Irisin recovers osteoarthritic chondrocytes: a muscle-cartilage cross-talk boosted by physical activity

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Abstract
Background Physical exercise favors weight loss and ameliorates both articular pain and function in patients suffering from osteoarthritis (OA). Irisin, a myokine released by skeletal muscles upon muscle contraction, has demonstrated to yield anabolic effects on different cell types. The study aimed to investigate the effect of irisin on human osteoarthritic chondrocytes (hOAC) in vitro. The hypothesis of this study was that irisin would improve hOAC metabolism and proliferation.

Methods hOAC were isolated from osteochondral tissues of 5 patients undergoing total knee joint replacement. Cells were cultured in growing media and then exposed to either phosphate-buffered saline (control group) or human recombinant irisin (experimental group). Cell proliferation (Picogreen assay), glycosaminoglycan content (dimethylmethylene blue), type II/X collagen gene expression (Real-Time polymerase chain reaction) and quantification (Western blot and densitometric analysis), p38/ERK MAPK and Akt involvement (Western blot and densitometric analysis) were evaluated in both groups.

Results Irisin increased hOAC proliferation (p < 0.001) and both type II collagen gene expression (p < 0.001) and protein levels (p < 0.01), while decreased type X collagen gene expression (p < 0.05) and protein levels (p < 0.001). These effects seemed to be mediated by the inactivation of the p38 MAPK and PI3K-Akt intracellular pathways, as irisin reduced phosphorylated p38 (p-p38), (p < 0.01) and phosphorylated Akt (p-Akt) (p < 0.001) protein levels.

Conclusion Irisin stimulated cell proliferation and anabolism in hOAC through p38 MAPK and PI3K-Akt inactivation in vitro, demonstrating for the first time the existence of a cross-talk between muscle and cartilage.

Background
Osteoarthritis (OA) is a degenerative joint disorder affecting more than 10% of adults older than 60 years of age. It is characterized by increasing joint pain and stiffness often leading to disability, with a tremendous negative impact on patients’ overall functionality and quality of life, as well as on healthcare expenditure\(^1\). Predominant features are articular cartilage damage and thinning, which are associated with chondrocyte hypertrophy, tissue inflammation and extracellular matrix (ECM)
degradation\textsuperscript{2-4}. Major risk factors for OA include genetic predisposition, female gender, joint injury and obesity\textsuperscript{5}. Apart from mechanical overloading, obesity appears to further impact on OA pathogenesis through the secretion of proinflammatory adipokines involved in cartilage degradation, synovial inflammation and osteophytes development\textsuperscript{6}. Weight loss and an active lifestyle are essential to reduce the risk of developing OA\textsuperscript{7} and improve joint pain and stiffness in patients already affected with knee\textsuperscript{8,9} and hip OA\textsuperscript{10}. Irisin is a myokine that is secreted into the serum by skeletal muscle after physical exercise\textsuperscript{11}. It was early recognized for its effects on glucose and fat metabolism, favoring thermogenesis and raising energy expenditure\textsuperscript{12}. These pleiotropic effects could explain the benefits of muscle training in numerous metabolic disorders including obesity, metabolic syndrome and diabetes\textsuperscript{13}. Palermo et al. recently showed that osteoporotic fractures were associated with lower irisin serum levels, independently of other factors\textsuperscript{14}. These data were supported by in vitro studies demonstrating that irisin can directly target osteoblasts and promote cell proliferation, differentiation and matrix mineralization via the p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinase (ERK) signaling pathways\textsuperscript{15}. Therefore, increasing evidence supports the role of skeletal muscle as an endocrine organ capable of secreting a wide range of myokines which communicate with other tissues and organs\textsuperscript{16}. In this regard, irisin may act as a messenger between muscle and bone during physical exercise\textsuperscript{17}. We hypothesized that irisin might maintain cartilage homeostasis through physical exercise acting as a cross-talk mechanism between muscle and cartilage. This novel idea is supported by a recent study reporting that serum and synovial fluid levels of irisin are negatively correlated with the severity of knee OA\textsuperscript{18}. In this study, we isolated human osteoarthritic chondrocytes (hOAC) from specimens obtained during total knee replacement procedures. hOAC were then cultured in presence of either recombinant irisin (r-irisin) or Dulbecco’s phosphate-buffered saline (DPBS) and evaluated for cell proliferation, glycosaminoglycan (GAG) production, type II and X collagen gene expression and
protein synthesis. Additional analyses were performed to assess p38 MAPK, ERK and PI-3 kinase-Akt pathways involvement.

