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Enhanced Mitochondrial Metabolism of Chondrocytes Promotes Diabetes with Osteoarthritis

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Abstract

Objective: The study sought to explore the effect of blood glucose on the metabolism of chondrocytes.

Methods: Chondrocytes from 7 patients, 2 from diabetes combined with KOA patients, 3 from simple KOA patients, 2 from femoral neck fracture without diabetes and KOA patients were extracted for metabolic analysis.

Results: Glycolysis metabolism of chondrocytes in diabetes combined with KOA group was the strongest, followed by simple KOA group and normal chondrocytes, while mitochondrial metabolism was the weakest in diabetes combined with KOA group, followed by simple KOA group and normal chondrocytes.

Conclusion: The decreased metabolism of mitochondria in chondrocytes leads to increased oxidative stress, which may be one of the promoting factors of diabetes complicated with osteoarthritis.

Keywords: Cartilage cells; Osteoarthritis; Oxidative stress; Glucose metabolism

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a highly prevalent complex disease with a genetic background and the intervention of environmental risk factors. The disease combines several defects, among which include a defect in insulin secretion by pancreatic beta-cells, and cellular insulin resistance mainly present in skeletal muscles and the liver but also in other tissues which is characterized by the failure of tissues to respond appropriately to insulin causing chronic hyperglycemia. According to the International Diabetes Alliance (IDF) [1], the number of people with diabetes worldwide will reach 463 million in 2019, and the total number of people with T2DM worldwide is expected to reach 700 million by 2045. The prevalence of this disease is age-related, and the incidence is expected to increase as life expectancy increases. Prolonged hyperglycemia, both in fasting and postprandial states, results in damage to the vessels, causes diabetic nephropathy, diabetic retinopathy and diabetic peripheral neuropathy [2]. In 2001, oxidative stress was first proposed as a unified mechanisms of hyperglycemia-induced damage by Michael Brownlee [3]. The four hypotheses are: Increased polyol pathway flux; increased Advanced Glycation End-Products (AGEs) formation; Activation of Protein Kinase C (PKC) isoforms; and increased hexosamine pathway flux. The four different pathogenic mechanisms reflect a common hyperglycemia-induced process: Overproduction of superoxide by the mitochondrial electron-transport chain.

OA is the most common joint disease and represents an enormous socioeconomic burden affecting millions of people worldwide. OA is a whole joint disease, involving structural alterations in the hyaline articular cartilage, sub chondral bone, ligaments, capsule, synovium, and periarticular muscles. Patients often present with pain, soft tissue mass, limited joint activity, which leads to a functional impairment, incapacity and disability in elderly people. With the combined effects of ageing, obesity and inflammation, along with increasing numbers of joint injuries, this is becoming more prevalent [4,5]. The complex pathogenesis of OA involves mechanical, inflammatory, and metabolic factors, which ultimately lead to structural destruction and failure of the synovial joint [6].

As age-related diseases, both T2DM and OA are expected to rise due to increase of life expectancy.

OA and T2DM frequently co-exist due to their high prevalence and shared risk factors. However, the pathological mechanism of why diabetes patients are more prone to osteoarthritis than healthy people is still unclear.

As the only cellular component of cartilage, the changes of biological characteristics of chondrocytes are closely related to the occurrence and development of OA. Compared with cartilage matrix, chondrocytes are fewer and have lower metabolic activity. Cartilage contains various enzymes of glycolysis metabolism, with low oxygen content, which mainly take the anaerobic glycolysis pathway to play a role [7,8]. OA is characterized by low-grape inflammatory reaction and cellular metabolic dysfunction. Under environmental stress, chondrocytes tend to adapt to changes in the microenvironment by transferring metabolic pathways, which is characterized by enhanced glycolysis metabolic pathways and mitochondrial metabolic dysfunction, which is also a process of cartilage aging [9,10]. Abnormal metabolism of chondrocytes is a response to changes in the inflammatory microenvironment. In OA chondrocytes, degenerative factors cause changes in mitochondrial structure, dynamics and genomic stability, resulting in decreased mitochondrial respiration and excessive production of Reactive Oxygen Species (ROS), which eventually leads to oxidative damage of chondrocytes, which is a sign of OA [11]. Here, we aim to explore the effect of hyperglycemia on chondrocytes from the perspective of cell metabolism by comparing the difference of oxygen metabolism and mitochondrial metabolism among patients with diabetes combined with KOA, simple KOA patients and healthy people.

