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RESEARCH ARTICLE
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STIMULATION OF DEACETYLATION OF B-CATENIN IN OSTEOARTHRITIS CHONDROCYTES CAN REVERSE CHON DROGENIC DEGENERATION CAUSED BY MITOCHONDRIAL DYSFUNCTION OF CHONDROCYTE

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Abstract
Obesity is an important contributing factor to osteoarthritis (OA), characterized by cartilage thinning and disintegration, inflammation and cartilage remodeling, accompanied by dysfunction of chondrocyte metabolism and mitochondrial biology. The aim of our study was to investigate the role of adipokine (leptin) -mediated β-catenin in the development and progression of OA, with a focus on the relationship between degenerative chondrocyte mitochondrial dysfunction and the Wnt/β-catenin pathway. Our data suggest that β-catenin acetylation modification is low in normal chondrocytes. However, in leptin mediated chondrocytes, down-regulated HDAC1 led to increased β-catenin acetylation of Lys-49, and increased downstream β-catenin phosphorylation promoted nuclear translocation and activated the β-catenin/WNT signaling pathway. The activation of mitochondrial dysfunction and cell death programs in OA chondrocytes. Functional analysis showed that leptin enhanced mitochondrial division, resulting in ROS overproduction, mitochondrial pro-apoptotic protein leakage, and Caspase-9-dependent cell death pathway activation. However, overexpression of HDAC1 blocks the progression of mitochondrial division while activating chondrocyte pro-survival signaling. At the molecular level, our data further suggest that the Wnt/β-catenin pathway is required for chondrocyte protective mechanisms resulting from HDAC1 overexpression: Inhibition of Wnt/β-catenin pathway by promoting β-catenin deacetylation can reduce mitochondrial fission, metabolic function changes and activation of apoptotic signals in chondrocytes. Overall, our data suggest that obesity-induced osteoarthritis is associated with HDAC1 downregulation, Wnt/β-catenin pathway deacetylation inactivation and mitochondrial dysfunction, and Caspase-9-dependent cell death activation. Based on this, enhancement of HDAC1 activity and inhibition of Wnt/β-catenin signaling pathway to stimulate deacetylation of β-catenin in osteoarthritis chondrocytes can reverse cartilage degeneration caused by mitochondrial dysfunction of chondrocytes..

Keywords:- leptin; β-catenin acetylation; Wnt/β-catenin; HDAC1

1 Introduction

Obesity is a major contributing factor to osteoarthritis (OA(Rihn et al., 2013; Luoma et al., 2000). It is well kown that non-physiological mechanical loads can accelerate arthrosis(Chan et al., 2011). The articular cartilage consists of the extracellular matrix (ECM) and chondrocytes(Freemont, 2009). The cartilage endplates (CEPs) is an important component of joint (Chan et al., 2011), which

is the predominant source of nutrients for the joint (Magnier et al., 2009). CEPs degeneration is considered the initiating factor of OA(Modic and Ross, 2007). During OA, cartilage endplate cells apoptosis, senescence and other pathological factors are involved(Alkhatib et al., 2014; Le Maitre et al., 2007), so inhibition of early CEP degeneration is very important to delay OA(Andersson, 1999).Cartilage endplate degeneration is usually accompanied by apoptosis of cartilage cellularity (Poole, 2012)which accelerates the process of OA(Alberton et al., 2015) . Apoptosis is thought to be a continuous process throughout OA progression.

Mitochondria produce energy by synthesizing adenosine triphosphate (ATP) to drive normal cellular physiological functions. The basic mechanisms of mitochondrial dysfunction in osteoarthritis include activation of chondrocyte apoptotic signaling, decreased chondrocyte biosynthesis, and cytokine induced chondrocyte inflammation. Mitochondrial dysfunction in OA chondrocytes may result from the direct effects of pro-inflammatory cytokines and reactive oxygen species (ROS) (Blanco et al., 2011).

Leptin is the product of the obese gene(Zhang et al., 1994). Serum leptin concentrations ar e positively associated with the amount of fat tissue(Dixon et al., 2010). As an important hor mone, leptin plays an important role in cell apoptosis and homeostasis. The leptin receptors are comprised of five forms, including long (OB-Rb), short (OB-Ra, -Rc, and -Rd), and soluble (OB-Re) receptors(Tartaglia, 1997).However, the OB-Rb receptor, mediates the most biologic al effects of leptin with the assistance of the other forms within the body. Binding of lepti n to its functional receptor activates multiple signal transduction pathways(Matarese et al., 2 005). Previous studies have shown that leptin plays a positive role in promoting mitochondr ial fusion in chondrocytes in hypoxic environments, but how leptin regulates mitochondrial f unction in chondrocytes and drives its normal function is rarely reported.

In this study, we found that in the obese model, the wnt/ β-catenin signaling pathway plays an important role in regulating the metabolism of chondrocytes by leptin, and the Wnt/β-catenin pathway is necessary for the protective mechanism of chondrocytes generated by HDAC1: HDAC1 inhibits the Wnt/β-catenin pathway by promoting β-catenin deacetylation, which can reduce the mitochondrial fission, metabolic function changes and the activation of apoptosis signal in chondrocytes. These results may provide a new direction for the study of the pathogenesis of osteoarthritis in obese individuals.

2Materials and Methods

2.1 Establishment of obese mice models with a high-fat diet

High-fat diet consumption promotes weight gain in rodents(Ozcan et al., 2009). Animals handling and experimental procedures were approved by the Animal Ethics Committee of the Second Affiliated Hospital of Soochow University. Four-weeks-old C57 male mice were housed at 24-26℃ with 12h light-12h dark cycles and provided with water and a chow diet (HFD, 58% kcal from fat, 26% kcal from sucrose). Weights of all mice were monitored once a week. Ratio of total leptin concentration to SOB-R act as a biomarker of leptin resistance(Sandhofer et al., 2003; Herrick et al., 2016) .

2.2 Serum biochemical measurements

Blood samples of the mice were collected and centrifuged at 3000rpm for 10min. The serum was collected and stored at-80 ℃ before use. The total leptin and SOB-R levels were determined following the instructions of the ELISA kits provided by the Nanjing Jiancheng Bioengineering Institute.