Methods

Cell isolation

All experiments were carried out in accordance with relevant guidelines and regulations approved by the Ethics Committee of Campus Bio-Medico University of Rome. hOAC were isolated from osteochondral tissues of five patients (n = 5) undergoing elective total knee joint replacement (Table 1). Informed consent was obtained from each subject. The age of the patients ranged from 58 to 85 years and knee OA severity was assessed using the Kellgren-Lawrence classification (grades 3 and 4). hOAC were isolated according to a standardized procedure\textsuperscript{19}. Specimens were minced and digested for 90' at 37 °C with gentle agitation in sterile Dulbecco’s Modification of Eagle’s Medium (DMEM; Corning) containing 1% penicillin/streptomycin (P/S; Sigma), 5% fetal bovine serum (FBS; Corning) and 0.2% pronase (Calbiochem). The remaining tissue was washed and digested overnight in DMEM with 1% P/S, 5% FBS, and 0.02% collagenase type II (Worthington). The digest was filtered through a 70-µm pore size nylon mesh, the cells washed, resuspended in DMEM with 10% FBS and 1% P/S, and incubated at 37 °C in a humidified atmosphere of 5% CO\textsubscript{2}. The culture media were changed twice weekly and cultures were allowed to grow until reaching 80–90% confluence. Passage 1-hOAC were used for the experiments.

Dose-Response Relationships

After trypsinization (Corning) and washing, $2.5 \times 10^5$ hOAC were resuspended in culture media and centrifuged at low speeds (2000 g) for 5' to form aggregates\textsuperscript{20}. Micromasses were treated either with DPBS (Euroclone) (Ctrl) or r-irisin (Sigma) for 7 days at a concentration of 25, 50, 75, and 100 ng/mL, which is the range between intraarticular\textsuperscript{18} and blood concentrations in humans\textsuperscript{15,21}. Media were changed three times during the week of culture. At the end of the experiment, micromasses were directly used to assess GAG content normalized to DNA as following. Micromasses were washed with PBS and digested with 100 µl of papain (Sigma) solution (0.25 mg/ml in 50 mM phosphate buffer, pH 6.5 containing 5 mM cysteine–hydrochloride and 5 mM ethylenediaminetetraacetic acid) overnight.
with gentle shaking at 65 °C. GAGs were measured by reaction with 1,9-dimethylmethylene blue (DMMB; Polysciences) using chondroitin sulfate (Sigma) as a standard. Measurements of absorption were performed at a wavelength of 530 nm (Tecan Infinite M200 PRO).

DNA content was assessed using PicoGreen Assay (Invitrogen) as described by the manufacturer’s guidelines on cells extracts. A standard curve based on known concentration of DNA was used to determine the DNA content. The sample fluorescence was measured using a microplate reader (Tecan Infinite M200 PRO) at 460 nm and 540 nm wavelengths respectively. Data were expressed as quantity of GAG normalized to DNA content of chondrocytes cultured in growing media for 7 days, comparing the percent variation between the control group and the experimental group.

**Cell proliferation**

Micromasses were treated either with DPBS (Ctrl) or r-irisin (Sigma) at a concentration of 25 ng/mL (concentration chosen after dose-response assay) for 14 days. At at 4, 10 and 14 days of culture DNA content was assessed using PicoGreen assay as described above. The assay was performed in triplicate for each donor. The total number of cells in the sample was determined by converting the total DNA to cell number using the conversion factor of 7.7 pg DNA/cell = 6 ng DNA = 1 x 10^3 cells as previously reported. Results were expressed as viable cells/mL.

**RNA extraction and gene expression analysis**

Micromasses were treated either with DPBS (Ctrl) or r-irisin (Sigma) at a concentration of 25 ng/mL. Total RNA was extracted from pellets after 7 days of culture using the TRlZol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was produced using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's instructions. mRNA levels were measured through qRT-PCR using TaqMan Gene Expression Assays and Taqman Universal Master Mix II with UNG-Real Time PCR System Instrument 7900HT FAST according to manufacturer’s instructions. Gene expression assays collagen X (Hs00166657), collagen 2A1 (Hs00264051) and GAPDH (Hs03929097) were used. The expression level of each gene has been normalized to the expression of GAPDH and calculated as 2^{-ΔΔCt}. Values in the experimental group were normalized to expression levels encountered in the control group, which was considered as a baseline. Reagents
were purchased from Applied Biosystems.

