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Review

# The myokine Irisin recapitulates the effect of physical activity on bone and muscle tissues

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The concomitant occurrence of osteoporosis and sarcopenia is very common during the process of aging and pathological conditions characterized by the disuse of the musculoskeletal system. However, to date there are no evidence about the mechanism responsible for the coupling of these two process.
During the last decade, studies on the interactions between muscle and bone have made remarkable steps forward, establishing that skeletal muscle is an endocrine organ producing and releasing myokines acting in a paracrine or endocrine fashion. Among these, the newly identified myokine Irisin, produced by skeletal muscle after physical exercise, plays a key role in the bone-muscle functional unit, with a major impact on the skeleton by increasing cortical bone mineral density, modifying its geometry and improving bone strength. Furthermore, *in vitro* and *in vivo* studies reported an autocrine effect of Irisin on skeletal muscle and highlighted the autocrine myogenic potential of this myokine.
This review summarizes new insights on the topic of Irisin action on bone and skeletal muscle, which support the hypothesis that Irisin may represent a novel molecular entity with exercise-mimetic properties. Hopefully future research may expand the knowledge of its ability to improve bone integrity and muscle activity and could pave the way for the use of Irisin as a new therapy for the prevention and treatment of musculoskeletal disorders, particularly useful for those patients that are not capable of performing physical activity, such as the elderly or bedridden patients.

**Key words**: Irisin, Bone, Muscle, Mechanical loading, Osteoporosis, Sarcopenia

## INTRODUCTION

Osteoporosis, the skeletal disease characterized by decrease of bone mass and deterioration of its microarchitecture, and Sarcopenia, the muscle disease characterized by progressive loss of muscle mass and strength, are often concurrent diseases affecting the elderly population. These associated pathologies represent one of the major threats that increase the risk of fall-related fracture during the aging process. In addition to its severity, further exacerbated by loss of independence, hospitalization and subsequent depression faced by elderly people, this issue generate high healthcare expenses, which were estimated at approximately 32 billion euros per year in the 27 EU countries [1] and 22 billion dollars per year in the United States [2]. Nevertheless, in light of the increased longevity of the population, the burden of concurrent osteoporosis and sarcopenia can further increase.
Despite the high healthcare burden, there is no widely accepted clinical definition of sarcopenia, even though several diagnostic criteria have been suggested. However, a significant step forward has been made at least for converging the diagnostic approaches [3].

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The characterization of osteoporosis is instead universally accepted and is currently diagnosed based on the bone mineral density compared with that of a young adult of the same sex and further refined through the fracture risk prediction algorithms, such as the Risk Assessment Tool fracture (FRAX®) [4]. Although sarcopenia has not a widely accepted diagnosis criteria, however numerous diagnostic tools, such as the dual energy x-ray absorptiometry (DXA) and the peripheral quantitative computed tomography (pQCT), made it possible to demonstrate association between the bone and muscle health conditions. Through the use of pQCT, it has been showed that bone size and strength are associated with muscle mass size and, although to a lesser extent, to the muscle strength. In addition, it has been found positive correlation between muscle size and cortical and trabecular bone mineral density [5]. Therefore, it has been observed, for example, that 58% of patients with hip fracture were also suffering from sarcopenia. Obviously, the diagnosis of concurrent sarcopenia implies reduced probability of hip fracture resolution and, often, these patients may also face increased risk of recurrent contralateral fractures [6].

During the last decade, an existing intimate relationship between skeletal muscle and bone has been established, not only because of mechanical force generated by muscle contraction that load the skeleton, but in particular for mounting evidence suggesting the existence of a bone-muscle functional unit in which these two tissues talk *via* paracrine signals [7 8]. Through this molecular communication, muscle and bone adapt to load and respond to damages occurring from childhood to the adult age. Albeit all the molecular messengers involved in musculoskeletal unit communication are not yet fully known, to date the skeletal muscle secretome accounts several factors, whose ability to affect the skeleton has been extensively described [9]. Since these molecules produced by skeletal muscle can also act in an endocrine fashion toward distant organs, they are also commonly referred as “myokines”. Among these myokines, the newly identified Irisin, highly secreted by skeletal muscle during physical activity, was originally described as a hormone-like protein capable of promoting the “browning response” in white fat depots (WAT), a program characterized by trans-differentiation of white to brown adipocytes, thus able of promoting thermogenesis and energy expenditure [10].