2.3 Primary cells isolation and leptin treatment

Primary chondrocytes were seeded in 6-well plate at a density of 2x106 cells/well and allowed to grow to 70% confluency. The Medium were then replaced with serum-free medium for overnight until leptin treatment. Various concentrations of leptin (0, 10, 50,100 ng/ml) were included for 48hrs

treatment.

2.4 Flow cytometry analysis of the CEP chondrocytes apoptosis

Flow cytometry was utilized for cell apoptosis analysis. After leptin treatment, cells were washed with phosphate buffered saline (PBS) and digested. 5 μl FITC-Annexin and 5 μl PI (250 μg/ml) were added into the cell suspensions, and incubated on ice in dark for 10 minutes. Next, cells were washed with PBS twice, and analyzed using a flow cytometry instrument (BD Bioscience, USA).

2.5 Western Blot

The frozen tissues were washed with cold PBS twice and centrifugation at 3000 rpm for 5 min at 4℃, then discard the supernatant. Total protein extracts were obtained by RIPA lysis buffer supplemented with protease inhibitor cocktail and phosphatase inhibitors. After centrifugation, the supernatant was collected and stored at -80℃. Protein concentration was determined by the BCA protein detection kit.

2.6 Immunostaining

Paraffin sections (5 μm thickness) were used for indirect immunostaining. Briefly, sections were deparaffined in methylcyclohexane for 10 min, hydrated in ethanol, and washed with PBS. Slides were then subjected to antigen retrieval in 0.01M citrate buffer (pH 6), and blocked for 1 h at room temperature with blocking buffer (10%normal goat serum, 5% BSA, and 0.1 Triton X-100 in PBS). The slides were then incubated with primary antibodies overnight at 4 ℃ followed by incubation with fluorescent anti-mouse or anti-rabbit antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove,PA). To quantify the number of immunolabeled cells, they have been counted under the microscope on well-oriented sections from at least six different animals per experimental condition.

2.7 TEM

Specimens were fixed with 2.5% glutaraldehyde at 4℃ for more than 4 hrs and then 1%OsO4 for 1-2hrs. Next, the specimens were dehydrated by an ethanol gradient, and then overnight acetone infiltration. Followed, the specimens were embedded in Spurr resin and sectioned in Leica EM UC7 Manufacturer (Leica, Wetzlar, Germany). The sections were stained with uranyl acetate and alkaline lead citrate, observed and pictured at ×30,000 magnification by Hitachi Model H-7650 TEM. Mitochondrial length was measured.

2.8 Statistical analysis

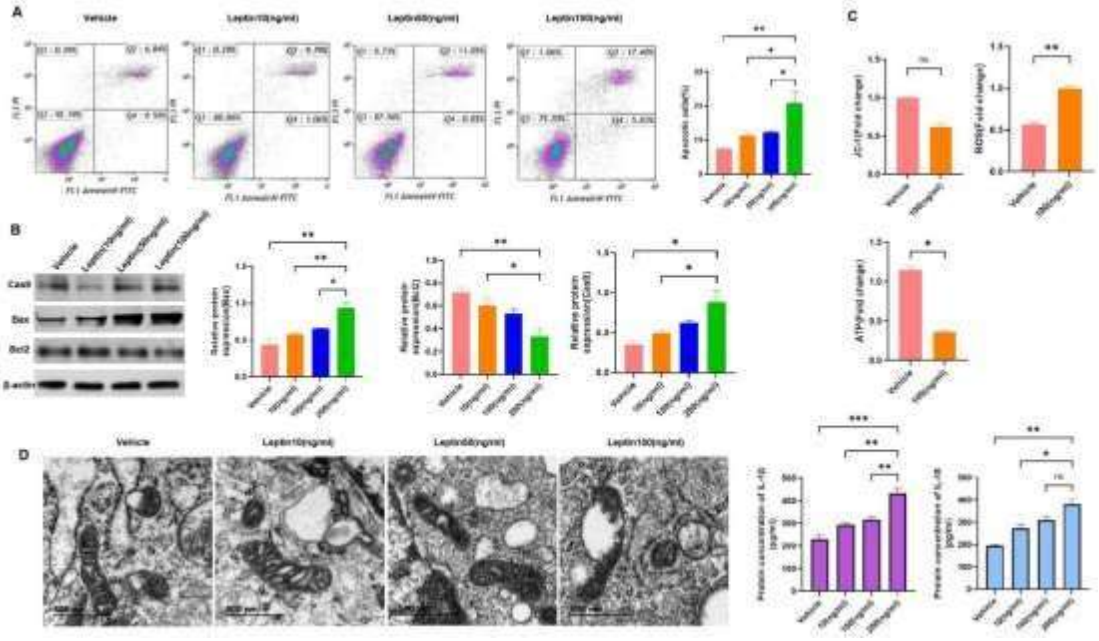
Data of normal distribution were presented as mean± SEM. Student's t-test was used for comparison of two sets of data; for more sets of data comparison, One-way analysis of variance was used and followed by Tukey's post test using SPSS (version 17.0) statistical software. $P<0.05$ was considered as statistically significant. All experiments were run at least three times. The results were analyzed by Graph Pad Prism.

