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Swim Exercise Augments the Protection of the Knee Joint Against Osteoarthritis Development in Diabetic Rats Treated with Insulin

El Ejercicio de Natación Aumenta la Protección de la Articulación de la Rodilla Contra el Desarrollo de Osteoartritis en Ratas Diabéticas Tratadas con Insulina

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SUMMARY: We recently reported that insulin can partially protect the knee joint against osteoarthritis (OA) development in a rat model of type 1 diabetes mellitus (T1DM). However, the combined protective effect of insulin and swim exercise against OA development secondary to diabetes has not been investigated before. Therefore, we hypothesized that swim exercise can augment the protection of the knee joint in diabetic rats treated with insulin. T1DM was induced in Sprague Dawley rats and treated with insulin and/or swim exercise. Tissues harvested from the articular cartilage of the knee joint were examined by light microscopy, and blood samples were assayed for biomarkers of oxidative stress and inflammation. Treatment of diabetic rats with insulin and swim exercise substantially protected the articular cartilage and significantly ($p<0.0001$) inhibited the inflammatory biomarkers, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and the oxidative stress biomarker, malondialdehyde (MDA) measured as thiobarbituric acid reactive substances (TBARS) comparable to control. Whereas, a lesser effective protection was observed by insulin or swim exercise alone. Thus, we demonstrate a substantial protection against OA development in rats treated with combined insulin and swim exercise possibly due to a complete inhibition of biomarkers of inflammation and oxidative stress.

KEY WORDS: Diabetes; Knee joint; Osteoarthritis; Swim exercise; Rat model.

INTRODUCTION

Diabetes is one of the leading global health care problems that claims the life of about 3.4 million every year (Danaei *et al*., 2011). Type 1 diabetes mellitus (T1DM) is believed to be caused by autoimmune antibodies targeting the β cells producing insulin in the pancreas that leads to the loss of insulin production and hence the appearance of diabetes symptoms in children, commonly between the ages of 7-19 years (Pugliese, 2004; Atkinson *et al*., 2014).

Osteoarthritis (OA) is a degenerative joint disease that involves degradation and destruction of the articular cartilage structure of the joint leading to pain, swelling and reduced joint movement; and there is increasing evidence to link metabolic disturbances such as diabetes to osteoarthritis (Pottie *et al*., 2006; Van Manen *et al*., 2012). OA of the knee and/or hip is regarded as one of the most prevalent conditions leading to disability particularly in the elderly population (Grazio & 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 (Hayami, 2008; Bliddal & Christensen, 2009).

Insulin and the insulin mimicking agent, vanadium were recently reported by our group to protect the knee joint from the deleterious effects of T1DM (El Karib *et al*., 2016). Diabetic limited joint mobility syndrome is seen in 8-50 % of T1DM patients. The lack of the bone anabolic actions of insulin and other pancreatic hormones could be the reason why T1DM affects the skeleton more severely than type 2 diabetes mellitus (T2DM) (Janghorbani *et al*., 2007; Hamann *et al*., 2012). In a study of patients with T2DM followed over a course of twenty years it was concluded that longstanding diabetes per se is detrimental for knee and hip joints, leading to progressive destruction and joint failure (Schett *et al*., 2013). Exercise and weight loss on the other hand, were reported to suppress TNF-a and IL-6 as it cause reduction in visceral fat mass with a subsequent decrease in

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adipokines release (Chidambaram & Carani Venkatraman, 2010; Sakr *et al*., 2014). In addition, swim exercise ameliorated hepatocyte ultrastructural damage and suppressed biomarkers of liver injury (Cakır *et al*., 2010) .

Biomarkers of inflammation such as IL-6 and TNF-α are significant predictors of knee OA (Livshits *et al*., 2009; Kou & Wu, 2014), and OA cartilages from DM patients showed increased responsiveness to IL-1ß-induced inflammation via oxidative stress and polyol pathway thus participate in the increased inflammation in OA.