Protein extraction and Western Blot analysis
Micromasses were treated with r-irisin (25 ng/ml) or PBS (Ctrl) either for 7 days or at 10’, 20’, and 1 h time points. Subsequently, protein extraction and Western Blot analyses were performed. Cell lysates were obtained using radioimmunoprecipitation assay buffer (RIPA buffer; Sigma) for 30’ on ice, cleared by centrifugation for 30’ at 12000 g at 4 °C for 30’ and quantified using detergent compatible (DC) protein assay kit (Bio-Rad). Total protein extracts (20 µg) from each sample were loaded on 4–12% SDS-PAGE gels, transferred onto nitrocellulose membranes through the Trans-Blot Turbo Transfer System (Bio-Rad) and incubated in a blocking buffer (TBST 1X with 5% non-fat dry milk) for one hour. Membranes were incubated with primary antibody overnight shaking at 4 °C in TBST 1X with 1% non-fat dry milk. Anti-p38 (rabbit, 1:1000, Cell Signaling), anti-phospho p38 Thr180/Tyr182 (rabbit, 1:1000, Cell Signaling), anti-phospho p44/42 ERK1/2 (rabbit, 1:1000, Cell Signaling), anti-phospho p44/42 ERK1/2 Thr202/Tyr204 (rabbit, 1:2000, Cell Signaling), anti-AKT (rabbit, 1:1000, Cell Signaling, anti-phospho AKT (rabbit, 1:1000, Cell Signaling), anti-Coll 2A1 (mouse, 1:500, Novus Biologicals, anti-Coll X (rabbit, 1:300, Abcam), anti-GAPDH (rabbit, 1:1000, Cell Signaling) were used. Anti-rabbit/mouse HRP-conjugated antibody (1:10000, Abcam) was used and the chemiluminescence signal detected using ChemiDoc (Bio-Rad) and Quantity One software (Bio-Rad) to quantify the signal intensity of different bands. Relative p-p38, p-ERK and p-AKT expression was estimated upon normalization to their respective unphosphorylated protein (p38, ERK and AKT).

Statistical analysis
All quantitative data are expressed as means ± SD. The statistical analysis of the results was performed using one-way analysis of variance (ANOVA) with Dunnett’s post-test and two-tailed t test where applicable. Statistical significance was set as p < 0.05 (*), p < 0.01 (**) and p < 0.001 (***)

Statistical analysis was done using Prism 7 (GraphPad, San Diego, CA, USA). Each experiment was repeated at least three times and representative experiments are shown.

Results
Irisin promotes GAG production by OA chondrocytes
Three-dimensional cell cultures (n = 4) treated with different concentrations of r-irisin (25, 50, 75 and
100 ng/mL) showed a significant increase in GAG synthesis normalized to DNA compared to the control cell cultures (Fig. 1) at the lowest concentration (25 ng/mL). Considering the GAG/DNA ratio in the control group as a baseline of 100%, hOAC exposed to 25 ng/mL irisin showed approximately a 3-fold increase of GAG/DNA ratio (278.38 ± 80.33%; p < 0.001). Although showing an increase in GAG content, treatment with higher doses of r-irisin did not reach statistical significance (125.15 ± 18.34, 159.26 ± 43.02, 144.51 ± 55.37 corresponding to 50, 75 and 100 ng/mL r-irisin, respectively).

Irisin enhances hOAC proliferation
Treating hOAC with 25 ng/mL r-irisin resulted in a significant increase in cell proliferation at 4, 10 and 14 days after starting three-dimensional cell culture (n = 4; Fig. 2). At day 4, exposure to r-irisin led to a 12% (62.59 ± 0.76 · 10^3 cells/mL) increase in cell proliferation compared to the control group (50.53 ± 0.98 · 10^3 cells/mL; p < 0.001). After 10 days of r-irisin treatment, the experimental group contained 105.5 ± 8.48 · 10^3 cells/ml while control hOAC cultured with DPBS had 75.02 ± 1.64 · 10^3 cells/ml (p < 0.001). At day 14, the mean hOAC number after r-irisin exposure still remained significantly higher (137.2 ± 1.24 · 10^3 cells/ml) in comparison to the control group (101.8 ± 3.21 · 10^3 cells/ml; p < 0.001).

