## Perspective

# Irisin and musculoskeletal health

Graziana Colaianni,[1] Saverio Cinti,[2] Silvia Colucci,[1,a] and Maria Grano[3,a]

[1]Department of Basic Medical Science, Neuroscience and Sense Organs, University of Bari, Bari, Italy. [2]Department of Experimental and Clinical Medicine, Center of Obesity, United Hospitals, University of Ancona, Ancona, Italy. [3]Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy

Address for correspondence: Maria Grano, Ph.D., Department of Emergency and Organ Transplantation, University of Bari, Piazza Giulio Cesare 11, 70124 Bari, Italy. maria.grano@uniba.it

Irisin is a hormone-like myokine produced in abundance by skeletal muscle in response to exercise, both in mice and humans. Once released into the circulation, irisin acts on white adipocytes to induce the browning response and subsequently activates nonshivering thermogenesis. We have examined the premise that irisin produced during exercise may subserve further functions in the musculoskeletal system. We review evidence for its possible skeletal effects, including the central role that irisin plays in the control of bone mass, with positive effects on cortical mineral density and geometry in mice. We also review the autocrine effects of irisin in skeletal muscle, in which it upregulates the expression of its precursor (FNDC5). Since loss of bone and muscle mass occurs with aging, immobility, and several metabolic diseases, future studies exploring the efficacy of irisin in restoring bone and reversing muscle wasting could be important to establishing irisin as a molecule that combines beneficial effects for treating osteoporosis and muscular atrophy. If the results from mice were confirmed in human studies, an irisin-based therapy could be developed for physically disabled or bedridden patients.

## Introduction

Regular physical activity helps to improve health status and can help prevent several diseases, such as obesity, diabetes, osteoporosis, and age-related muscle wasting.[1,2]

It is widely accepted that muscle and bone are tightly coupled, not only in their anatomical proximity and mechanical interaction but also in terms of paracrine and endocrine signals in both physiological and pathological conditions.[3,4]

Studies in humans and animal models have extensively demonstrated that physical activity, immobility, aging, and several diseases can cause parallel changes in bone and muscle mass. Osteoporosis, a systemic bone disease characterized by low bone mass with occurrence of spontaneous fractures, and sarcopenia, characterized by muscle wasting and progressive loss of physical performance, are twin conditions of aging, showing many shared pathways, including reduction in anabolic molecule release or increased secretion of inflammatory molecules.[5] Although the anabolic effects due to increased exercise and mechanical load and the catabolic responses due to unloading or immobility states have been well documented, the molecular mechanisms through which bone–muscle cross talk occurs are still under investigation. Studies have shown that different bone-derived signals affect skeletal muscle; some of

---

[a]These authors contributed equally to this manuscript.

them, such as osteocalcin, insulin-like growth factor 1 (IGF-1), prostaglandin E2, and FGF-23, display positive effects, while others, such as activin or TGF-β, cause negative responses.[6] Additionally, some muscle-derived signals, such as interleukins (IL-6, IL-8, IL-15), indirectly target bone through actions on other tissues, while other myokines, such as irisin, can affect bone directly.[7–11]

Irisin is released by the cleavage of the extracellular domain of the transmembrane receptor fibronectin type III domain–containing 5 (FNDC5), expressed in skeletal muscle as well as other tissues. Structural and biochemical data have shown that irisin is a preformed dimer and can bind an as-yet unknown receptor. Following the initial studies, many authors have tried to identify the physiological role of irisin.[7] In 2012, irisin, secreted abundantly by skeletal muscle during exercise, was described as a hormone-like molecule that elicits the browning response in white fat (WAT), causing its transdifferentiation into brown fat.[7] This elegant study suggested that irisin could have beneficial effects in the treatment of obesity and metabolic disorders. Here, we provide an overview of the role of irisin on bone and suggest that this molecule is an additional link in bone–muscle cross talk.

## The osteogenic potential of the myokine irisin

Regular exercise, through its systemic action, has beneficial effects for an organism and is a potent stimulus for new bone formation. Therefore, we postulated that irisin, an exercised-induced myokine, might have beneficial effects on bone accrual and contribute to the protective effect of muscle on bone tissue. Our hypothesis was confirmed by the finding that the medium collected from muscle cells from exercised mice, which contains irisin, enhanced the differentiation of bone marrow stromal cells into osteoblasts. We have shown that, in these cell cultures, osteoblastic parameters, such as the number of alkaline phosphatase (ALP)$^+$ colonies, as well as the gene levels of *ALP* and collagen type I, were higher in the presence of conditioned media from exercised mice compared with myoblasts from sedentary mice. These effects were irisin dependent, since they were blunted when the culture medium was spiked with a neutralizing antibody against irisin.[8] Moreover, the occurrence of Erk

