similar to the control group (Figure 1A), by restoring the chondrocytes spherical intact nucleus and vaculated cytoplasms with regular lacunae.

Exercise inhibits T1DM-induced IL-6 release

To test the hypothesis that exercise suppresses the release of the proinflammatory cytokine, IL-6 that is known to be involved in the pathology of diabetes (Suthem *et al.* 1990; Senn *et al.* 2002) and OA (Doss *et al.* 2007; Stannus *et al.* 2010), we measured, by ELISA, the blood level of IL-6, 8 weeks after T1DM induction. T1DM caused a four-fold increase in IL-6 that was significantly inhibited by swim exercise (Figure 2).

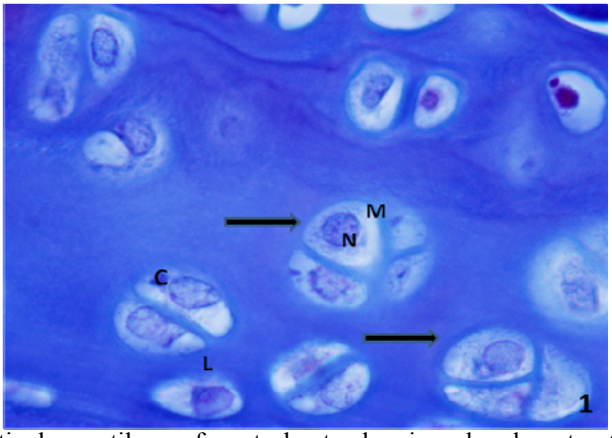

Fig. 1A: A photomicrograph of articular cartilage of control rats showing chondrocytes (C) lying centrally within its lacunae (L) that are arranged in groups (arrows). The cells show finely granular cytoplasm that contains discrete vacuoles and are surrounded by cartilage matrix (M) which is faint in many areas around the chondrocytes are clearly seen. Active euchromatic nuclei (N) are seen. Chondrocytes have little intercellular substance that is associated with collagenous fibers (arrows). (Masson's Trichrome counter-stained with Haematoxylin X1000).

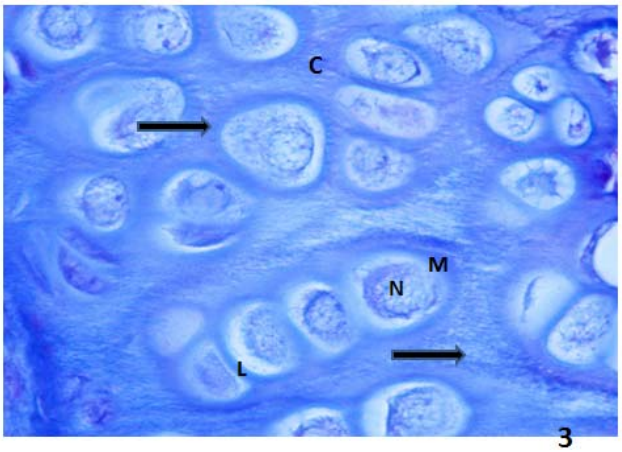

Fig. 1B: A photomicrograph of articular cartilage of type 1 diabetic rats showing enlarged chondrocytes (C) with enlarged nuclei (N) in most of them with disrupted lacunae (L), shrunken cytoplasm that lose their vacuoles and surrounded by a condensed faint matrix (M). Atrophic and shrunken chondrocytes in the right corner of the figure. Note condensed collagen fibers (arrows) in between and around most of the chondrocytes. (Masson's Trichrome counter-stained with Haematoxylin X1000).

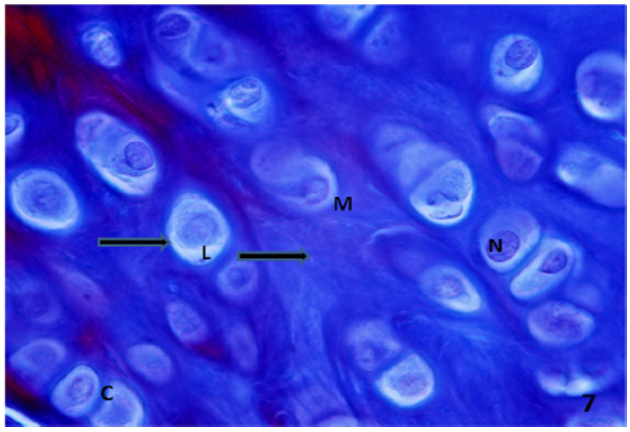

Fig. 1C: A photomicrograph of articular cartilage of diabetic rats plus swim exercise showing chondrocytes (C) with spherical intact nuclei (N) and a vacuolated cytoplasm with lacuna (L) and separated by little intercellular substance and are associated with lesser concentrations of collagenous fibers (arrows) that are destructed around some of the chondrocyte matrix (M). (Masson's Trichrome counter-stained with Haematoxylin X1000).

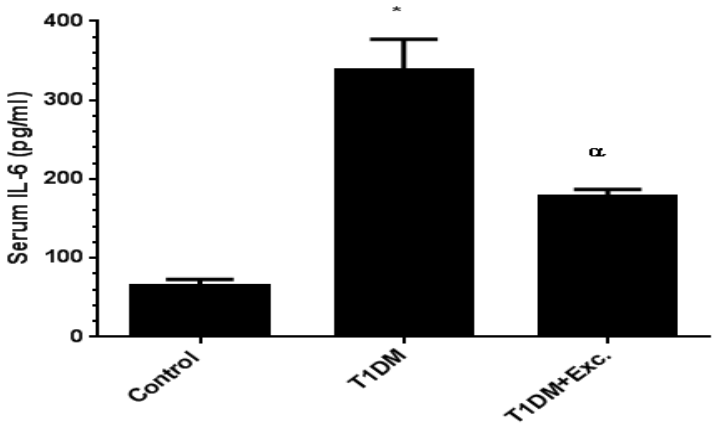

Fig. 2: Serum IL 6 in control, diabetic and contro plus swimming.

- * Significant in comparison to control group.
- α Significant in comparison to diabetic group.

DISCUSSION

The main outcomes of our study were that swim exercise ameliorates the OA induced by T1DM and suppressed one of the biomarker of T1DM and OA, IL-6. To the best of our knowledge, this is the first report that swim exercise restored the normal integrity of T1DM articular cartilage in rats. Histological staining of the articular cartilage of the knee joint, which is a known target by OA (Pottie *et al.* 2006; Musumeci *et al.* 2014), confirmed the development of OA secondary to T1DM (Figure 1B). The previously reported diabetic rat model for metabolism-induced arthritis (Niethard FU 1986) studied different parameters than ours such as crystal deposit below the cartilage and biochemical analysis of proteoglycan and collagen metabolism that confirmed the animal model of arthritis. Our data with the significant inhibition of IL-6 by swim exercise in T1DM rats together with the exercise restored the diabetic articular knee joint cartilage histological picture similar to controls point to IL-6 as a very important proinflammatory biomarker to monitor the healing progress in OA secondary to diabetes. These data are in agreement with the previously published work on the role of IL-6 in the pathogenesis of inflammation-induced bone loss (Korczowska and Lacki 2005) and the report that proposed IL-6 as a significant predictor of knee OA (Livshits *et al.* 2009).

CONCLUSIONS

In conclusion, using T1DM-induced OA animal model, our data points to the importance of exercise as a recommended method to alleviate OA induced by T1DM. Also, our data promotes IL-6 as an important candidate to monitor the disease.

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