3 Results

3.1 High concentrations of leptin (≥ 10 ng/mL) enhance CEP chondrocytes apoptosis

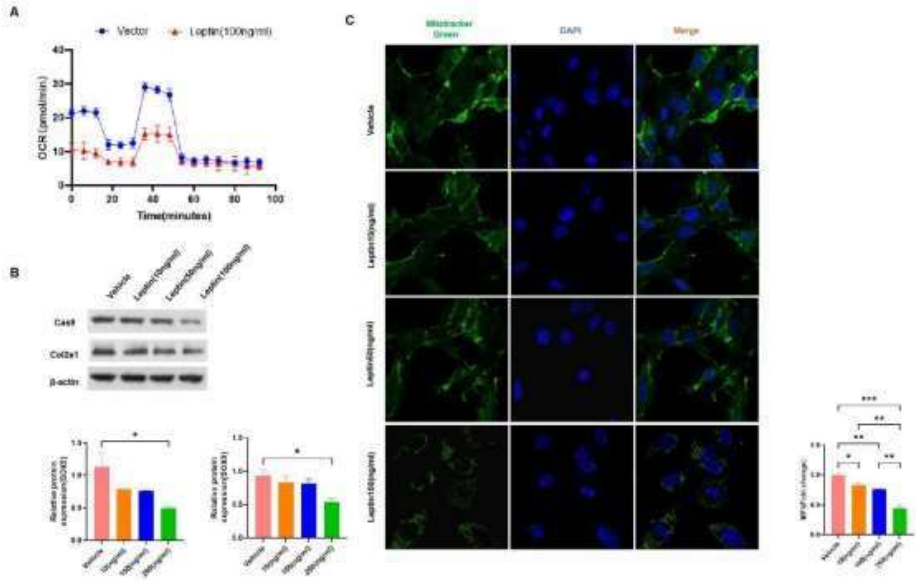
Articular chondrocytes were isolated from 2 weeks old male mice and cultured them in serum-free media. Chondrocytes were treated with different concentrations (0,10,50,100 ng/mL) of leptin for 48 hrs. Apoptosis were measured by flow cytometry. The amounts of apoptotic cells in the low dose treatment group were significantly lower than that in the control groups, and this difference was dose-dependent. However, the effect was significant change after 100ng/mL leptin-treatment (Figure 1A).Wsetern Blot analysis of the key indicators of apoptosis Caspase9,Bax,Bcl2 showed that apoptosis expression was significantly increased when the concentration of search factor was 100ng/mL. The apoptosis of chondrocytes was markedly appeared at the doses of >50 ng/mL.

Consistently, Western blot results showed the cleaved form of caspase-9 increased in the leptin(10-100ng/ml) treatment groups; furthermore, Bax was increased in a dose-dependent manner, whereas the Bcl-2 reduced at the doses of >100 ng/ml ,In addition, ATP,ROS. membrane potential JC-1 were also evaluated for changes related to mitochondrial metabolism (Figure1B). Compared with the control group, the expression of inflammatory factors in leptin-stimulated cells was significantly increased with the increase of dose (Figure1C). Electron microscope observation showed that with the increase of leptin concentration, chondrocyte mitochondrial damage was aggravated and mitochondrial damage appeared (Figure1D).

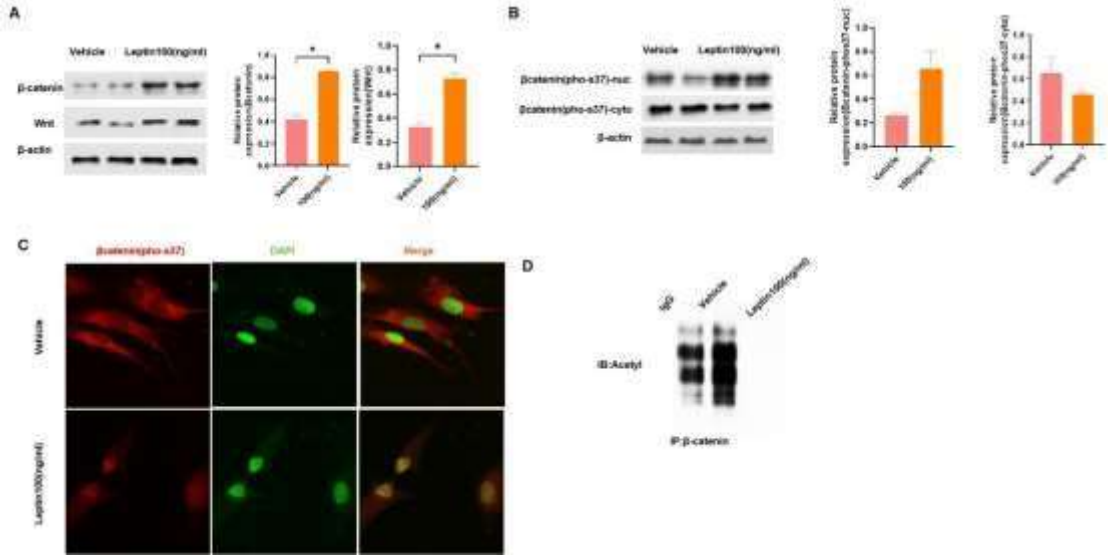


3.2 Active mitochondria are necessary for osteogenesis

To determine which metabolic pathways are used during mitochondrial OxPhos in cartilage, we performed a hippocampal metabolic profile analysis. Using metabolic inhibitors, we deterined the relative contribution of mitochondrial OxPhos. Leptin inhibits mitochondrial OxPh os function. SOX9/Col2a1 are significant markers of chondrogenesis formation. The levels of SOX9 and Col2a1 were increased in the low leptin(0-20ng/mL) groups,and further increas ed after pretreatment with leptin (≥ 100 ng/mL) (Figures 2B); In addition, the cell mitocho ndriaultrastructure changed: the fluorescent staining area of mitotrcker green（Mitochondrial dy） was increased in the High doses of leptin treatment groups compared with controls , this result was reversed in the high-dose groups (Figures 2C). These results indicated that h igh dose of leptin inhibit activity of chondrocytes.



3.3 The role of leptin on βcatenin/Wnt signal pathy way



βcatenin/ WNT plays an important role in the regulation of chondrocytes and osteoblasts (Bo oth et al., 2014). To explore the relationship between leptin and apoptosis upon leptin stimul ation, The expression levels of both βcatenin and WNT proteins were detected at 100ng/m L leptin stimulation, and it was found that the protein scalars of βcatenin and WNT protein s increased significantly during 100ul/Ml leptin stimulation (Figure 3A). In addition, the pho sphorylation of βcatenin at S37 site was significantly increased under leptin stimulation (Fig ure 3B). The phosphorylation of βcatenin increased the nuclear translocation of protein, and the immunofluorescence results consistently observed that Leptin increased the phosphorylati on of βcatenin (Figure 3C). After immunoprecipitation of β-Catenin protein complex under 100ng/mL leptin stimulation with β-Catenin antibody, acetylation antibody detection was perf ormed and it was found that the acetylation modification was enhanced in chondrocytes und er leptin intervention (Figure 3D).These results suggested that Phosphorylation and acetylatio n of βcatenin may play a pivotal role in leptin-induced chondrocytes apoptosis.