Materials and Methods

Experiments subjects

The study was approved by the Ethics Committee of the First Affiliated Hospital of Jinan University. A total of 196 patients who underwent conservative or surgical treatment for KOA in the spinal joint Surgery Department of the First Affiliated Hospital of Jinan University from January 2019 to December 2020 were selected and analyzed. The diagnostic criteria proposed by the American College of Rheumatology in 1995 were used as the diagnostic criteria for KOA [12], excluding reactive arthritis, infectious arthritis, aseptic necrosis of the femoral head, bone tuberculosis, and secondary arthritis caused by sequelae of fracture or rheumatic system diseases.

Human cartilage tissues

KOA chondrocytes were extracted from cartilage tissue of patients undergoing total knee arthroplasty due to KOA, and normal cartilage cells were extracted from hip cartilage tissue of patients undergoing hip arthroplasty due to femoral neck fractures, totaling 7 patients. Informed consent was obtained before participation in the study.

Experiments design

Patients were divided into two groups: diabetes combined with KOA group and simple KOA group. Cells were divided into 3 groups: Diabetes combined with KOA group, Simple KOA group and Normal group. Chondrocytes were extracted and cultured for metabolic analysis.

Human articular chondrocytes isolation and culture

Chondrocytes were isolated from knee joint tissues. The cartilage was cut into thin slices and then washed with PBS three times, and add 0.25% trypsin 3 ml in water bath for shock digestion for 20 min. The cartilage slices were washed with PBS again to remove residual trypsin (BI technology, China) and shaking digested with 0.2% collagenase type II (Gibco, USA) in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, USA) for 4 h in a 3°C shanking bath, Digested cartilage was collected and centrifuged. The pellet was resuspended in DMEM and filtered through 200-μm nylon mesh. chondrocytes were cultured in 6-well plates with DMEM supplement with 10% fetal bovine serum (Gibco, USA) and 1% Penicillin-Streptomycin-Neomycin (Gibco, USA) in a 5% CO_2 incubator at 37°C about 72 h. And cells were sub-cultured when the degree of cell fusion is 80% to 90%.

Metabolic assays and analyses

OCR and ECAR with Seahorse XF96 analysis: Agilent seahorse XF technology relies on the energy metabolism detection system to measure the two energy metabolism pathways of living cells in real time and dynamically, and provide information about glycolysis function, mitochondrial function, oxidative stress and metabolic dysfunction of living cells by measuring Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) [13]. For measurements of human cells using an XF96 Analyzer (Seahorse Bioscience, Agilent Technologies, USA). In this study, OCR and ECAR of three groups of chondrocytes were measured using Agilent's mitochondrial Pressure Kit (102015-100, Agilent, USA) and glycolysis Kit (103020-100, Agilent, USA). In OCR determination, the concentration of FCCP was 1.0 μmol/l, and the concentration of oligomycin was 1.5 μmol/L; In ECAR determination, the concentration of oligomycin was 1.0 μmol/L.

Chondrocytes were plated in XF96 seahorse plate at 5,000 cells per well according to experimental designs. Isolation and culture were followed as described previously. The day before the experiment, cells were switched to Seahorse XF base medium supplemented with 1 ml glucose, 1 ml pyruvate and 1 ml glutamine and further incubated in CO_2-free incubator for 1 h. Oligomycin, FCCP and Rotenone/Antimycin A were prepared in XF assay medium with final concentration of 100 μM, 100 μM and 50 μM.