The combined effect of exercise and insulin on diabetes-induced OA in animal models has not been addressed before. Therefore, we aim to study the potential protective effect of insulin and exercise against OA of the knee joint secondary to T1DM, and to see if exercise adjuvant treatment with insulin can augment the protection.

MATERIAL AND METHOD

Animals. The experiments were performed on 30 male Sprague Dawley rats of 10 weeks old and weighting 150 - 250 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 Research Ethical Committee of King Khalid University (Abha, KSA) and conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Animal Protocol. After one week adaptations, animals were classified and randomly allocated into 5 groups (n= 6) as follows: Control group (Control): non- treated rats injected intraperitoneally (i.p.) once with citrate buffer (0.1 M, pH 4.5); Diabetic type 1 group (T1DM):DM was induced in rats by a single i.p. injection of streptozotocin (STZ) in a dose of 65 mg/kg (Haidara *et al*., 2004). Rats showed glucose level above 300 mg/dl were considered to be diabetic and included in the experiments (Haidara *et al*., 2004; El Karib, 2014); T1DM and insulin group (T1DM+ Ins.): rats were made type 1 diabetic as in T1DM group and received mixtard insulin subcutaneously in a dose of 0.75 IU/100 gm weight in 0.75 ml volume once daily (Haidara *et al*., 2015), after 48 h of diabetic induction; T1DM and Exercise group (T1DM +Exc.): T1DM rats start swimming training program after 48 h of induction of T1DM; T1DM and Exercise and insulin group (T1DM+ Exc.+Ins.): T1DM rats received insulin with the same doses as in previous groups in addition to swimming training program.

At the end of the 8th weeks of the experiment, blood was collected into plain tubes, then allowed to clot for 20 min then centrifuged at 14000 rpm for 10 min for serum separation. 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 formal saline for histological examinations.

Measurement of IL-6 and TNF-α cytokines. Rat Interleukin-6 ELISA kit, IL-6 for serum, plasma and tissue culture supplements, (Ray Biotech Inc., Mfr. No. ELR-IL6-001) and Rat tumour necrosis factor alpha, (TNF-α ELISA kit BIOTANF INC, Cat. No. R6365) were used as recommended by the manufacturer.

Measurements of SOD and TBARS. Superoxide dismutase assay kit (SOD), rat, Cayman Chemical, Cat. No. 706002 and Thiobarbituric Acid Reactive Substances, TBARS Assay kit, Cayman Chemical Item Number 10009055 were used as recommended by the manufacturer.

Histopathological studies. Specimens from the articular cartilage of the knee joints were fixed in 10 % neutral buffered formalin and then prepared using standard procedures for Hematoxylin and Eosin staining.

Statistical Analysis. Data are presented as mean ± SD. Comparison of data was analysed using Graph pad Prism software, version 5. Comparison between the groups were performed by analysis of variance (one way ANOVA), followed by Turkeys's post-hoc test. Probability (P) values of <0.05 were considered to be significant.

RESULTS

Swim exercise potentiates insulin action on the suppression of inflammatory biomarkers induced by diabetes. To investigate whether swim exercise can potentiate the effect of the insulin hormone in lowering the levels of inflammatory biomarkers induced by diabetes, we measured the blood levels of TNF-α and IL-6 in all animal groups after 8 weeks. Diabetes caused a two-fold increase in TNF-α and five-fold increase in IL-6 compared to the control group. Altered cytokines were significantly inhibited by insulin, swim exercise, and insulin plus swim exercise (Fig. 1A and 1B). The inhibition by insulin plus swim exercise was incomparable to control. The relative degree of inhibition of both inflammatory biomarkers by these methods of treatment was: insulin plus swim exercise > insulin > swim exercise.

Swim exercise potentiates insulin action on the modulation of oxidative and anti-oxidative stress biomarkers levels affected by diabetes. To investigate whether swim exercise can potentiate the effect of the insulin hormone in lowering the level of the oxidative stress biomarker, TBARS induced by diabetes, and upregulation of the anti-oxidative stress enzyme, superoxide dismutase (SOD) inhibited by diabetes, we measured the blood levels of these biomarkers in all animal groups after 8 weeks. Diabetes caused a three-fold increase in TBARS and two-fold decrease in SOD compared to the control group that were significantly modulated by insulin, swim exercise, and insulin plus swim exercise (Figs. 2A and 2B). The effect of insulin plus swim exercise was incomparable to control. The relative degree of inhibition of TBARS and augmentation of SOD by these methods of treatment was: insulin plus swim exercise > insulin > swim exercise.