phosphorylation in osteoblasts within 5 min after irisin treatment has demonstrated that the action of the myokine on osteoblasts is receptor mediated. We have demonstrated that irisin plays a role in all the stages of osteoblast differentiation: it increases the number of ALP$^+$ colonies, a parameter of the early phase of osteoblastogenesis, and enhanced the formation of mineralized nodules, a crucial feature of the late stage of the differentiation process. These effects were associated with irisin-induced overexpression of mRNA levels of crucial transcription factors involved in osteoblast differentiation, such as Atf4, RUNX2, and osterix, as well as the upregulation of gene levels of lipoprotein receptor–related protein 5 and β-catenin, critical in the activation of the Wnt pathway, which is the key signal in the process of osteoblastogenesis.[9]

Many studies have shown that, in addition to the quality, the strength of bone depends not only on its density and mass but also on the geometrical organization of cortical bone and its distribution in space. It has been well established that increased bone strength, accompanied by enlarged cross-sectional area, which is responsible of distributing bone mass farther from the bending axis, can improve skeletal resistance.[12] Exercise also strengthens bone by modifying its geometry, as observed in tennis players who develop increased bone mass and bone perimeter in the playing arm compared with the non-playing arm.[13]

Therefore, on the basis of these findings and our *in vitro* data on the osteogenic potential of irisin, we investigated whether the myokine could induce beneficial effects on the skeleton *in vivo*.[9] We treated healthy young mice with recombinant irisin (r-irisin) for 4 weeks, and micro–computed tomography analysis of the tibia showed that cortical bone mineral density, periosteal circumference, and polar moment of inertia were increased.[9] The increased polar moment of inertia and index of long bone resistance to torsion, together with the enlarged bone perimeter, suggest that irisin treatment modifies bone geometry to make long bones more efficient at resisting torsional forces. Likewise, bending strength and energy to fracture were increased in irisin-treated mice, as demonstrated by a 3-point bending test, suggesting that irisin treatment might make bone less susceptible to fracture. We have also shown an increase in osteoblast number and a parallel decrease in osteoclast number in irisin-treated

mice, indicating that irisin can contribute to the improvement in bone quality.[9]

Although irisin clearly mimics the effects of physical activity, and its efficacy on bone loss has not yet been investigated, our future studies will explore its effect on the skeleton in animal models of osteoporosis in which the resorption activity exceeds the formation of bone, such as hindlimb-suspended mice.[14] These mice are also a widely accepted murine model for simulating weightlessness, in which hindlimb weight bearing is prevented but the hindlimbs are not immobilized; thus, the passive muscular forces remain functional. Interestingly, in these animals, bone loss is accompanied by muscle wasting; therefore, the effect of the myokine can be studied at the same time in both osteoporosis and sarcopenia.

## The myogenic potential of the myokine irisin

The physiological roles of irisin in bone–muscle cross talk and the question of whether irisin can reciprocally affect both tissues are topics that need to be investigated *in vivo*. We have hitherto demonstrated *in vivo* that mice treated with r-irisin displayed a higher number of FNDC5$^+$ fibers than mice injected with vehicle. This finding suggests that irisin synthesis, by enhancing the expression of the precursors from whose cleavage it derives, may exert its autocrine action.[9] Moreover, it supports *in vitro* findings of other authors who treated C2C12 myotubes with 3.1–12.4 ng/mL of r-irisin for 24 h and observed an increased expression of specific mitochondrial transcription factors, such as peroxisome proliferator–activated receptor γ coactivator-1α, nuclear respiratory factor 1, and mitochondrial transcription factor A, all involved in increasing mitochondrial content and oxygen consumption.[15] It has also been demonstrated that *FNDC5* mRNA and irisin synthesis were increased during myogenic differentiation of human myocytes *in vitro*, supporting the idea of the myogenic potential of irisin.[16] Moreover, human myocytes treated with r-irisin expressed elevated levels of IGF-1 and low levels of myostatin, the expression of which were modulated by r-irisin through an ERK-dependent pathway.[16]

Interestingly, irisin and myostatin are both synthetized by skeletal muscle, and their secretion is inversely regulated by physical activity.[17] Indeed, it has been demonstrated that myostatin negatively regulates skeletal muscle mass, since myostatin deficiency leads to increased cortical area and bone mineral content.[18] Importantly, data showing that irisin and its precursor were highly expressed in skeletal muscle of myostatin knock-out mice[19] suggests that the increased muscle mass observed in these mice could also be dependent on increased irisin synthesis.

