*Original Article*
# Influence of swimming exercise on the expression of apoptotic gene caspase-3 in chondrocytes in osteoarthritis

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**Abstract:** Objective: To study swimming exercise's effect on caspase-3 expression in chondrocytes in osteoarthritis (OA). Methods: 36 SD rats were randomly separated into normal group (n = 12), OA model group (n = 12) and swimming exercise group (n = 12). After modeling, rats received no intervention in model group and swimming exercise once a day (15 min/time) for 4 consecutive weeks in swimming exercise group. After intervention for 4 weeks, specimens were taken to analyze tissue morphology by H&E staining, caspase-3 expression by Western blot and qPCR, chondrocytes apoptosis by TUNEL assay. Results: HE staining revealed abnormal bone tissue morphology in model group and swimming exercise group with improved morphology after swimming exercise. Model group and swimming exercise group all showed significantly higher Caspase-3 protein level than normal group with lower level after swimming exercise (P < 0.05). Consistently, qPCR showed similar expression profile of caspase-3 mRNA level to the protein level. Conclusion: Swimming exercise can inhibit caspase-3 level and chondrocytes apoptosis in OA, thus improving the joint morphology.

**Keywords:** Swimming exercise, osteoarthritis, chondrocyte, caspase-3, apoptosis

## Introduction

Osteoarthritis (OA) is a kind of common arthritic disease in clinic, which frequently occurs in the finger joint, shoulder joint, knee joint, etc. [1]. The major pathological changes of OA are cartilage destruction and osteoproliferation, and arthralgia and activity limitation are the most common clinical manifestations of patients, seriously affecting the quality of life of patients [2, 3]. Currently, the exact cause and pathogenesis of OA remain unclear, but age, genetic factors and mechanical force are considered as major risk factors for OA [4]. Studies have demonstrated that [5, 6] apoptosis of articular chondrocytes is observed in OA, and there is a close correlation between them. Caspase-3 is an important participant and executor of the apoptotic process [7]. Moreover, studies have revealed that [8] caspase-3 is highly expressed in cartilages in OA, and is positively correlated with the severity of OA. Previous study indicated that age, but not short-term intensive swimming, affects chondrocyte turnover in zebrafish vertebral cartilage [9]. The role of swimming exercise in chondrocyte remains to be further determined. Swimming exercise is a commonly-used rehabilitation method, and its advantages include the higher accuracy of biological regulation in active exercise and rehabilitation via underwater exercise, which is often clinically used in the rehabilitation therapy of OA with an excellent curative effect. This study aims to assess whether swimming exercise affects caspase-3 level in chondrocytes in OA, so as to clarify the mechanism of swimming exercise.

## Materials and methods

### Laboratory animals and grouping

A total of 36 adult SD rats purchased from Shanghai SLAC Laboratory Animal Co., Ltd. were equally and randomly separated into normal group, model group and swimming exercise group using a random number table. All opera-

**Table 1.** Primer sequences

| Name | Primer sequence |
| --- | --- |
| Caspase-3 | Forward: 5'-TATTCCACAGCACCTGGTTA-3' |
| | Reverse: 5'-CAATACATGGAATCTGTTTCTT-3' |
| GADPH | Forward: 5'-ACGGCAAGTTCAACGGCACAG-3' |
| | Reverse: 5'-GAAGACGCCAGTAGACTCCACGAC-3' |

tions and protocols on animals were approved by the Laboratory Animal Ethics Committee of the First Hospital of Jilin University.

*Experimental reagents and instruments*

Primary antibodies: anti-caspase-3 antibody (Abcam, USA) and anti-β-actin antibody, immunohistochemistry kit (Maxim, Fuzhou), hematoxylin-eosin (HE) staining kit (Solarbio, Beijing), and fluorescence qPCR instrument (ABI 7500, USA).

*Construction of rat model of OA*

After anesthesia with 7% chloral hydrate (5 mL/kg), rats were fixed on the operating table under a supine position, the knee joint was exposed, the hair was shaved off and the joint was disinfected. Then 0.1 mL 2% papain and 0.05 mL 0.05 mmol/L L-cysteine were injected once into the knee joint, and they were injected again after 4 days to construct the rat model of OA.

*Treatment in each group*

Rats in normal group were fed normally without any treatment. The OA model was prepared using the above method in model group without any intervention. The OA model was also prepared using the above method in swimming exercise group, and rats were treated with swimming exercise at 3 days after modeling, and the specific method is as follows: the rats were placed in a 36 cm-deep pool at 32-36°C, and swam at a speed of 3 cm/s once a day (15 min/time) for 4 consecutive weeks.