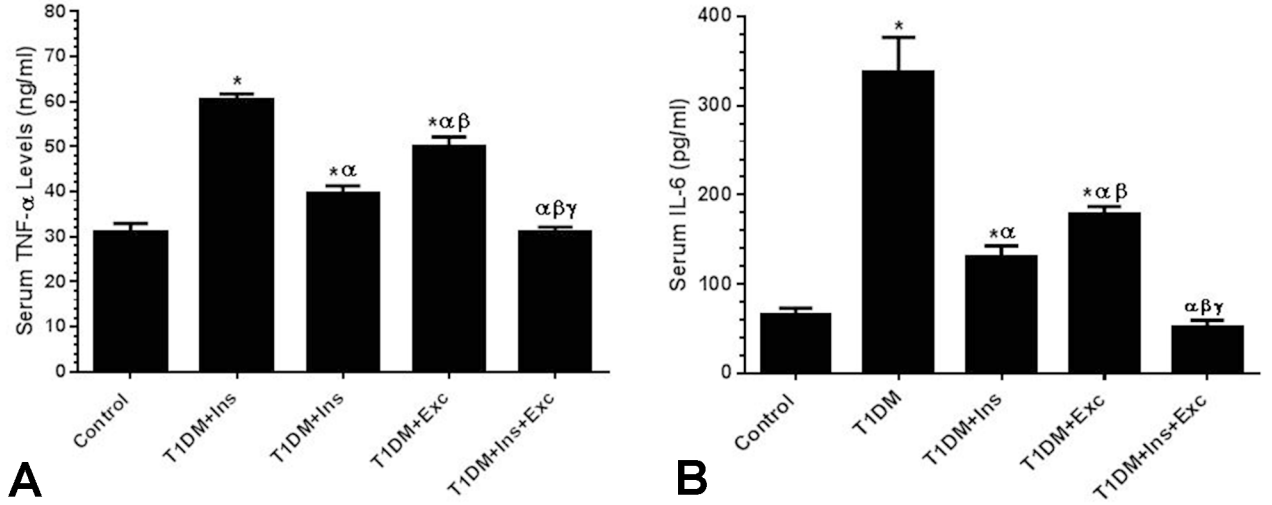

Fig. 1. Insulin and swim exercise inhibit TNF-a and IL-6 in T1DM treated rats. (A and B). TNF-a and IL-6 blood levels from the five groups of rats (n=6 each) were measured using ELISA after 8 weeks. Control, vehicle injected rats; T1DM, streptozotocin injected rats (type 1 diabetic group, or the model group); T1DM+Ins, diabetic group treated with insulin; T1DM+Exc, diabetic group "treated" with swim exercise; T1DM+Ins.+Exc, diabetic group treated with insulin and swim exercise. All results are the mean (±SD) of three experiments. Significance indicated: $p<0.05$. *: Significant in comparison to control group, a: Significant in comparison to diabetic group, b: Significant in comparison to T1DM+Ins group, and g: Significant in comparison to T1DM+Exc group.