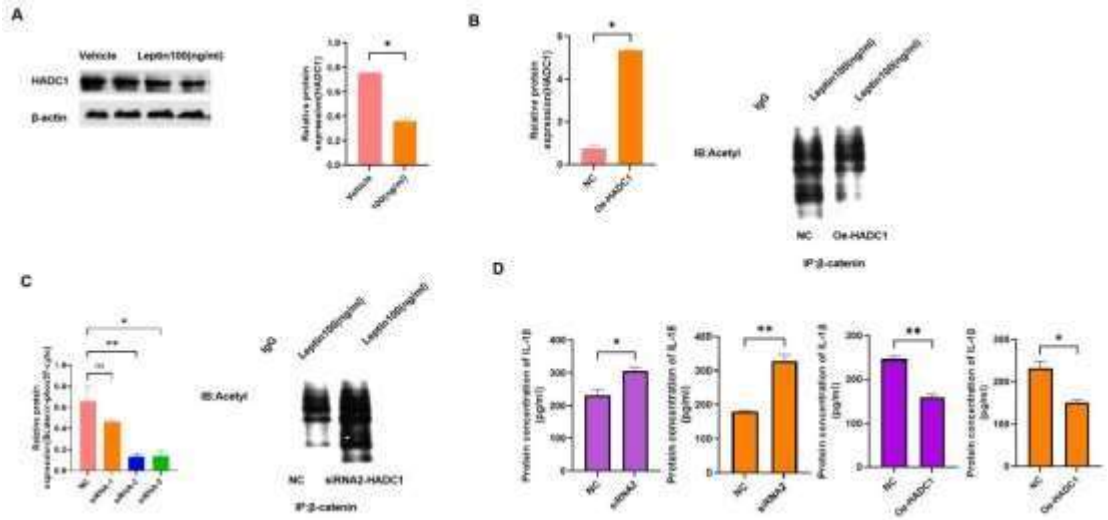
3.4 Leptin regulates the apoptosis of chondrocytes through the deacetylase HADC1 med iated β-catenin deacetylation

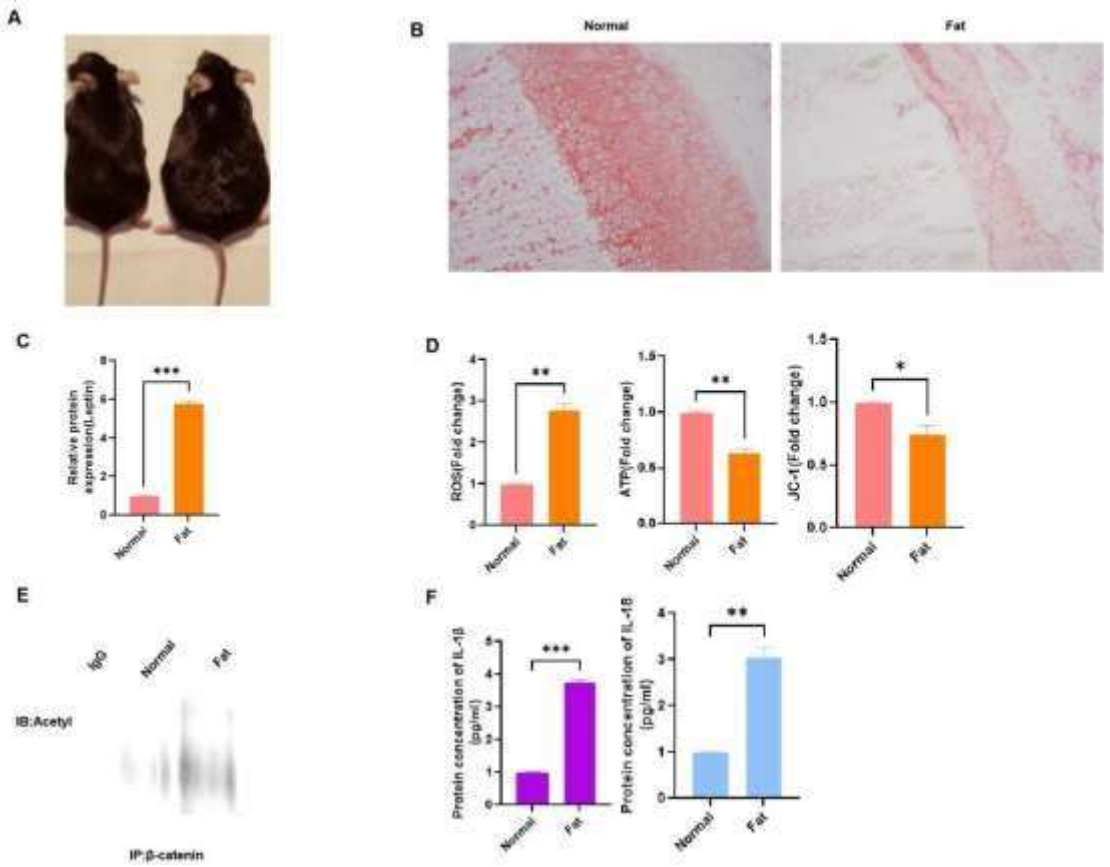
Our data suggest that β-catenin acetylation modification is low in normal chondrocytes (sup

plemental material). However, HDAC1 protein levels were significantly reduced in leptin-me diated chondrocytes (Figure 4A). However, after interfering HADC1 expression by siRNA, d own-regulated HDAC1 led to increased β-catenin acetylation of Lys-49, and increased downs tream β-catenin phosphorylation to promote nuclear translocation (Figure 4B). However, ove rexpression of HDAC1 resulted in decreased β-catenin acetylation level of Lys-49 (Figure 4 C). Chondrocytes with decreased HDAC1 expression activated the β-catenin/WNT signaling pathway under Leptin stimulation. Increased expression of inflammatory cytokines (Figure 4 D). These results suggested that leptin may regulate the activation of inflammatory signals a nd cell death procedures in OA chondrocytes through the regulation of β-catenin deacetylati on mediated by HDAC1.

3.5 CEP chondrocytes deterioration in obese mouse models

We found that in obese mouse models, Serene O staining demonstrated cartilage degeneration in obese models, the results showed that articular chondrocytes were significantly reduced in obese mice (Figure 5B). ELISA analysis for components of Leptin, It was performed on an obese mouse model and found that plasma Leptin content was significantly increased in obese individual mice (Figure 5C). in which a marked increase expression of ROS appeared to decrease in the obesity mice compared with the control mice. In addition, we detected the acetylation level of the protein under immunoprecipitation with anti-β catenin antibody, which was consistent with the results of Leptin stimulation of chondrocytes (Figure 5E). Similarly, the expression of IL-1β/IL-18, an inflammation-related protein, also increased significantly in the plasma of obese mice. In vivo experimental results showed that the increase of leptin level in obese mice led to the increase of β-catenin acetylation level in chondrocytes and stimulated the activation of βcatenin signaling pathway. Regulates the activation of inflammatory signals and cell death programs in OA chondrocytes.