Statistics

Agilent WAVE software was used for metabolic analysis, and PRISM software (8.0, GraphPad) was used for statistical analysis. Chi-square test was used to analyze gender between groups, T test was used to analyze age, and p value <0.05 was considered as statistically significant difference or correlation.

Results

General conditions between diabetes combined with KOA group and simple KOA group

A total of 196 patients were included in this study. There were 24 patients diagnosed diabetes combined with KOA. The proportion of diabetes combined with KOA was 12.2%. There were significantly more females than males in two groups, but there was no significant difference in gender composition ratio between the two groups (χ^2=2.51, p=0.20, p>0.05) (Figure 1A). The age of simple KOA group was slightly lower than that of diabetes combined with KOA group, but the difference was not statistically significant too (t=1.62, p=0.11, p>0.05) (Figure 1B).

K-L classification difference between diabetes combined with KOA group and simple KOA group

In order to analyze the difference of K-L classification between patients with diabetes combined with KOA and simple KOA, we

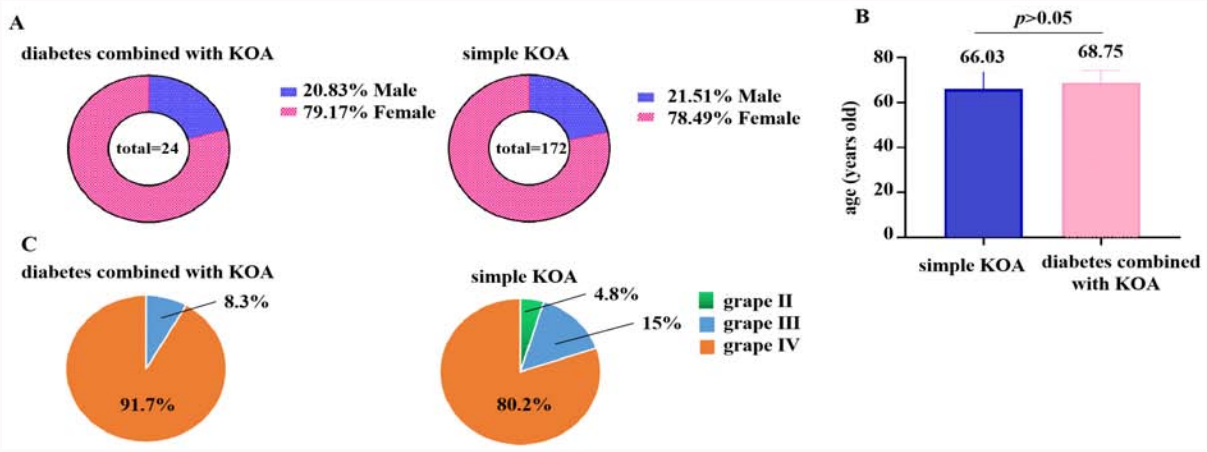

Figure 1: General condition and K-L grade of diabetes combined with KOA group and simple KOA group: A) The proportion of men and women in diabetes combined with KOA group is similar to that in simple KOA group, with similar age and K-L rating. B) The age of diabetes combined with KOA group is similar to that of simple KOA group. C) The K-L population composition ratio of diabetes combined with KOA group was similar to that of simple KOA group.

made statistics on the K-L classification of 196 patients. Taking the side with high score for statistics, the results showed that there were 2 cases with K-L grade III and 22 cases with grade IV in diabetes combined with KOA group. In the simple KOA group, there were 7 cases with K-L grade II, 22 cases with grade III and 118 cases with grade IV. Chi square test was used to analyze the constituent ratio of K-L classification. The results showed that there was no difference in the constituent ratio of K-L classification between diabetes combined with KOA group and simple KOA group (χ^2=3.67, P =0.24, P>0.05) (Figure 1 C).