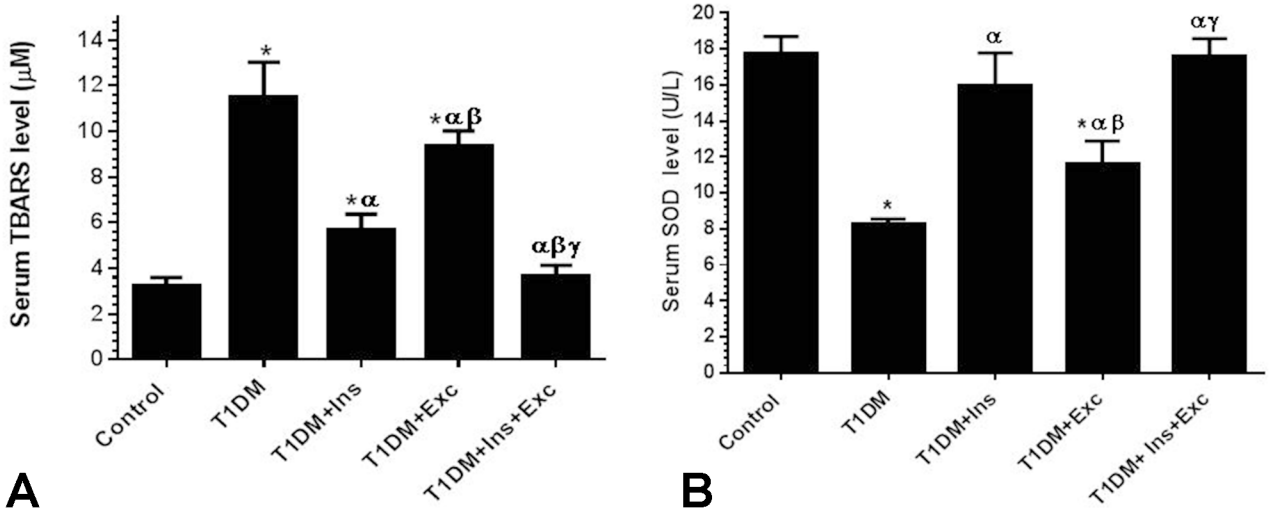

Fig. 2. Different effects of insulin and swim exercise on inhibition of TBARS and activation of SOD in T1DM treated rats. (A and B). TBARS and SOD levels in blood from the 5 rat groups were measured after 8 weeks treatment by TBARS assay and ELISA. Control, vehicle injected rats; T1DM, streptozotocin injected rats (type 1 diabetic group, or the model group); T1DM+Ins, diabetic group treated with insulin; T1DM+Exc, diabetic group "treated" with swim exercise; T1DM+Ins.+Exc, diabetic group treated with insulin and swim exercise. All results are the mean (±SD) of three experiments. Significance indicated: $p<0.05$. *: Significant in comparison to control group, a: Significant in comparison to diabetic group, b: Significant in comparison to T1DM+Ins group, and g: Significant in comparison to T1DM+Exc group.

Swim exercise potentiates insulin protection of the knee joint against diabetes-induced osteoarthritis. Tissue preparation for histological examination under light microscopy from the articular cartilage of the knee joint of the model group (Fig. 3C) revealed damaged chondrocytes, disrupted lacunae and condensed collagen fibre compared to a normal histology in the non-diabetic healthy control rats (Fig. 3A and 3B). In control group, chondrocytes are lying centrally within their lacunae (L) and arranged in groups (arrows). Normal chondrocytes contain active euchromatic nuclei (N) and fine granular