4 Discussion

Our previous study showed that physiological dose of leptin lead to the apoptosis of chondrocytes. In obese individuals, however, we found an increased risk of arthrosis. Thus far, obesity is a major risk factor for intervertebral arthrosis (OA). Obesity substantially increases the risk of the development of OA(Vo et al., 2016). Leptin is a protein-related product of obesity gene, which increases as obesity increases, and more and more evidences show that leptin plays a vital role in chondrocytes growth and development. However, the role of high leptin levels in leading OA was less reported. In this study， In leptin mediated chondrocytes, down-regulated HDAC1 led to increased β-catenin acetylation of Lys-49, and increased downstream β-catenin phosphorylation promoted nuclear translocation and activated the β-catenin/WNT signaling pathway. The activation of mitochondrial dysfunction and cell death programs in OA chondrocytes.

Leptin has been shown to play an important role in apoptosis and senescence (Lee et al., 2011).We also demonstrated the pro-survival effects of leptin in chondrocytes in earlier studies. Recent studies have reported the autophagy regulation effect of leptin in cells. Our results showed that pathological dose of leptin led to high expression of apoptotic proteins and decreased autophagy activity in particular chondrocytes, while physiological dose of leptin had the opposite effect on autophagy and apoptosis.

In this study, we found that leptin activated b-catenin signaling, but did not significantly affect the level of b-catenin protein, suggesting that leptin regulates the modification of b-catenin, which usually includes phosphorylation and acetylation. Our data show that: In the chondrocytes mediated by leptin, leptin down-regulates the expression of HDAC1 in chondrocytes, resulting in increased β-catenin acetylation of Lys-49 and activation of downstream phosphorylation. Nuclear translocation of phosphorylated β-catenin activates the β-catenin/WNT signaling pathway, leading to mitochondrial dysfunction and activation of cell death programs in OA chondrocytes. In addition, mitochondrial function analysis showed that leptin enhanced mitochondrial division, resulting in ROS overproduction, mitochondrial pro-apoptotic protein leakage, and Caspase-9-dependent cell death

pathway activation. However, overexpression of HDAC1 blocks the progression of mitochondrial division while activating chondrocyte pro-survival signaling. At the molecular level, our data further suggest that the Wnt/β-catenin pathway is required for chondrocyte protective mechanisms resulting from HDAC1 overexpression: Inhibition of Wnt/β-catenin pathway by promoting β-catenin deacetylation can reduce mitochondrial fission, metabolic function changes and activation of apoptotic signals in chondrocytes. Overall, our data suggest that obesity-induced osteoarthritis is associated with HDAC1 downregulation, Wnt/β-catenin pathway deacetylation inactivation and mitochondrial dysfunction, and Caspase-9-dependent cell death activation. Based on this, enhancement of HDAC1 activity and inhibition of Wnt/β-catenin signaling pathway to stimulate deacetylation of β-catenin in osteoarthritis chondrocytes can reverse cartilage degeneration caused by mitochondrial dysfunction of chondrocytes.

5 Conclusion

Our findings suggest that leptin may play an important role in articular cartilage. In ob ese individuals, due to the high expression of leptin in the blood, the activity of deacetylas e HADC1 in CEP chondrocytes is inhibited, resulting in changes in the modification level of βcatenin in the βcatenin/WNT signaling pathway, leading to the occurrence of chondrocyt e apoptosis procedures. The findings will help find new treatments for osteoarthritis caused by obesity.

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Competing Interests

The authors have declared that no competing interest exists.

References

1. Alberton P, Dex S, Popov C, Shukunami C, Schieker M, Docheva D (2015) Loss of tenom odulin results in reduced self-renewal and augmented senescence of tendon stem/ progenitor c ells. Stem Cells Dev 24(5): 597-609. https://10.1089/scd.2014.0314.
2. Alkhatib B, Rosenzweig DH, Krock E, Roughley PJ, Beckman L, Steffen T,... Haglund L (2014) Acute mechanical injury of the human intervertebral disc: Link to degeneration and pain. Eur Cell Mater 28: 98-110, 110-1. https://10.22203/ecm.v028a08.
3. Andersson GB (1999) Epidemiological features of chronic low-back pain. Lancet 354 (9178): 581-5. https://10.1016/S0140-6736(99)01312-4.
4. Basu S, Rajakaruna S, Reyes B, Van Bockstaele E, Menko AS (2014) Suppression of MAP K/JNK-MTORC1 signaling leads to premature loss of organelles and nuclei by autophagy d uring terminal differentiation of lens fiber cells. Autophagy 10(7): 1193-211. https://10.4161/ auto.28768.
5. Bergknut N, Auriemma E, Wijsman S, Voorhout G, Hagman R, Lagerstedt AS,... Meij BP (2011) Evaluation of intervertebral disk degeneration in chondrodystrophic and nonchondrody strophic dogs by use of Pfirrmann grading of images obtained with low-field magnetic reso nance imaging. Am J Vet Res 72(7): 893-8. https://10.2460/ajvr.72.7.893.
6. Booth LA, Tavallai S, Hamed HA, Cruickshanks N, Dent P (2014) The role of cell signalli ng in the crosstalk between autophagy and apoptosis. Cell Signal 26(3): 549-55.