Irisin restored the normal ECM gene expression profile of hOAC
Irisin treatment resulted in the increase of mRNA expression of type II collagen (Fig. 3A): the relative mRNA expression level of this gene was 12.94 ± 2.283 in the experimental group compared to controls (n = 5; p < 0.001). We also found a significant decreased mRNA expression of the hypertrophic chondrocyte-related gene encoding type X collagen (Fig. 3A): the mRNA expression level was 0.474 ± 0.315 in the r-irisin group compared to the control group (p < 0.001).

We confirmed these data by quantifying the gene product synthesis using Western Blot (Fig. 3B). Irisin increased the protein levels of type II collagen and decreased the levels of type X collagen after 7 days of exposure (n = 3). These results were confirmed by densitometric analysis of protein bands (Fig. 3C). Indeed, relative type II collagen expression normalized to GAPDH expression was 7.750 ± 1.422 in the control group, while r-irisin exposure increased this ratio to 54.11 ± 9.924 after 7 days (p = 0.009). Conversely, relative type X collagen expression normalized to GAPDH expression was 1.290
± 0.149 in the control group, whereas r-irisin treatment diminished the ratio to 0.722 ± 0.091 after 7 days (p = 0.031).

Irisin mitigates OA-related changes via the p38 MAPK and Akt signaling pathways

A decreased amount of phosphorylated p38 (p-p38) and phosphorylated Akt (p-Akt) in hOAC was detected by Western Blot from 10, 20, and 60 minutes after treatment with r-irisin (n = 3; Fig. 4A). The decreased phosphorylation of p38 and Akt was statistically significant, as confirmed by densitometry (Fig. 4B). p-p38 relative protein expression was 1.072 ± 0.1084 in the control group, 1.082 ± 0.085 at 10 minutes, 0.918 ± 0.027 at 20 minutes, and 0.634 ± 0.054 after 60 minutes of r-irisin exposure (p < 0.001). Regarding p-Akt, relative protein expression was 8.501 ± 0.4955 in the control group, 6.309 ± 0.0258 at 10 minutes (p < 0.001), 2.451 ± 0.4163 after 20 minutes (p < 0.001) and 1.466 ± 0.220 at 60 minutes (p < 0.001). Conversely, p-ERK protein levels did not show significant changes.

Discussion

In this study we report for the first time that irisin can directly target hOAC and promote cell proliferation and GAG and type II collagen synthesis, while reducing type X collagen expression through inactivation of p38 MAPK and Akt signaling pathways. This is the first study showing that irisin can directly act on chondrocytes and attenuate OA-related cartilage degeneration in vitro, suggesting the existence of a cross-talk mechanism between muscle and cartilage. Irisin is secreted by skeletal muscle in response to physical exercise and may theoretically promote chondrocyte anabolism so that cartilage can better adapt to increased load and friction during prolonged exercise. While irisin first reported effect was to promote adipocyte transdifferentiation and energy metabolism, irisin-induced proliferation, differentiation and anabolic effects were also observed with other cell types, including osteoblasts, bone marrow stromal cells, and human umbilical vein endothelial cells. Recent research efforts have described the wide biological activity of such myokine, whose effects are pleiotropically exerted on several organs, namely the brain, the pancreas, the liver, the bone and the skeletal muscle. Our data expands the knowledge
base for irisin, reporting its role in promoting chondrocyte anabolism. We tested the anabolic effects of irisin by treating primary hOAC in a three-dimensional culture system with r-irisin for 7 days. As irisin effect on articular chondrocytes has not been reported before, we performed a dose-response experiment to assess the most effective concentration on GAG synthesis by using increasing doses within a range including intraarticular\textsuperscript{18} and serum irisin concentration\textsuperscript{15,21} as reported by previous studies. Our results showed that irisin increased the expression of type II collagen while reducing the expression of type X collagen, a marker of chondrocyte hypertrophy in osteoarthritic cartilage. In addition, we demonstrated that irisin was able to increase hOAC proliferation at all considered timepoints by disabling the PI3K-Akt pathways which plays a role in cartilage anabolic as well as catabolic processes in response to the activation of inflammatory processes of various origins. The reduced number of chondrocytes within osteoarthritic cartilage reduces the capacity of the tissue to counteract exogenous stresses and to maintain the original ECM composition. In this regard, increasing chondrocyte proliferation would enhance cartilage metabolism and capacity to react to stressful stimuli.