*Sampling*

After anesthesia, cartilage tissues were collected from 6 rats in each group, fixed in 4% paraformaldehyde at 4°C, and decalcified with ethylene diaminetetraacetic acid (EDTA) decalcifying solution. The decalcifying solution was replaced once every 3 d till the complete de-

calcification of tissues. Then tissues were prepared into paraffin sections used for immunohistochemical detection. The knee cartilage tissues were isolated directly from the remaining 6 rats for measuring caspase-3 level.

*Detection method*

*HE staining:* The paraffin sections (5 μm-thick) were routinely dewaxed and soaked in water, followed by HE staining using the HE staining kit according to the instructions to observe the morphology of knee joint of rats.

*TUNEL assay:* The paraffin sections were routinely dewaxed and soaked in water, followed by analysis of chondrocytes apoptosis by TUNEL kit.

*Immunohistochemistry:* The paraffin sections were routinely dewaxed and soaked in water, and the citric acid buffer was added and heated for antigen retrieval followed by endogenous peroxidase blockage and addition of anti-caspase-3 antibody (1:200) for overnight incubation at 4°C and then secondary antibody for 10 min. After incubation with streptavidin-peroxidase solution and DAB addition for development, the sections were counterstained with hematoxylin followed by observation under a microscope.

*Western blot:* The tissue protein was isolated using lysis solution and quantified by BCA assay followed by separation on SDS-PAGE for western blot using anti-caspase-3 antibody (1:2000 dilution). The membrane was developed after addition of chemiluminescence reagent.

*qPCR:* RNA was extracted for cDNA synthesis followed by qPCR with conditions: 96°C 10 min, 40 cycles of 96°C 10 s, 60°C 30 s. Gene expression was analyzed using GAPDH as a reference. The primer sequences were shown in **Table 1**.

*Statistical methods*

Data in this study were presented as mean ± standard deviation; Statistical Product and Service Solutions (SPSS) 19.0 software (SPSS Inc., Chicago, IL, USA) was used for data processing. Continuous data from multiple groups were analyzed by using one-way ANOVA, with the Tukey's post hoc test. $P$-values < 0.05 were considered statistically significant.
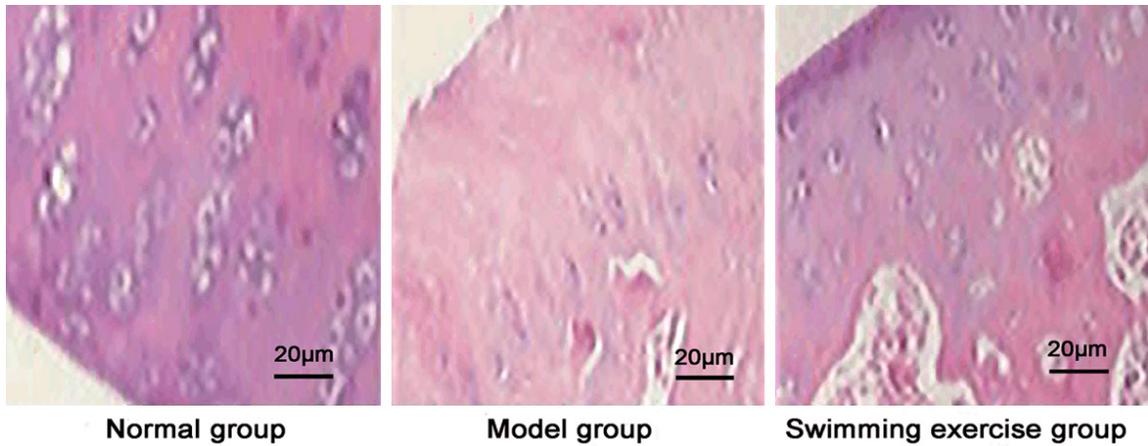
**Figure 1.** Observation of tissue morphology via HE staining (scale bar 20 μm).
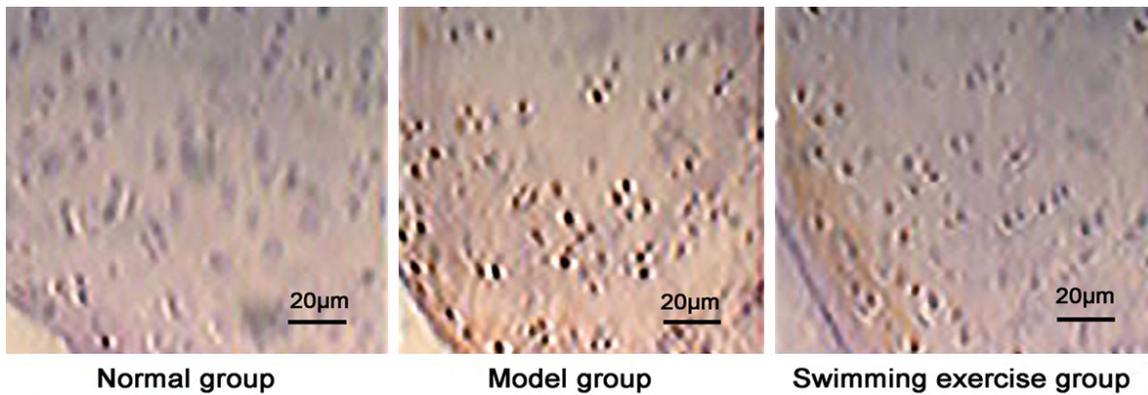