Mitochondrial stress test

Chondrocytes adapt to environmental changes by changing metabolic pathways. In many disease states, glycolysis is enhanced and mitochondrial respiration is weakened, such as rheumatoid arthritis, hyperthyroidism, malignant tumors, etc. The weakening of mitochondrial respiration and the excessive production of ROS eventually cause oxidative damage to chondrocytes. In order to understand whether there is a difference in mitochondrial metabolism between chondrocytes of patients with diabetes combined with KOA, patients with simple KOA and healthy person, we used Agilent seahorse XF96 mitochondrial stress tests to compare OCR of three groups of chondrocytes. The results showed that whether the basal respiration, or the maximum respiration after adding the uncoupling agent FCCP, or the standby respiration representing the potential adaptability of cells to energy demand, the chondrocytes in the diabetes combined with KOA group were lower than those in simple KOA group. At the same time, the OCR of chondrocytes in the diabetes combined with KOA group was also significantly lower than that in simple KOA group, suggesting that in the state of low-grape chronic inflammation, basic respiration, maximum respiration and respiratory reserve capacity of chondrocytes in patients with diabetes combined with KOA are weaker than those in patients with simple KOA, indicating that the chondrocytes have a poor adaptability to changes in energy metabolism and are more prone to oxidative damage (Figures 2A-2D).

Glycolysis metabolism test

Under normal conditions, glycolysis is the main metabolic pathway of chondrocytes. However, under environmental stress, chondrocytes will adapt to the changes of microenvironment by enhancing glycolysis and weakening mitochondrial respiration. In order to analyze the possible differences in glycolysis metabolism between diabetes combined with KOA group, simple KOA group and normal chondrocytes, we used Agilent seahorse XF96 glycolysis stress test to compare the glycolysis rate, glycolysis capacity and glycolysis reserve capacity of chondrocytes in the three groups. The results showed that after adding saturated glucose, the ECAR of chondrocytes in the diabetes combined KOA group was significantly higher than that in the simple KOA group and the normal group, suggesting that the glycolysis rate of cartilage in the basic state of the diabetes combined KOA group was higher than that in the simple KOA group, and the basic glycolysis rate of cartilage in the simple KOA group was also higher than that in the normal group. However, after adding 2-deoxyglucose, the ECAR of chondrocytes in the simple KOA group was higher than that in the diabetes combined KOA group, and the glycolysis reserve capacity was also higher than that in the diabetes combined KOA group, suggesting that the glycolysis capacity of chondrocytes in simple KOA group was higher than that in the diabetes combined KOA group, and under stress conditions, the ability of chondrocytes in simple KOA group to respond to energy demand was stronger than that in diabetes combined KOA group. The results showed that the reserve capacity of chondrocytes in diabetes combined with KOA group was worse than that in simple KOA group (Figures 2E-2H).

Discussion and Conclusions

Mariely [14] conducted a follow-up study and enrolled 100 diabetic patients and 102 non-diabetic patients in 2013, the results show that the incidence rate of OA was 49% in diabetic patients, while the incidence rate of OA was only 26.5% in people without diabetes. The proportion of patients with diabetes in OA population is 11%. Our data showed that the proportion of patients with diabetes in OA is 12.2%, there was no significant difference in gender composition ratio between the two groups. The results were similar to previous studies.

The increase of oxidative stress may be the common pathogenesis of chronic complications of diabetes and diabetes with KOA. Long term hyperglycemia can enhance oxidative stress in multiple ways, then weaken mitochondrial function, cause oxidative damage to chondrocytes, and then lead to chondrocyte degeneration and KOA.