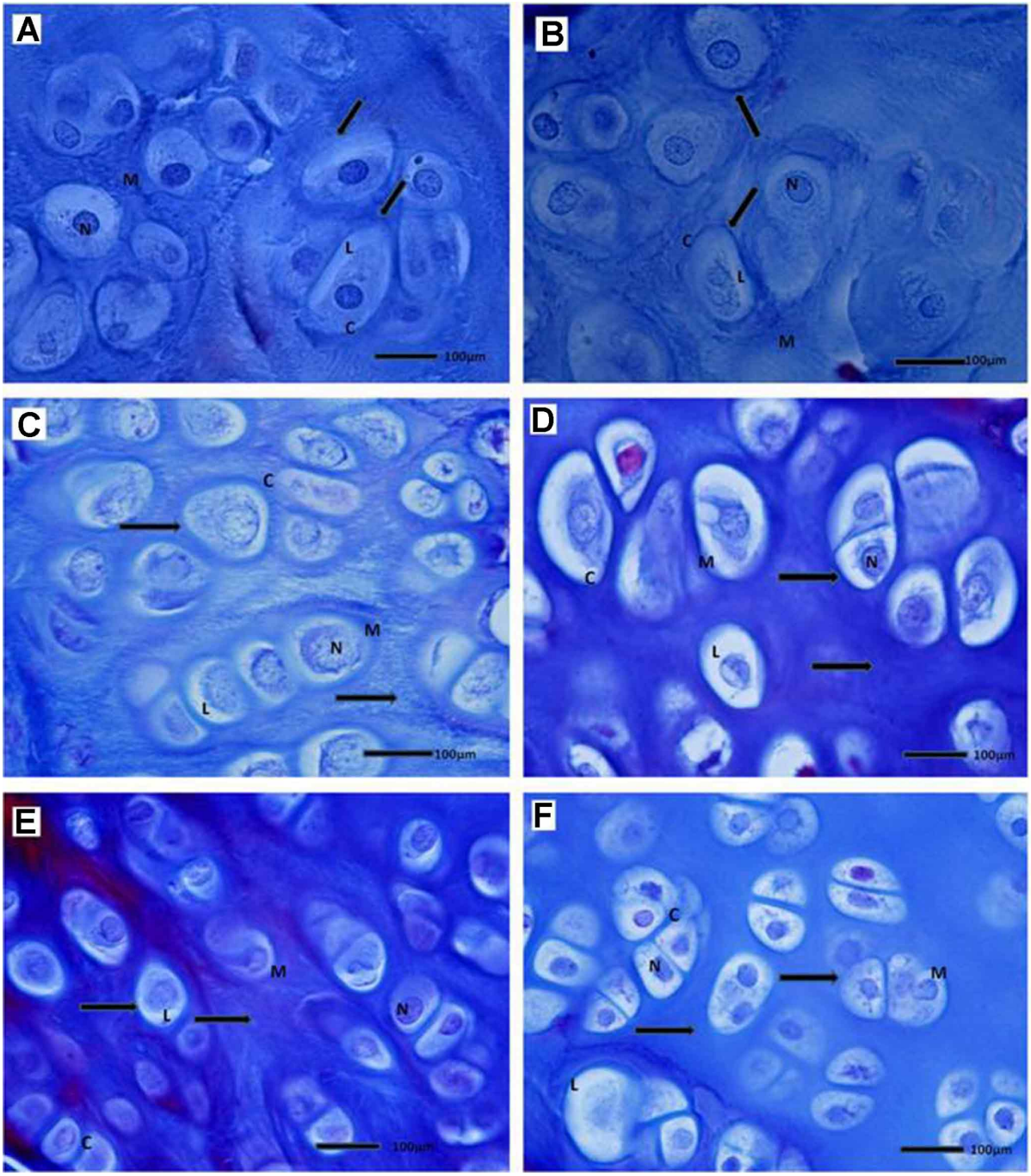

Fig. 3. Insulin and swim exercise protected the knee joint against OA in diabetic rats. Masson's Trichrome counter-stained with Haematoxylin images (x1000) of rats' articular cartilage from the knee joint 8 weeks post diabetic induction. A and B. Control group. C. Diabetic group. D. Diabetic group treated with insulin. E. Diabetic group treated with swim exercise. F. Diabetic group treated with insulin and swim exercise. Abbreviations: C, chondrocyte; L, leaving lacunae; N, nuclei; M, cartilage matrix. Note that arrows point to collagen fibers.

cytoplasm with discrete vacuoles, which are surrounded by cartilage matrix (M). Both insulin (Fig. 3D) and swim exercise (Fig. 3E) treatment comparably improved the histology of the articular cartilage, showing spherical intact nuclei and vaculated cytoplasm within normal lacunae. A substantial protection was obtained when insulin plus swim exercise was applied (Fig. 3F) that showed intact chondrocytes arranged in groups comparable to control.