**Figure 2.** Immunohistochemical detection of caspase-3 expression (scale bar 20 μm).

## Results

*Observation of tissue morphology via HE staining*

In normal group, the knee joint had normal morphology, complete structure and normal chondrocytes arranged closely and in order, and there were complete tidal lines a little far away from the joint surface. In model group, the knee cartilages were damaged with abnormal morphology, incomplete structure and partial loss, the number of chondrocytes was reduced and disorganized, and tidal lines were incomplete and near to the joint surface. In swimming exercise group, the knee cartilages were partially damaged, but the morphology and structure were improved, the number of chondrocytes was larger and they were arranged in well order, and there were complete tidal lines which were a little far away from the joint surface (**Figure 1**).

*Immunohistochemical staining*

Caspase-3 positive expression showed dark brown color and was lower in normal group and higher in model group and swimming exercise group (**Figure 2**) with a significantly reduced level after swimming exercise than model group ($P < 0.05$) (**Figure 3**).

*Caspase-3 protein expression*

Caspase-3 protein expression was significantly higher in model group and swimming exercise group than normal group (**Figures 4**, **5**) and it was significantly decreased after swimming exercise in comparison to the model group ($P < 0.05$) (**Figure 5**).
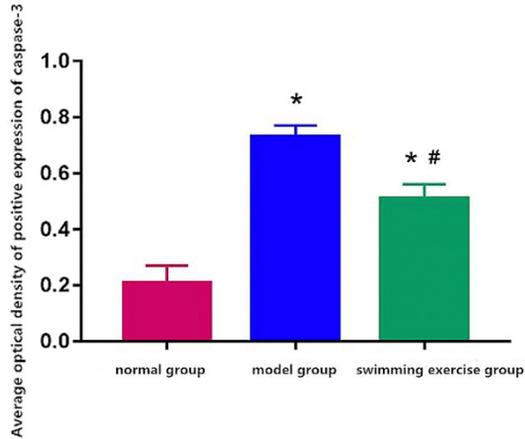
**Figure 3.** Average optical density of positive expression of caspase-3. Note: $^*P < 0.05$ vs. normal group, $^#P < 0.05$ vs. model group.
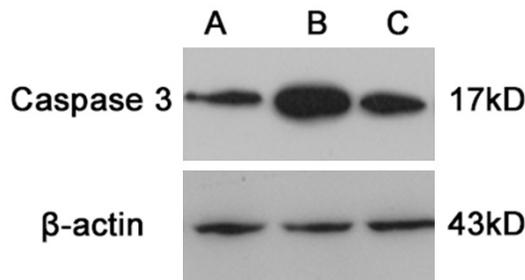


**Figure 4.** Detection of caspase-3 protein expression via Western blotting. A. normal group. B. model group. C. swimming exercise group.
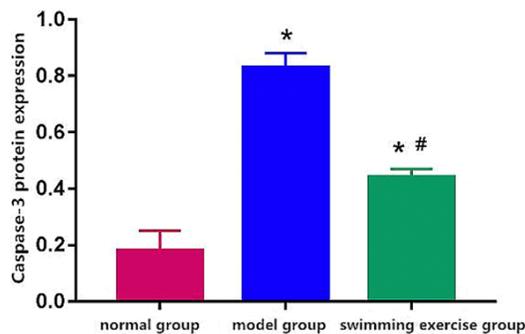


**Figure 5.** Caspase-3 protein expression. Note: $^*P < 0.05$ vs. normal group, $^#P < 0.05$ vs. model group.

*Caspase-3 mRNA expression*

Caspase-3 mRNA level was lower in normal group and higher in model group and swimming exercise group with a declined level after swimming exercise in comparison to model group ($P < 0.05$) (**Figure 6**).
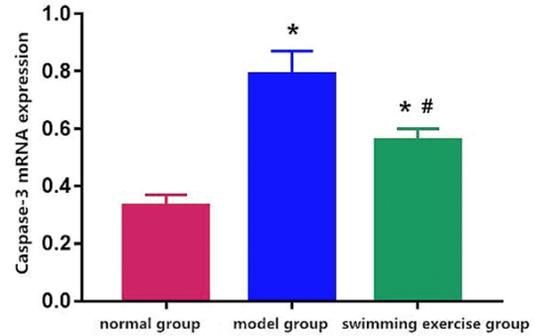