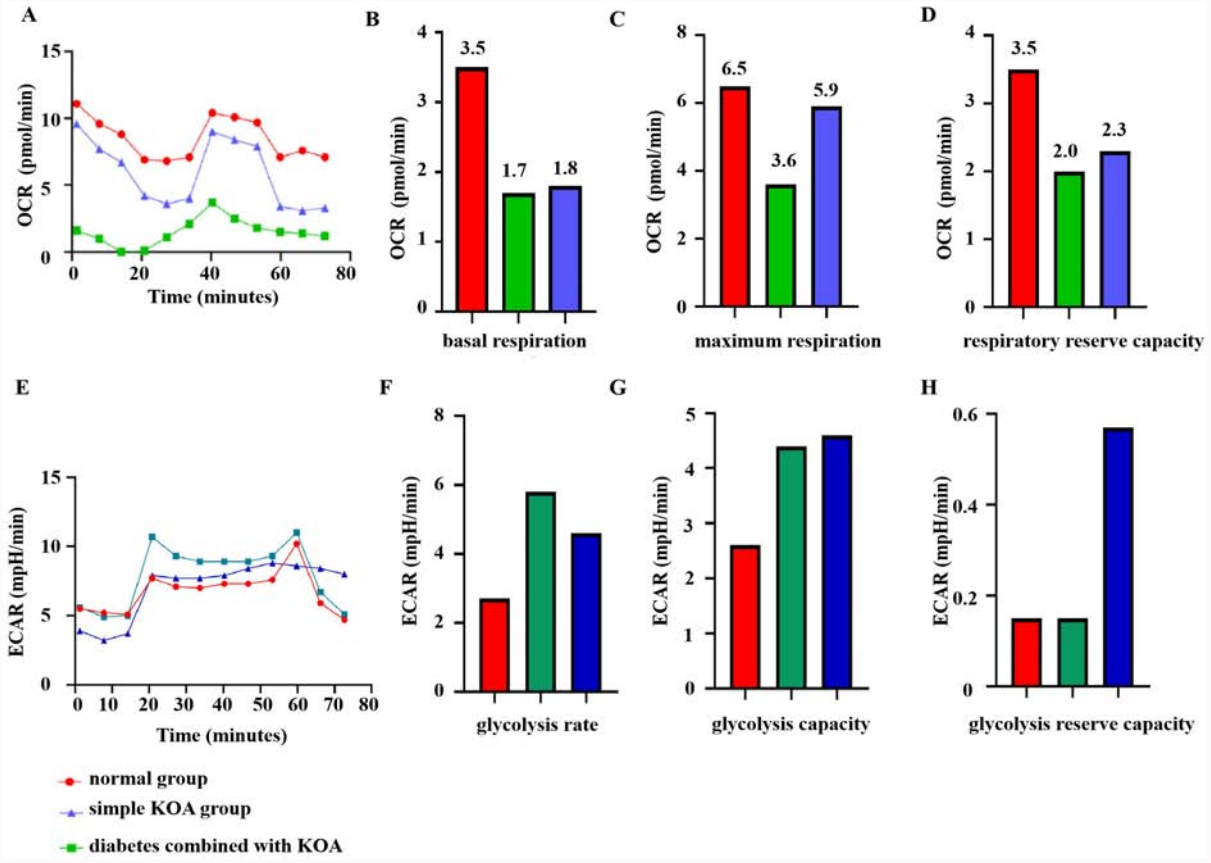

Figure 2: OCR and ECAR with Seahorse XF96 analysis: A) The OCR of chondrocytes in the diabetes combined with KOA group was significantly lower than that in simple KOA group and normal group. B) The basal respiration of diabetes combined with KOA group was similar to that of simple KOA group, both of which were significantly lower than normal group. C) The maximum respiration in diabetes combined with KOA group was significantly lower than that in simple KOA group and normal group, and the maximum respiration in simple KOA group was slightly lower than that in normal group, too. D) The respiratory reserve capacity of diabetes combined with KOA group and simple KOA group was significantly lower than that of normal group. However, diabetes combined with KOA group was similar to that of simple KOA group, and that of diabetes combined with KOA group was slightly lower than that of simple KOA group. E) The ECAR of chondrocytes in the diabetes combined KOA group was significantly higher than that in the simple KOA group and the normal group. F) Glycolysis rate of cartilage was the highest in diabetes with KOA group, followed by simple KOA group, and the lowest in normal group. G) Glycolysis capacity of diabetes combined with KOA group was similar to that of simple KOA group, both of which were significantly higher than that of normal group. H) Glycolysis reserve capacity of the simple KOA group was the highest, and the diabetes combined KOA group was similar to normal group.