Our findings have demonstrated that irisin promotes chondrocyte anabolism by inhibiting the phosphorylation of p38 and Akt. Thus, we suggest that p38 and Akt signaling pathways may play a critical role in the chondrogenic effect of irisin.

p38 and ERK signaling pathways are crucial to cell proliferation and differentiation\textsuperscript{30} and may be the main pathways mediating irisin effects. Indeed, irisin can stimulate browning of white adipocytes through p38 and ERK MAPK\textsuperscript{31}, promotes human umbilical vein endothelial cell proliferation through the ERK signaling pathway\textsuperscript{32} and osteoblast proliferation and differentiation via activating the phosphorylation of p38 and ERK\textsuperscript{15}. Moreover, these pathways have been directly implied in OA pathogenesis. In osteoarthritic cartilage, excessive amounts of basic fibroblast growth factor are released upon mechanical loading and activate several transduction pathways involving different MAPK, including ERK and p38. This ultimately leads to upregulation of metalloproteinases, namely ADAMTS-5 and MMP-13, resulting in type II collagen degradation and aggrecan fragmentation\textsuperscript{33}. 
Furthermore, p38 seems to be involved in promoting chondrocyte hypertrophy and apoptosis, inhibiting cartilage synthesis and downregulating chondrocyte autophagy\textsuperscript{34}. Extensive studies have revealed the function of Akt pathway in chondrocytes during endochondral ossification. Deletion of Akt1 results in delayed calcification\textsuperscript{35}, while Akt activation in embryonic chondrocytes promotes chondrocyte proliferation and inhibits hypertrophic differentiation\textsuperscript{36}. However, the in vivo function of Akt signaling in the maintenance of articular cartilage homeostasis and in OA development is largely undefined, with different in vitro studies reporting contradictory results. PI3K/Akt signaling has been shown to play a chondroprotective role by regulating chondrocyte survival, proliferation and extracellular matrix synthesis\textsuperscript{37,38}. In contrast, some studies have reported a detrimental effect of PI3K/Akt pathway on OA, which might be achieved through transduction of procatabolic stimuli or inhibition of articular chondrocyte autophagy\textsuperscript{39,40}. Our findings demonstrated that increased cell proliferation and ECM anabolism in chondrocytes treated with irisin were associated with a reduction of p-Akt and thus with a downregulation of the PI3K/Akt pathway. This is consistent with previous data regarding PI3K/Akt pathway involvement in irisin signaling on distinct cell types\textsuperscript{41}, although other studies reported increased levels of p-Akt\textsuperscript{42}. Contrariwise, no significant change in ERK activity was recorded in this study. This suggest that ERK and PI3K/Akt role in irisin signaling is probably cell-specific and conditioned by local stimuli.

Physical training yields recognized benefits in preserving joints health and is one of the main conservative approaches for preventing and treating OA\textsuperscript{43}. Exercise, by strengthening periarticular muscles along with general aerobic conditioning can improve joint stability, reduce pain and ameliorate quality of life\textsuperscript{8,10}. Moreover, the administration of physiological dynamic loads, as during physical exercise, enhances the production of ECM components, including collagens, proteoglycans and oligomers by articular chondrocytes\textsuperscript{44}. Conversely, disuse and limited movement due to severe illness, cachexia and muscular diseases can favor joint degeneration and rigidity\textsuperscript{45}. However, the effect of physical training on both joint health and irisin serum concentration strictly
depends on the type of exercise\textsuperscript{46}. Several past studies have reported that resistance, anaerobic and high intensity exercise can increase irisin levels in the bloodstream\textsuperscript{47}, while aerobic exercise and reduced load training do not significantly influence irisin concentration\textsuperscript{48}. Duration of exercise training and environmental factors both influence the levels of circulating irisin. A large meta-analysis reported a decrease of circulating irisin in healthy individuals undergoing either endurance or resistance chronic exercise (> 8 weeks)\textsuperscript{49}, whilst another study showed a reduction in irisin levels after two weeks of climbing at high altitude-hypoxia\textsuperscript{50}. To date, no evidence concerning the ideal type of exercise or training protocol for osteoarthritic joints is available. A large meta-analysis comparing high-intensity versus low-intensity exercise for knee and hip OA was inconclusive\textsuperscript{51}, although it is widely accepted that improving muscle strength, aerobic capacity and lowering body weight benefits joint maintenance and cardiovascular health\textsuperscript{5}.