**Figure 6.** Caspase-3 mRNA expression. Note: $^*P < 0.05$ vs. normal group, $^#P < 0.05$ vs. model group.
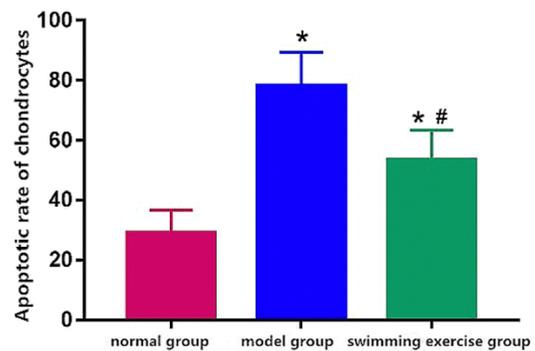


**Figure 7.** Apoptotic rate of chondrocytes. Note: $^*P < 0.05$ vs. normal group, $^#P < 0.05$ vs. model group.

*TUNEL apoptosis detection*

The apoptotic rate was lower in normal group and higher in model group and swimming exercise group with a decreased level in swimming exercise group in comparison to model group ($P < 0.05$) (**Figure 7**).

**Discussion**

The major pathological reactions of OA, a common degenerative joint disease in clinic, are degradation of articular cartilage and chondrocyte apoptosis [10]. OA is thought to be related to a variety of factors, including chondrocyte apoptosis, genetic factors and immune response [11, 12]. Studies have demonstrated that [13, 14] the apoptosis degree of chondrocytes in OA patients is significantly increased compared with normal chondrocytes, indicating a close correlation between chondrocyte apoptosis and OA. At the same time, some studies have further confirmed [15, 16] that the results of in-situ hybridization are consistent

between experimental rabbit model of OA and human OA, and TUNEL assay has shown that the degree of chondrocyte apoptosis in OA model was significantly higher than that of normal chondrocytes, further suggesting that chondrocyte apoptosis is one of the important pathological reactions of OA. Moreover, studies have confirmed [17, 18] that the progression of OA is accelerated by chondrocyte apoptosis after OA occurs, so chondrocyte apoptosis is involved in the process of OA progression. Capase-3-induced apoptosis, one of the known important components of death receptor signal transduction, is known to have a close correlation with OA [19, 20]. Caspase-3 inhibitors can effectively reduce the expression of caspase-3 in chondrocytes in OA and inhibit the chondrocyte apoptosis, indicating caspase-3's role in OA and chondrocyte apoptosis [21, 22]. Currently, it has been confirmed that the high expression of caspase-3 and chondrocyte apoptosis can be found in OA patients and rodent OA caused by modeling [23, 24]. Therefore, caspase-3 can serve as one of the important targets for inhibiting the chondrocyte apoptosis in OA. It has been indicated that reduced Rspo-2 levels in OA osteoblasts are responsible, at least in part, for their reduced Wnt/beta-catenin signaling and abnormal mineralization [25]. Spermidine activates RIP1 deubiquitination to inhibit TNF-alpha-induced NF-kappaB/p65 signaling pathway in osteoarthritis [26]. The limitation of the study therefore exists that the mechanisms of swimming exercise need further investigation besides caspase-3, which may lead to potential new avenues of combined treatment of OA.

The advantages of swimming exercise are as follows: (1) the buoyancy of water reduces the weight load on the joint during exercise, (2) the resistance of water against joint movement enhances the exercise, and (3) the appropriate water temperature can alleviate the muscle spasm, ease the pain and improve the blood circulation in the joint. At the same time, studies have revealed that [27, 28] the effects of exercise on the body's metabolism and structural morphology are highly specific, and only the exercise load of the specific type, intensity, frequency and duration can exert a positive effect on maintaining the normal function of articular cartilage. Swimming exercise is a typical cyclic motion of limbs, which has an excel-

lent rehabilitation effect in clinic. During swimming exercise, the complex biological resistance and discomfort in active motion of joint in OA can be perceived by the fine proprioceptive sensation, thus adjusting the body motion in time, so that the body function and pathological changes can be compatible with the swimming status [29, 30].

## Conclusion

Results of this study indicate that swimming exercise can significantly inhibit the expression of caspase-3, an apoptotic gene in OA, thus inhibiting chondrocyte apoptosis in OA, which may be one of the reasons for the good rehabilitation effect of swimming exercise on OA.

## Disclosure of conflict of interest

None.

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