ROS production increases under oxidative stress. ROS is one of the most intuitive indicators in response to oxidative stress, which is often used to reflect the strength of oxidative stress in clinic. Its biomarkers include Catalase (CAT), Glutathione (GSH), Superoxide Dismutase (SOD) And Lipid Peroxidase (LPO). Research have found that the levels of serum and joint fluid oxidative stress indicators in patients with OA at different clinical stages are not the same, including SOD, GSH, Malondialdehyde (MDA). These indicators can be used as an important basis for evaluating the condition and prognosis of patients with OA [15]. Studies have shown that enhanced oxidative stress leads to the increase of Nitric Oxide (NO) produced by inducible NO Synthase (iNOS). As a pro-inflammatory and destructive mediator of OA, NO can increase the production of prostaglandin E2 and inflammatory cytokines which lead to cartilage damage by inducing inflammatory response. In addition, NO plays a regulatory role in the activation of MMP9, through inhibiting the activity of metalloproteinase inhibitor 1 to up regulate MMP9 expression and inducing OA [16].

Under normal circumstances, glycolysis metabolism is the main metabolic pathway of chondrocytes. However, under oxidative stress, chondrocytes will adapt to changes in the microenvironment by enhancing glycolysis metabolism and weakening mitochondrial respiration [17]. Chondrocytes rapidly acquire ATP by enhancing glycolysis, so as to obtain enough energy to adapt to low-level chronic inflammation, which is a compensatory protective response of chondrocyte energy supply. When mitochondrial respiration is weakened, oxygen produced by aerobic oxidation cannot undergo oxidative phosphorylation, resulting in excessive ROS production and enhanced oxidative stress. Combined with our results of seahorse, compared with the chondrocytes of patients with simple KOA and normal chondrocytes, the chondrocytes of patients with diabetes combined with KOA have decreased mitochondrial respiration and increased RO increased the production, then enhanced oxidative stress, which may affect the integrity of chondrocytes through inflammation promotion and the effect on MMPs, leading to oxidative damage of chondrocytes and OA. On the other hand, the glycolysis rate of chondrocytes in patients with diabetes combined with KOA group was higher than that in patients with simple KOA, but the glycolysis ability and reserve ability were weaker than that in patients with simple KOA. We speculate that the low-level inflammatory reaction *in vivo* caused by long-term high glucose status may be one of the reasons for the decrease of chondrocyte reserve reaction in patients with diabetes combined with KOA.

To sum up, in the environment of chronic low-level inflammation, the metabolism of chondrocytes changes, which is manifested by enhanced glycolysis metabolism, reduced intracellular oxidative phosphorylation effect, weakened mitochondrial respiration, increased ROS production, aggravated the oxidative damage of chondrocytes, and then caused chondrocyte degeneration, leading to the occurrence and development of OA. By analyzing the possible differences in the energy metabolism of chondrocytes between diabetes with KOA and simple KOA, the study provides the basic data and phenotypic trend of cell metabolism for the pathogenesis of diabetes KOA. However, due to the small number of cases of cell experiment, it cannot well represent the metabolism of chondrocytes in these two disease states, which still needs to be further verified by subsequent experiments.

Authors' Contribution

Simin Luo and Qiping Shiare responsible for designing experiments and writing guidance articles. Shuangshuang Li and Hongxing Chen are responsible for collecting samples and cell tests. Qiu Dong is responsible for the surgical part and result analysis, Qing Liu is responsible for sample collection, cell tests and writing, and Lan Qin and Jian Liu are responsible for helping to sort out clinical data.

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