DISCUSSION

The main objective of this study was to further extend our previous findings that showed giving either insulin or swim exercise training post diabetes induction partially protected the knee joint of rats from the deleterious effects of diabetes (El Karib *et al*., 2016; Al-Hashem *et al*., 2017) to determine whether combining insulin with a non-conventional treatment method, swim exercise can effectively treat OA secondary to diabetes. Therefore, we induced diabetes in rats using STZ and then exposed these animals to insulin injection and swim exercise training. The principal findings in our study were that insulin in combination with swim exercise completely blocked the rise in biomarkers of inflammation and oxidative stress beyond the control levels and substantially protected the integrity of the articular cartilage histology. These conclusions were supported by the data indicating that streptozotocin induced T1DM in rats caused a significant modulation in TNF-α, IL-6, TBARS, and SOD levelsconcomitant with a substantial destruction to the articular cartilage of the knee joint that were inhibited by a combination of insulin and swim exercise comparable to control untreated group (Figs. 1-3).

The articular cartilage of the knee joint is known to be targeted by diabetes (Niethard, 1986; Pottie *et al*.; El Karib, 2014; Musumeci *et al*., 2014) and prolonged hyperglycemia and accumulation of glucose derived advanced glycation end products (AGEs) were proposed to contribute to OA development and insulin is reported to have anabolic effects on bone (Yan & Li, 2013). In addition, insulin was found to increase proteoglycan synthesis in primary chondrocytes tissue culture and increased proteoglycan and matrix synthesis in articular cartilage explants obtained from healthy pigs and diabetic human and mice (Cai *et al*., 2002). On the other hand, exercise is recommended to treat or manage both diabetes and OA (Ivy, 1997; Messier *et al*., 2000). Therefore, our approach to combine insulin with swim exercise to reach effective treatment is justified. Indeed, our biochemical and histological data showed that when both insulin and swim exercise were given together, they produced the most effective results, which were similar to the non-diabetic controls.

The importance of the measured biomarkers here demonstrated again to their importance in monitoring both, the prognosis and the healing progress of the disease. Indeed, our findings are in agreement with the previously published work on the role of TNF-α and IL-6 in the pathogenesis of inflammation induced bone loss, osteoclastic bone resorption and cartilage destruction (Fuller *et al*., 2002; Korczowska & Lacki, 2005; Kapoor *et al*., 2011) and the reports that proposed IL-6 and TNF-α as significant predictors of knee OA (Livshits *et al*.; Kou & Wu).

In conclusion, our data demonstrates that in a rat animal model of T1DM and OA, using swim exercise training together with the only drug available to treat this type of diabetes, insulin protect against development of OA and inhibited biomarkers of inflammation and oxidative stress comparable to control non diabetic animals.

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RESUMEN: Recientemente informamos que la insulina puede proteger parcialmente la articulación de la rodilla contra el desarrollo de osteoartritis (OA) en un modelo de diabetes tipo 1 (DM1) en ratas. Sin embargo, el efecto protector combinado de la insulina y el ejercicio de natación contra el desarrollo de OA secundario a la diabetes no se ha investigado. Por lo tanto, planteamos la hipótesis de que el ejercicio de natación puede aumentar la protección de la articulación de la rodilla en ratas diabéticas tratadas con insulina. La DM1 se indujo en ratas Sprague Dawley y se trataron con insulina y/o ejercicio de natación. Los tejidos recogidos del cartílago articular de la articulación de la rodilla se examinaron mediante microscopía óptica, y las muestras de sangre se analizaron en busca de biomarcadores de estrés oxidativo e inflamación. El tratamiento de ratas diabéticas con insulina y ejercicio de natación protegió sustancialmente el cartílago articular y significativamente ($p < 0,0001$) inhibió los biomarcadores inflamatorios, factor de necrosis tumoral alfa (TNF-α) e interleucina-6 (IL-6) y el biomarcador de estrés oxidativo, el malondialdehído (MDA) fue medido como sustancia reactivas al ácido tiobarbitúrico (TBARS), comparable al control. Se observó una menor protección efectiva mediante la insulina o el ejercicio

de natación solo. Por lo tanto, demostramos una protección sustancial contra el desarrollo de OA en ratas tratadas con insulina combinada con el ejercicio de natación, posiblemente debido a una inhibición completa de biomarcadores de inflamación y estrés oxidativo.