Skeletal muscle tissue rapidly adapts to mechanical load.[20] Resistance exercise increases load through muscle, which in turn results in increased muscle size and strength, mainly attributable to hypertrophy (growth of preexisting muscle cells) rather than hyperplasia (increase in cell number).[20] Conversely, the increase in muscle mass due to myostatin inhibition leads to both hypertrophy and hyperplasia.[21] Therefore, even though myostatin unequivocally regulates muscle mass, its role in skeletal muscle hypertrophy triggered by mechanical loading has been unclear for a long time. In defining the role of myostatin as a predictor of muscle growth, some questions remained elusive. For example, although exercise training decreased *myostatin* mRNA proportionally to the loading applied,[22] this effect was more evident in subjects unresponsive to physical exercise, in whom muscle mass did not increase.[23] Additionally, it was not clear why only the muscles subjected to load increased their mass compared with all the muscles throughout the body, as it was expected that exercise-induced decrease of myostatin levels in the circulation should affect the whole body.[24] Taken together, these results suggest that another yet-unidentified soluble factor, produced by skeletal muscle following exercise, could be responsible for the positive effect on muscle growth in response to load. Future studies are needed to verify if this unknown molecule is irisin.

On the other hand, during aging and immobility, the physiological changes caused by skeletal unloading determine the onset of sarcopenia, which is characterized by a decrease in muscle fiber size (atrophy) and number (hypoplasia). Numerous pharmacological and nutritional approaches have been attempted to protect against musculoskeletal damage caused by mechanical unloading, but conclusive results have not been obtained. Therefore, further investigations should be aimed at determining whether disuse-induced sarcopenia could be prevented by r-irisin treatment.

## Irisin and the inducible brown adipose tissue are anabolic for the skeleton

External stimuli, such as cold exposure or physical activity through irisin action,[9] stimulate the expansion of inducible brown adipose tissue, whose cells show morphological characteristics similar to classical brown adipose tissue, such as the presence of multilocular lipid droplets and numerous mitochondria.[7] Considering the tight relationship between skeletal metabolism and energy homeostasis, researchers are placing great importance on the role of BAT in bone metabolism. Transgenic mice overexpressing forkhead box protein C2 in adipose tissue are a well-established model for BAT induction. These mice displayed elevated bone mass owing to increased bone formation, triggered by wingless-related MMTV integration site 10b (WNT10b) and insulin-like growth factor–binding protein 2, two bone anabolic molecules produced by BAT.[25]

Previous work showed that therapeutic treatment of normal and obese mice with r-irisin at a dose of 3500 mg/kg/week activated the browning response in white adipose tissue.[26] The effect was confirmed by a significant increase of uncoupling protein 1 expression (>25-fold change) in mice receiving r-irisin and was accompanied by total body weight reduction. In our study, when mice were treated with a lower dose of r-irisin (100 mg/kg/week) to rule out an indirect action of irisin on bone mass via BAT expansion, we did not observe transdifferentiation of white to brown adipose tissue.[11] Hence, we demonstrated that the low dose of r-irisin was effective to reinforce bone but not sufficient to induce the browning response; this suggests that bone tissue is more sensitive than adipose tissue to the action of irisin.[9] However, further investigations may explore the synergistic effect of both direct and indirect irisin action by injecting mice with a higher dose of irisin. Addressing this topic could be relevant, considering the well-known inverse relationships between BAT in aging[27] and muscle activity and bone integrity.[9]

## Conclusions

In view of the findings reported here, the exercise-mimetic activity of r-irisin seems evident. However, further studies on murine models of osteoporosis and muscle atrophy would allow us to assess whether irisin is also effective at preventing or reversing bone loss and muscle wasting. Equally important will be future human studies that could encourage the use of irisin as therapeutic strategy for the prevention and treatment of osteoporosis, sarcopenia, or both, particularly in those patients who are unable to perform physical activity, such as the aged population or bedridden patients.

## Competing interests

M. Grano, S. Cinti, S. Colucci, and G. Colaianni are named inventors of patent application related to the work described.