The major limitation of this study is that results have been obtained using an in vitro experimental design, even though human primary cells have been used. Currently, no reports are available correlating irisin synovial fluid concentration with the type of physical activity performed in either healthy subjects or patients with OA. Therefore, these data need to be further confirmed in an experimental animal model of OA exposed to physical exercise. In addition, as the effect of irisin on articular chondrocytes under physiological conditions has not been described yet, our understanding of its biological role on hOAC might not encompass all the effects that the myokine would have on the healthy tissue. A further consideration limiting this study is related to the posttranslational glycosylation of irisin after secretion that enhances its biological function\textsuperscript{31}. Indeed, most of commercial r-irisin derived from Escherichia Coli, including the one used in this study, is non-glycosylated. Therefore, the biological activity may not exactly reflect the myokine action on chondrocytes in vivo, which may be even stronger.

**Conclusion**

Our results indicate that irisin may be one of the mediators by which physical exercise and muscle tissues modulate cartilage metabolism, demonstrating the existence of a biological cross-talk
mechanism between muscle and cartilage. Taken together, our data demonstrate the role of irisin in chondrocyte metabolism and suggest that irisin can be used as a cartilage-regulating factor, which directly targets chondrocytes and enhances cell anabolism, suggesting a potential therapeutic role in treating OA.

**Abbreviations**

ADAMTS: A Disintegrin and Metalloproteinase with Thrombospondin motifs; Ctrl: control; DPBS: Dulbecco's phosphate-buffered saline; ECM: extracellular matrix; ERK: extracellular signal-regulated kinase; GAG: glycosaminoglycan; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; hOAC: human osteoarthritic chondrocytes; MAPK: mitogen-activated protein kinase; MMP: matrix metalloproteinase; OA: osteoarthritis; p-Akt: phosphorylated Akt; p-ERK: phosphorylated ERK; p-p38: phosphorylated p38; PCR: polymerase chain reaction; PI3K: phosphoinositide 3-kinase; r-irisin: recombinant irisin.

**Declarations**

**Ethics approval and consent to participate**

Osteochondral specimens were acquired from OA patients undergoing total knee replacement at the Campus Bio-Medico University Hospital. This study was approved by the Clinical Research Ethics Committees of the Campus Bio-Medico University of Rome. All patients participated in this study provided written informed consent.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.
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Author’s contributions

GV and GDG conceptualized and designed the study. GDG performed the experiments, with the assistance of LA, CC, VT and FR. GV, FC, LA wrote the manuscript and prepared the figures. RP and VD supervised the study.

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**Table 1**
Due to technical limitations, table 1 is only available as a download in the supplemental files section.

**Figures**
Figure 1

Irisin increases GAG content in treated hOAC. GAG/DNA content in hOAC after irisin treatment demonstrated a significative increase in the experimental group treated with 25 ng/mL. n = 4, ***p < 0.001 compared to the control group.
Figure 2

Irisin increases hOAC proliferation. Cell proliferation after treatment with 25 ng/mL irisin at day 0, 4, 10 and 14, as compared with the control group. N = 4, ***p < 0.001 compared to the control group at each timepoint. Ctrl, control group.
Irisin restores ECM composition. (A) Type II collagen relative mRNA expression was significantly higher after 7 days of irisin exposure, as compared with the control group. Conversely, type X collagen relative mRNA expression was diminished upon irisin treatment compared to the control group. N = 5. (B) Western blot analysis confirmed the same trends, as type II collagen levels resulted higher whereas type X collagen levels were lower after irisin exposure at 7 days. N = 3. (C) Densitometric analysis of protein bands attested that these findings were statistically significant: type II collagen relative protein expression was increased (left chart), while type X collagen relative protein expression resulted to be lower at both intervals. Results were normalized based on GAPDH expression. *p = 0.031; **p = 0.009; ***p < 0.001. Ctrl, control group. Coll II, collagen type II. Coll X, collagen type X.

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Irisin inactivates p38 MAPK and Akt pathways within hOAC. (A) Western blot analysis showed a reduction in p-p38, p-ERK and p-Akt levels after irisin exposure at 10, 20 and 60 minutes. N = 3. (B) Densitometric analysis of protein bands demonstrated a significant decrease of p-p38 levels at 60’ minutes. Similarly, p-Akt levels were significantly decreased at each time point. p-ERK levels were not significantly decremented at each time point. N = 3. **p = 0.007; ***p < 0.001.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
Table 1.docx