https://10.1 016/j.cellsig.2013.11.028.
7. Chan SC, Ferguson SJ, Gantenbein-Ritter B (2011) The effects of dynamic loading on the i ntervertebral disc. Eur Spine J 20(11): 1796-812. https://10.1007/s00586-011-1827-1.
8. Chan WC, Sze KL, Samartzis D, Leung VY, Chan D (2011) Structure and biology of the i ntervertebral disk in health and disease. Orthop Clin North Am 42(4): 447-64. https://10.101 6/j.ocl.2011.07.012.
9. Chen N, Karantza-Wadsworth V (2009) Role and regulation of autophagy in cancer. Biochi m Biophys Acta 1793(9): 1516-23. https://10.1016/j.bbamcr.2008.12.013.
10. Deng X, Ruvolo P, Carr B, May WJ (2000) Survival function of ERK1/2 as IL-3-activated, staurosporine-resistant Bcl2 kinases. Proc Natl Acad Sci U S A 97(4): 1578-83. https://10.1 073/pnas.97.4.1578.
11. Dixon AE, Holguin F, Sood A, Salome CM, Pratley RE, Beuther DA,... Shore SA (2010) An official American Thoracic Society Workshop report: Obesity and asthma. Proc Am Tho rac Soc 7(5): 325-35. https://10.1513/pats.200903-013ST.
12. Fan S, Zhang B, Luan P, Gu B, Wan Q, Huang X,... Liu J (2015) PI3K/ AKT/ mTOR/ p70S 6K pathway is involved in Abeta25-35-Induced autophagy. Biomed Res Int 2015: 161020. h ttps://10.1155/2015/161020.
13. Freemont AJ (2009) The cellular pathobiology of the degenerate intervertebral disc and disc ogenic back pain. Rheumatology (Oxford) 48(1): 5-10. https://10.1093/rheumatology/ken396.
14. Herrick JE, Panza GS, Gollie JM (2016a) Leptin, leptin soluble receptor, and the free leptin index following a diet and physical activity lifestyle intervention in obese males and femal es. J Obes 2016: 8375828. https://10.1155/2016/8375828.
15. Herrick JE, Panza GS, Gollie JM (2016b) Leptin, leptin soluble receptor, and the free lepti n index following a diet and physical activity lifestyle intervention in obese males and fem ales. J Obes 2016: 8375828. https://10.1155/2016/8375828.
16. Huang L, Li C (2000) Leptin: A multifunctional hormone. Cell Res 10(2): 81-92. https://10. 1038/sj.cr.7290038.
17. Le Maitre CL, Freemont AJ, Hoyland JA (2007) Accelerated cellular senescence in degener ate intervertebral discs: A possible role in the pathogenesis of intervertebral arthrosis. Arthri tis Res Ther 9(3): R45. https://10.1186/ar2198.
18. Lee YH, Magkos F, Mantzoros CS, Kang ES (2011) Effects of leptin and adiponectin on p ancreatic beta-cell function. Metabolism 60(12): 1664-72. https://10.1016/j.metabol.2011.04.00 8.
19. Levine B, Klionsky DJ (2004) Development by self-digestion: Molecular mechanisms and bi ological functions of autophagy. Dev Cell 6(4): 463-77. https://10.1016/s1534-5807 (04) 00099- 1.
20. Lockshin RA, Zakeri Z (2004) Apoptosis, autophagy, and more. Int J Biochem Cell Biol 3 6(12): 2405-19. https://10.1016/j.biocel.2004.04.011.
21. Luoma K, Riihimaki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A (2000) Low back pain in relation to lumbar arthrosis. Spine (Phila Pa 1976) 25(4): 487-92. https:// 10.1097/00007632-200002150-00016.
22. Magnier C, Boiron O, Wendling-Mansuy S, Chabrand P, Deplano V (2009) Nutrient distrib ution and metabolism in the intervertebral disc in the unloaded state: A parametric study. J Biomech 42(2): 100-8. https://10.1016/j.jbiomech.2008.10.034.
23. Matarese G, Moschos S, Mantzoros CS (2005) Leptin in immunology. J Immunol 174(6): 3 137-42. https://10.4049/jimmunol.174.6.3137.
24. Mizushima N, Levine B, Cuervo AM, Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. Nature 451(7182): 1069-75. https://10.1038/nature06639.
25. Mobasheri A (2002) Role of chondrocyte death and hypocellularity in ageing human articula r cartilage and the pathogenesis of osteoarthritis. Med Hypotheses 58(3): 193-7.

https://10.10 54/mehy.2000.1180.
26. Modic MT, Ross JS (2007) Lumbar degenerative disk disease. Radiology 245(1): 43-61. htt ps://10.1148/radiol.2451051706.
27. Ogier-Denis E, Codogno P (2003) Autophagy: A barrier or an adaptive response to cancer. Biochim Biophys Acta 1603(2): 113-28. https://10.1016/s0304-419x(03)00004-0.
28. Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D,... Ozcan U (2009) Endoplasmic retic ulum stress plays a central role in development of leptin resistance. Cell Metab 9(1): 35-51. https://10.1016/j.cmet.2008.12.004.
29. Poole AR (2012) Osteoarthritis as a whole joint disease. HSS J 8(1): 4-6. https://10.1007/s1 1420-011-9248-6.
30. Rihn JA, Kurd M, Hilibrand AS, Lurie J, Zhao W, Albert T, Weinstein J (2013) The influ ence of obesity on the outcome of treatment of lumbar disc herniation: Analysis of the Spi ne Patient Outcomes Research Trial (SPORT). J Bone Joint Surg Am 95(1): 1-8. https://10. 2106/JBJS.K.01558.
31. Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR (2003a) Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. Ob es Res 11(6): 760-8. https://10.1038/oby.2003.106.
32. Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR (2003b) Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. Ob es Res 11(6): 760-8. https://10.1038/oby.2003.106.
33. Santoro A, Mattace RG, Meli R (2015) Drug targeting of leptin resistance. Life Sci 140: 6 4-74. https://10.1016/j.lfs.2015.05.012.
34. Tartaglia LA (1997) The leptin receptor. J Biol Chem 272(10): 6093-6. https://10.1074/jbc.2 72.10.6093.
35. Vo NV, Hartman RA, Patil PR, Risbud MV, Kletsas D, Iatridis JC,... Kang JD (2016) Mol ecular mechanisms of biological aging in intervertebral discs. J Orthop Res 34(8): 1289-306. https://10.1002/jor.23195.
36. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional clo ning of the mouse obese gene and its human homologue. Nature 372(6505): 425-32. https:// 10.1038/372425a0.