## References

1. Dunstan, D. 2011. Diabetes: exercise and T2DM-move muscles more often! *Nat. Rev. Endocrinol.* **7:** 189–190.
2. Egan, B. & J.R. Zierath. 2013. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab.* **17:** 162–184.
3. Girgis, C.M., N. Mokbel & D.J. Digirolamo. 2014. Therapies for musculoskeletal disease: can we treat two birds with one stone? *Curr. Osteoporos. Rep.* **12:** 142–153.
4. Tagliaferri, C., Y. Wittrant, M.J. Davicco, *et al.* 2015. Muscle and bone, two interconnected tissues. *Ageing Res. Rev.* **21:** 55–70.
5. Reginster, J.Y., C. Beaudart, F. Buckinx & O. Bruyère 2016. Osteoporosis and sarcopenia: two diseases or one? *Curr. Opin. Clin. Nutr. Metab. Care* **19:** 31–36.
6. Regan, J.N., T. Trivedi, T.A. Guise & D.L. Waning 2017. The role of TGFβ in bone–muscle crosstalk. *Curr. Osteoporos. Rep.* **15:** 18–23.
7. Boström, P., J. Wu, M.P. Jedrychowski, *et al.* 2012. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* **481:** 463–468.
8. Colaianni, G., C. Cuscito, T. Mongelli, *et al.* 2014. Irisin enhances osteoblast differentiation *in vitro*. *Int. J. Endocrinol.* **2014:** 902186.
9. Colaianni, G., C. Cuscito, T. Mongelli, *et al.* 2015. The myokine irisin increases cortical bone mass. *Proc. Natl. Acad. Sci. U.S.A.* **112:** 12157–12162.
10. Colaianni, G. & M. Grano. 2015. Role of irisin on the bone–muscle functional unit. *BoneKEy Rep.* **4:** 765.
11. Holmes, D. 2015. Bone: irisin boosts bone mass. *Nat. Rev. Endocrinol.* **11:** 689.
12. Lieberman, D.E., J.D. Polk & B. Demes. 2004. Predicting long bone loading from cross-sectional geometry. *Am. J. Phys. Anthropol.* **123:** 156–171.
13. Jones, H.H., J.D. Priest, W.C. Hayes, *et al.* 1977. Humeral hypertrophy in response to exercise. *J. Bone Joint Surg. Am.* **59:** 204–208.

14. Morey-Holton, E., R.K. Globus, A. Kaplansky & G. Durnova. 2005. The hindlimb unloading rat model: literature overview, technique update and comparison with space flight data. *Adv. Space Biol. Med.* **10:** 7–40.

15. Vaughan, R.A., N.P. Gannon, C.M. Mermier & C.A. Conn. 2015. Irisin, a unique non-inflammatory myokine in stimulating skeletal muscle metabolism. *J. Physiol. Biochem.* **71:** 679–689.

16. Huh, J.Y., F. Dincer, E. Mesfum & C.S. Mantzoros. 2014. Irisin stimulates muscle growth-related genes and regulates adipocyte differentiation and metabolism in humans. *Int. J. Obes. (Lond.)* **38:** 1538–1544.

17. MacKenzie, M.G., D.L. Hamilton, M. Pepin, *et al.* 2013. Inhibition of myostatin signaling through Notch activation following acute resistance exercise. *PLoS One* **8:** e68743.

18. Elkasrawy, M.N. & M.W. Hamrick. 2010. Myostatin (GDF-8) as a key factor linking muscle mass and bone structure. *J. Musculoskelet. Neuronal Interact.* **10:** 56–63.

19. Shan, T., X. Liang, P. Bi & S. Kuang. 2013. Myostatin knock-out drives browning of white adipose tissue through activating the AMPK–PGC1α–Fndc5 pathway in muscle. *FASEB J.* **27:** 1981–1989.

20. Goodman, C.A., J.W. Frey, D.M. Mabrey, *et al.* 2011. The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth. *J. Physiol.* **589:** 5485–5501.

21. Lee, S.J. 2010. Extracellular regulation of myostatin: a molecular rheostat for muscle mass. *Immunol. Endocr. Metab. Agents Med. Chem.* **10:** 183–194.

22. Heinemeier, K.M., J.L. Olesen, P. Schjerling, *et al.* 2007. Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types. *J. Appl. Physiol.* **102:** 573–581.

23. Kim, J.S., J.K. Petrella, J.M. Cross & M.M. Bamman. 2007. Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis. *J. Appl. Physiol.* **103:** 1488–1495.

24. Wong, T.S. & F.W. Booth. 1988. Skeletal muscle enlargement with weight-lifting exercise by rats. *J. Appl. Physiol.* **65:** 950–954.

25. Rahman, S., Y. Lu, P.J. Czernik, *et al.* 2013. Inducible brown adipose tissue, or beige fat, is anabolic for the skeleton. *Endocrinology* **154:** 2687–2701.

26. Zhang, Y., R. Li, Y. Meng, *et al.* 2014. Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. *Diabetes* **63:** 514–525.

27. Zingaretti, M.C., F. Crosta, A. Vitali, *et al.* 2009. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* **23:** 3113–3120.