## THE EXERCISE-LIKE MYOKINE IRISIN

The Irisin discovery had received acclaim from the scientific community since exercise-induced benefits are known to be exerted on many organs, so much that engaging in regular physical activity is recommended as the best non-pharmacological treatment for the prevention of obesity, osteoporosis, sarcopenia, metabolic disorders, cardiovascular and brain disease [11 12]. For instance, it has been observed that Irisin plays role in the central nervous system, as showed by the expression of its precursor in rat and mice cerebellar Purkinje cells [13]. Irisin is also required for a proper neural differentiation of embryonic stem cells [14] and modulates hippocampal neurogenesis in a dose-dependent manner [15].

So far, few studies have tried to assess the efficacy of recombinant Irisin, as exercise-mimetic molecule, in murine models *in vivo*. Zhang et colleagues demonstrated that normal and obese mice, treated with 3500 μg/kg/week of recombinant Irisin (r-Irisin), injected every day for two weeks, showed a 25-fold change increase of uncopling protein 1 (UCP1) expression in white fat depots, thus confirming Irisin ability to promote the browning trans-differentiation of white adipocyte. The effect was accompanied by decreased body weight and enhanced glucose homeostasis, as proved by the higher expression of betatrophin and increased pancreatic b-cell proliferation in r-Irisin treated animals [16].

Interestingly, it has been shown that a considerable lower dose of r-Irisin (100 μg/kg/week), injected in normal mice once a week for four weeks, significantly increased cortical bone mineral density and improved cortical geometry and bone strength, but was not sufficient to activate the trans-differentiation of white to brown adipose tissue [17]. This result, not only revealed one of the molecular messengers responsible for muscle-bone crosstalk during physical activity, but also pointed out that the skeleton is a more sensitive target to Irisin action compared with adipose tissue [17].

## THE BONE ANABOLIC ACTION OF IRISIN

Physical activity is a vigorous stimulus for increasing bone mass and it has been extensively documented that exercise has positive effects on bone mineral density [18]. In order to investigate if Irisin was responsible for the protective effect that exercised muscles exert on bone tissue, healthy young mice were treated with a low dose of recombinant Irisin for four weeks. By microCT analysis of the tibia, it has been observed a marked effect on cortical bone mineral density (BMD) and bone perimeter [17]. Furthermore, the 20% increase of polar moment of inertia, an index of resistance to torsional forces, supported the idea that Irisin, modifying bone geometry, would increase bone strength. In fact, mechanical tests assessed on tibia confirmed that bending strength and energy to fracture were strongly increased in Irisin-injected mice.

The effect of Irisin on bone is mostly exerted on bone forming cells, as demonstrated by increased number and size of osteoblasts on cortical bone and by elevated expression of Activating transcription factor 4 (*Atf4*) in bone marrow, signifying commitment of mesenchymal stem cell toward an osteoblastogenic phenotype [17 19]. In addition, long bones of r-Irisin treated mice expressed high level of osteopontin (OPN), one of the most abundant protein of bone matrix that is also known to be a mechanically responsive molecule [20], and strongly reduced expression of sclerostin, one of the inhibitors of the bone anabolic Wnt pathway [21].

Although Irisin receptor has not been identified yet, its action on osteoblast is receptor-mediated, as demonstrated by MAP kinase Erk activation upon r-Irisin administration *in vitro* [17]. The r-Irisin-activated ERK-mediated intracellular signaling was also supported by data obtained by other researchers, which showed in parallel a significant increase of phosphorylated p38 in both primary rat osteoblast and MC3T3-E1 osteoblast cell line after r-Irisin treatment [22].

## THE MYOGENIC POTENTIAL OF IRISIN

In skeletal muscle of mice treated with r-Irisin it was observed a high number of fibers expressing the Irisin precursor, thus intriguingly suggesting that Irisin production may be enhanced by an autocrine action [17]. This result was also confirmed *in vitro* by treating murine myotubes with r-Irisin for 24 hours that, upon treatment, expressed high levels of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), the transcription factor responsible for Irisin synthesis. Additionally, muscle cells treated with r-Irisin also expressed higher levels of nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM), indicating increased mitochondrial content and oxygen consumption [23].

The effect of r-Irisin has been also tested in human skeletal muscle cells *in vitro*, in which, through an ERK-dependent mechanism, insulin-like growth factor 1 (IGF-1) and myostatin expressions were increased and decreased, respectively [24]. Excitingly, Irisin and myostatin are both produced by skeletal muscle and their synthesis is inversely regulated by physical exercise [25]. Moreover, myostatin knock-out mice highly express