PALABRAS CLAVE: Diabetes; Articulación de la rodilla; Osteoartritis; Ejercicio de natación; Modelo de rata.

REFERENCES

Al-Hashem, F.; El Karib, A. O.; Bin-Jaliah, I.; Dallak, M.; Sakr, H. F.; Eid, R. A.; Zaki, M. S. A.; Al-Shamsi, M.; Haidara, M. A. & Al-Ani, B. Exercise protects against insulin-dependent diabetes-induced osteoarthritis in rats: A scanning electron microscopy study. *Ultrastruct. Pathol., 41(3)*:252-7, 2017.

Atkinson, M. A.; Eisenbarth, G. S. & Michels, A. W. Type 1 diabetes. *Lancet, 383(9911)*:69-82, 2014.

Bliddal, H. & Christensen, R. The treatment and prevention of knee osteoarthritis: a tool for clinical decision-making. *Expert Opin. Pharmacother, 10(11)*:1793-804, 2009.

Cai, L.; Okumu, F. W.; Cleland, J. L.; Beresini, M.; Hogue, D.; Lin, Z. & Filvaroff, E. H. A slow release formulation of insulin as a treatment for osteoarthritis. *Osteoarthritis Cartilage, 10(9)*:692-706, 2002.

Cakır, B.; Kasımay, O.; Kolgazi, M.; Ersoy, Y.; Ercan, F. & Yeg˘en, B. C. Stress-induced multiple organ damage in rats is ameliorated by the antioxidant and anxiolytic effects of regular exercise. *Cell Biochem. Funct., 28(6)*:469-79, 2010.

Chidambaram, J. & Carani Venkatraman, A. Cissus quadrangularis stem alleviates insulin resistance, oxidative injury and fatty liver disease in rats fed high fat plus fructose diet. *Food Chem. Toxicol, 48(8-9)*:2021-9, 2010.

Danaei, G.; Finucane, M. M.; Lu, Y.; Singh, G. M.; Cowan, M. J.; Paciorek, C. J.; Lin, J. K.; Farzadfar, F.; Khang, Y. H.; Stevens, G. A.; Rao, M.; Ali, M. K.; Riley, L. M.; Robinson, C. A.; Ezzati, M. & Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Glucose). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet, 378(9785)*:31-40, 2011.

El Karib, A. O. Exercise ameliorates diabetes-induced osteoarthritis in rats. *Egypt. Acad. J. Biol. Sci., 6(2)*:93-9, 2014.

El Karib, A. O.; Al-Ani, B.; Al-Hashem, F.; Dallak, M.; Bin-Jaliah, I.; El-Gamal, B.; Bashir, S. O.; Eid, R. A. & Haidara, M. A. Insulin and vanadium protect against osteoarthritis development secondary to diabetes mellitus in rats. *Arch. Physiol. Biochem., 122(3)*:148-54, 2016.

Fuller, K.; Murphy, C.; Kirstein, B.; Fox, S. W. & Chambers, T. J. TNFalpha potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. *Endocrinology, 143(3)*:1108-18, 2002.

Grazio, S. & Balen, D. Obesity: risk factor and predictor of osteoarthritis. *Lijec. Vjesn., 131(1-2)*:22-6, 2009.

Haidara, M. A.; Assiri, A. S.; Youssef, M. A.; Mahmoud, M. M.; Ahmed, M. S. E.; Al-Hakami, A. & Chandramoorthy, H. C. Differentiated mesenchymal stem cells ameliorate cardiovascular complications in diabetic rats. *Cell Tissue Res., 359(2)*:565-75, 2015.

Haidara, M. A.; Khloussy, H.; Ammar, H. & Aal Kassem, L. A. Impact of alpha-tocopherol and vitamin C on endothelial markers in rats with streptozotocin-induced diabetes. *Med. Sci. Monit., 10(2)*:BR41-6, 2004.