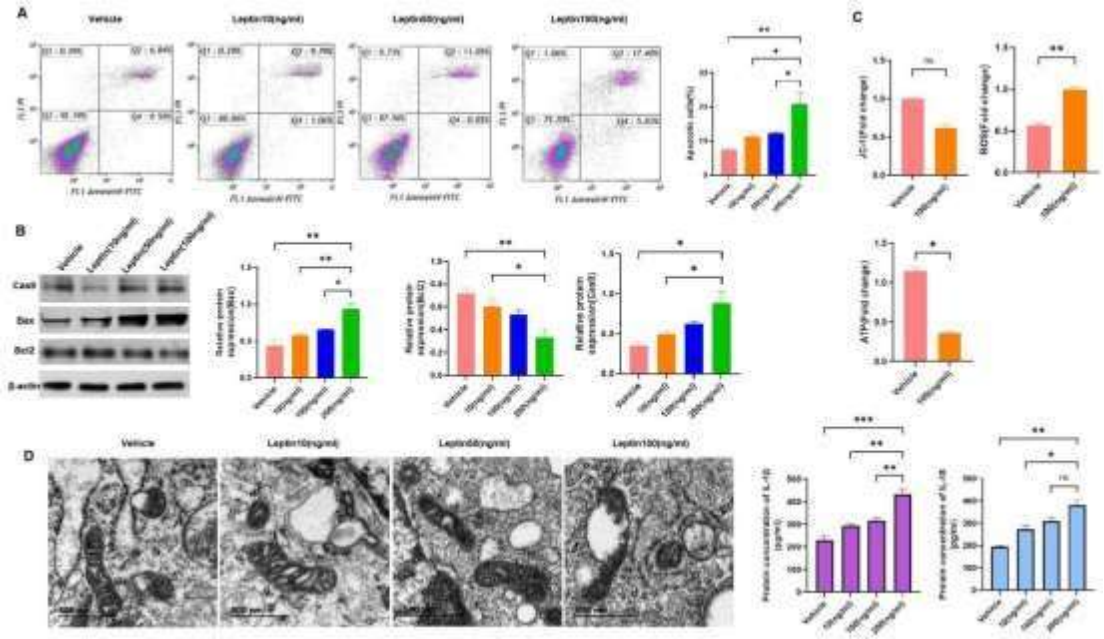

Figure 1 The apoptosis of chondrocytes increased upon high doses of leptin (A) Annexin V/PI staining and cell cycle was analyzed by FACS for cell apoptosis and necrosis at different doses. (B) Apoptosis related proteins Caspase 9/Bcl2/Bax expression level of whole-cell protein

lysates was measured by western blot, and β-actin served as a loading control.(C) Changes in oxidative stress ROS, membrane potential JC-1, and cellular energy ATP in mitochondrial metabolism. (D) Transmission electron microscopy was used to observe the mitochondrial structure in chondrocytes treated with different leptin doses. (E) ELISA was used to detect the expression of inflammatory proteins in chondrocytes stimulated with different leptin doses (48hr).

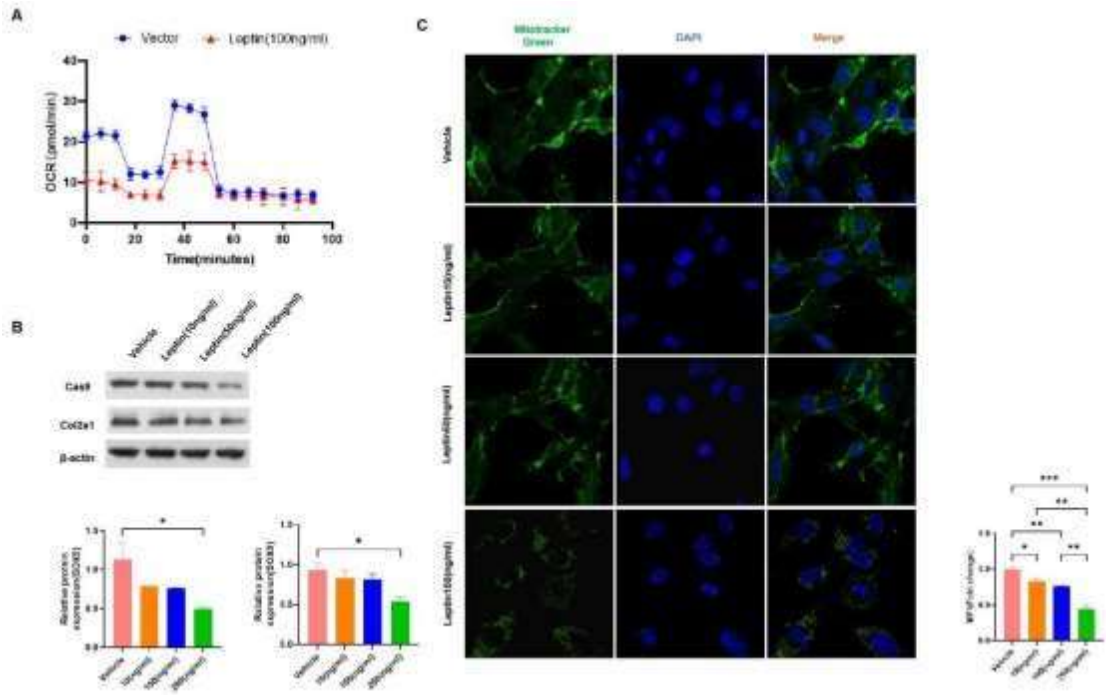

Figure 2 Influence of different doses of leptin on mitochondrial activity of CEP chondr ocytes Mitochondrial energy metabolism and mitochondrial biological changes after treatment of ch ondrocytes with different doses of leptin.(A) Effect of 100ng/mL leptin on mitochondrial me tabolism OCAR in chondrocytes.(B)Western blotting was performed to assess SOX9and Col2 a1 protein levels in cells, βactin was used as the loading control. (C) The changes of mito chondrial dye Mitotracker Green were detected by immunofluorescence.