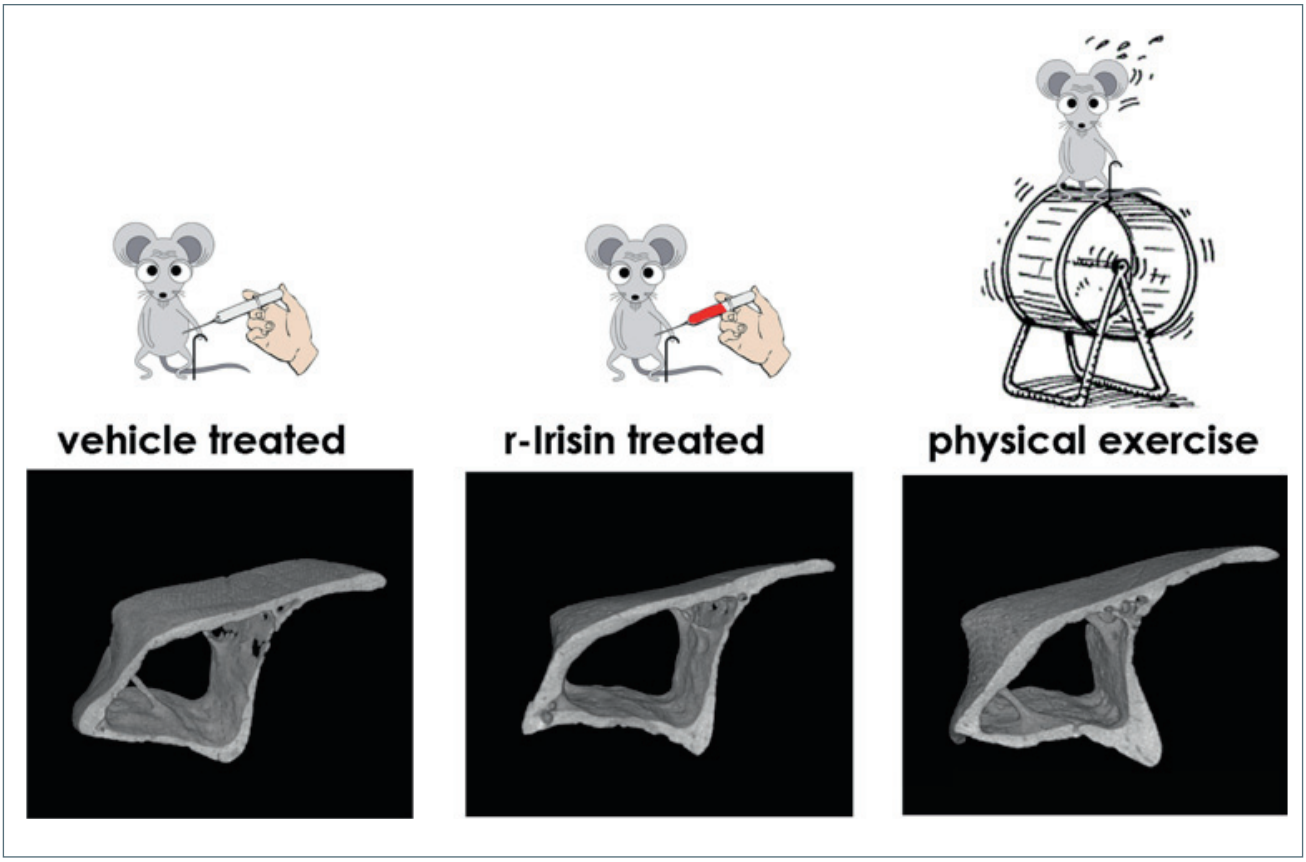


**Figure 1.** Representative micro-CT-generated section images of tibia harvested from 12-month-old mice treated with vehicle or r-Irisin (100 μg/Kg/weekly) or subjected to physical activity for 4 weeks.

Irisin and its precursor [26], thus supporting the idea that the increase of muscle mass observed in these mice could be also induced by Irisin.

## IRISIN FOR THE PREVENTION AND TREATMENT OF OSTEOPOROSIS AND SARCOPENIA: A BRIGHT FUTURE LIES AHEAD?

A better understanding of the molecular entities involved in muscle and bone communication can shift the paradigm for the simultaneous treatment of osteoporosis and sarcopenia. To date, no randomized controlled trials that evaluated the combined effects of chemical molecule on both bone and muscle tissue have been reported.

The research findings on the effects of Irisin on the bone-muscle functional unit, altogether support the idea that Irisin is a regulatory hormone-like molecule with key functions for the metabolism of the musculoskeletal system. However, further studies on osteoporotic and sarcopenic murine models would allow to evaluate if Irisin is effective in preventing or retrieving bone and muscle loss. If remarkable results will be achieved, future studies could lead to the assessment of r-Irisin in human clinical trials. An Irisin-based therapeutic strategy should be particularly useful in those patient that cannot perform physical activity, such as elderly people or bedridden patients (Fig. 1).

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