Hamann, C.; Kirschner, S.; Günther, K. P. & Hofbauer, L. C. Bone, sweet bone--osteoporotic fractures in diabetes mellitus. *Nat. Rev. Endocrinol., 8(5)*:297-305, 2012.

Hayami, T. Osteoarthritis of the knee joint as a cause of musculoskeletal ambulation disability symptom complex (MADS). *Clin. Calcium, 18(11)*:1574-80, 2008.

Ivy, J. L. Role of exercise training in the prevention and treatment of insulin resistance and non-insulin-dependent diabetes mellitus. *Sports Med., 24(5)*:321-36, 1997.

Janghorbani, M.; Van Dam, R. M.; Willett, W. C. & Hu, F. B. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am. J. Epidemiol., 166(5)*:495-505, 2007.

Kapoor, M.; Martel-Pelletier, J.; Lajeunesse, D.; Pelletier, J. P. & Fahmi, H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat. Rev. Rheumatol., 7(1)*:33-42, 2011.

Korczowska, I. & Lacki, J. K. Changes in certain biochemical markers of bone turnover in rheumatoid arthritis patients treated with short-term low dose glucocorticosteroids. *Przegl. Lek., 62(12)*:1384-6, 2005.

Kou, S. & Wu, Y. Meta-analysis of tumor necrosis factor alpha -308 polymorphism and knee osteoarthritis risk. *B. M. C. Musculoskelet. Disord., 15*:373, 2014.

Livshits, G.; Zhai, G.; Hart, D. J.; Kato, B. S.; Wang, H.; Williams, F. M. & Spector, T. D. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: The Chingford Study. *Arthritis Rheum., 60(7)*:2037-45, 2009.

Messier, S. P.; Loeser, R. F.; Mitchell, M. N.; Valle, G.; Morgan, T. P.; Rejeski, W. J. & Ettinger, W. H. Exercise and weight loss in obese older adults with knee osteoarthritis: a preliminary study. *J. Am. Geriatr. Soc., 48(9)*:1062-72, 2000.

Musumeci, G.; Castrogiovanni, P.; Leonardi, R.; Trovato, F. M.; Szychlinska, M. A.; Di Giunta, A.; Loreto, C. & Castorina, S. New perspectives for articular cartilage repair treatment through tissue engineering: A contemporary review. *World J. Orthop., 5(2)*:80-8, 2014.

Niethard, F. U. Morphological and biochemical studies of the cartilage in the diabetic rat--a model for metabolism-induced arthritis. *Z. Orthop. Ihre Grenzgeb., 124(2)*:125-39, 1986.

Pottie, P.; Presle, N.; Terlain, B.; Netter, P.; Mainard, D. & Berenbaum, F. Obesity and osteoarthritis: more complex than predicted! *Ann. Rheum. Dis., 65(11)*:1403-5, 2006.

Pugliese, A. Genetics of type 1 diabetes. *Endocrinol. Metab. Clin. North Am., 33(1)*:1-16, 2004.

Sakr, H. F.; Al-Hashem, F. H.; El-Naby, W. M.; Alkhateeb, M. A.; Zaki, M. S.; Refaey, H. M. & Morsy, M. D. Preventive roles of swimming exercise and pioglitazone treatment on hepatic dysfunction in a rat model of metabolic syndrome. *Can. J. Physiol. Pharmacol., 92(2)*:162-70, 2014.

Schett, G.; Kleyer, A.; Perricone, C.; Sahinbegovic, E.; Iagnocco, A.; Zwerina, J.; Lorenzini, R.; Aschenbrenner, F.; Berenbaum, F.; D'Agostino, M. A.; Willeit, J. & Kiechl, S. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. *Diabetes Care, 36(2)*:403-9, 2013.

Van Manen, M. D.; Nace, J. & Mont, M. A. Management of primary knee osteoarthritis and indications for total knee arthroplasty for general practitioners. *J. Am. Osteopath. Assoc., 112(11)*:709-15, 2012.

Yan, W. & Li, X. Impact of diabetes and its treatments on skeletal diseases. *Front. Med., 7(1)*:81-90, 2013.

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