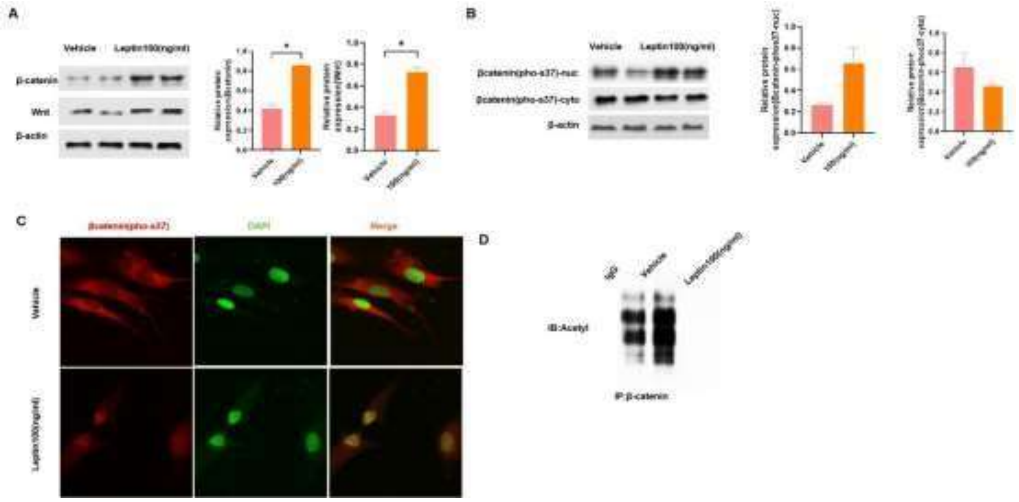

Figure 3 Effect of βcatenin/WNT Pathway on leptin treatment chondrocytes βcatenin/ WNT plays an important role in the regulation of chondrocytes and osteoblasts. (A) Comparing the vehicle groups, βcatenin/WNT protein of the leptin groups was significantly increased.

(B) Comparing with the leptin (100 ng/ml) group, the phosphorylation level of the vehicle group was significantly reduced.C) Phosphorylation immunofluorescence staining of the vehicle group. these

results indicate that leptin regulates apoptosis through its effects on autophagy.

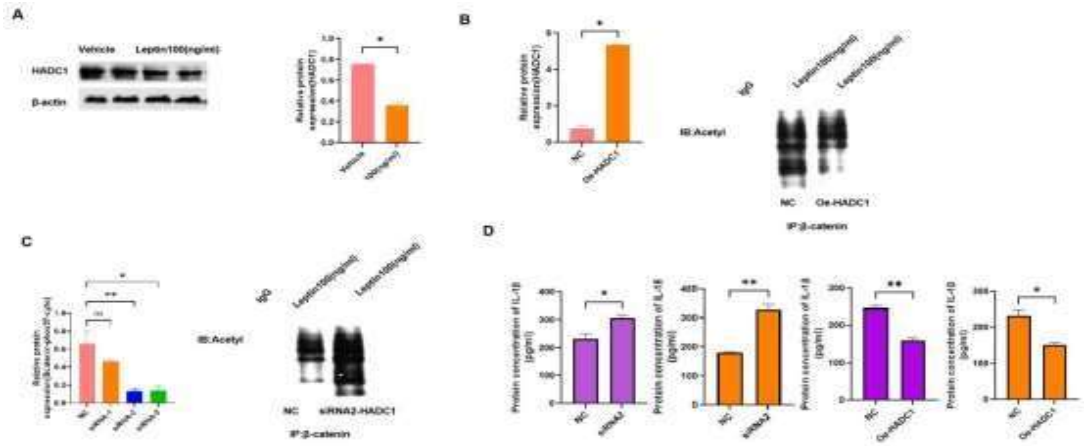

Figure 4 Effect of HADC1 Pathway on leptin treatment chondrocytes (A)HADC1 expression level of whole-cell protein lysates was measured by western blot, and beta- actin served as a loading control.(B) Changes in acetylation level of chondrocytes with over expression of HADC1 under 100ng/mL leptin.(B) Changes in acetylation level of chondrocytes with siRNA knockdown of HADC1 under 100ng/mL leptin. ELISA was used to detect the expression of inflammatory protein in chondrocytes (48hr) stimulated by different doses of leptin.(A)

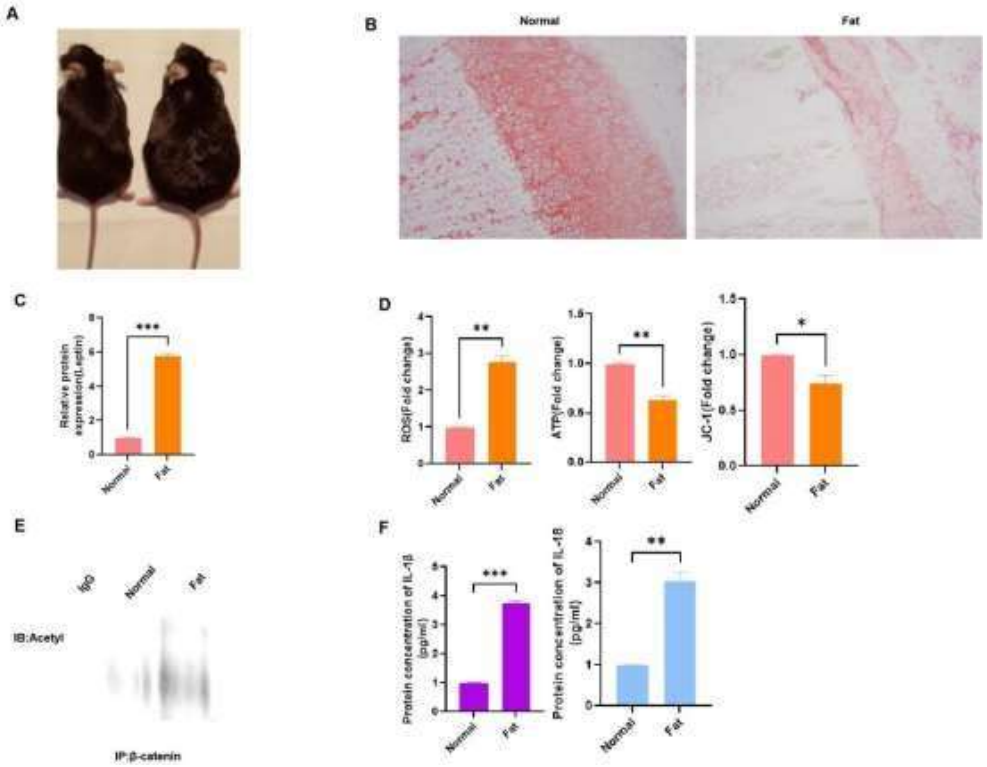

Figure 5. Leptin effects on obese mouse models. (A) High-fat diet obese mouse models (right). (B)Safranin O staining was used to visualize the chondrocytes distribution. (C) The le vel of plasma leptin in mice. (D) The fraction of C h a n g e s in oxidative stress ROS, memb rane potential JC-1, and cellular energy ATP in mitochondrial metabolism.. (F)The relative expression of IL-1β and IL-18 in the obese mice.