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Irisin: good or bad for the bone? A new path forward after the reported discovery of irisin receptor?

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Irisin is a protein derived from C-terminal cleavage of the fibronectin type III domain containing (FNDC)5 transmembrane protein, a process induced by the peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1a [1]. Irisin is mainly produced by the skeletal muscle in response to exercise in mice [2] and possibly in humans [3], and it may be beneficial in several metabolic disorders [4], especially those known to improve with exercise [5]. Exercise is a potent stimulus of bone formation, improving bone mineral density (BMD) mainly through loading on the skeleton, thus reducing fracture risk [6]. It has been recently suggested that irisin might be a major regulator of the cross-talk between muscle and bone [7].

There are several *in vitro*, preclinical and clinical studies supporting an osteogenic role for irisin. In *in vitro* studies, irisin increased osteoblast differentiation through the Wingless (Wnt)/β-catenin pathway [8], and also induced the proliferation, differentiation, alkaline phosphatase activity, and mineralization of cultured osteoblasts through activation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) [9]. Moreover, in RAW264.7 cells, irisin suppressed the formation of osteoclasts through inhibition of the receptor activator of nuclear factor-kappa B ligand (RANKL) [10]. In 5-week-old mice, FNDC5 protein expression and irisin increased over 6-fold in bone tissue after exercise [11]. Furthermore, intraperitoneal irisin administration resulted in increased trabecular and cortical bone thickness and osteoblasts numbers [11]. Similarly, in male mice, recombinant irisin administration upregulated the expression of osteogenic genes, including Runx2, Osterix, low-density lipoprotein-related protein 5, β-catenin, alkaline phosphatase and type I collagen in bone marrow stromal cells and increased bone formation, with a parallel reduction in osteoclast number, thereby increasing cortical bone mass and strength [12]. Finally, in hind-limb suspended mice, treatment with recombinant irisin preserved both cortical and trabecular BMD, while also prevented the anticipated decrease of the trabecular bone volume due to immobilization [13]. In human studies, irisin levels were inversely correlated with serum levels of sclerostin, the negative regulator of osteoblastic activity [14] and positively associated with BMD and strength in young athletes [15] and with bone mineral status evaluated by quantitative ultrasound (QUS) in healthy children [16]. Furthermore, circulating irisin levels were inversely associated with previous osteoporotic fractures in postmenopausal women [17–19], independently from other factors associated with bone fragility, including BMD and bone turnover markers [17]. However, the results in clinical studies should be interpreted with caution, given that controversies and obscurities regarding the characterization and identification of irisin have arisen based on quantification inconsistencies and doubts about the validity of the currently available

Abbreviations: FNDC5, fibronectin type III domain containing 5; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; PPAR-γ, peroxisome proliferator-activated receptor-γ; PGC-1a, peroxisome proliferator-activated receptor-γ coactivator-1a; BMD, bone mineral density; RANKL, receptor activator of nuclear factor-kappa B ligand; Wnt, Wingless.

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assays. The best validated assay [20] is not commercially available any more. Fortunately, better assays are under development and are expected to further propel the field forward in terms of clinical studies [21]. Measurements using mass spectrometry [22] have shown that irisin circulates in the blood stream of humans. However, this study needs to be confirmed and needs to be used in the validation of future assays currently under development.

On the contrary to all the above, a recent study suggests a not so favorable skeletal role for irisin [23]. In this study, Kim et al. identified integrins, particularly αV/β5, as the receptors for irisin on the osteocytes. Osteocytes, being the mechano-sensor of the bone and the main source of sclerostin and RANKL production, control osteoblastic and osteoclastic activity, respectively, and orchestrate their coupling by modifying the secretion of these two molecules [24–26]. Increased mechanical stimuli, as in exercise, suppress sclerostin production, thus stimulating canonical Wnt/β-catenin signaling and osteoblast differentiation and activity [24]. However, in this study, irisin treatment induced the expression of sclerostin from osteocyte-like cells *in vitro* and its expression from mouse osteocyte-enriched bones *in vivo* in a dose dependent manner, as well as sclerostin circulating levels. These changes imply osteoblast suppression and are in contrast to the above reported inverse correlation between circulating irisin and sclerostin levels in humans [14] and the osteogenic effects of irisin shown in previous studies [8,9,12]. Additionally, in the same study, *FNDC5* knock-out mice exhibited reduced levels of RANKL, a major inducer of osteoclast maturation, activity and survival [23]. Furthermore, these knockout mice had increased femoral trabecular bone mass and increased connectivity density, probably due to the ensuing reduction in bone resorption. Additionally, *FNDC5* knock-out mice were resistant to ovariectomy-induced bone loss. The latter was attributed to inactivation of both osteoclastic bone resorption and osteocytic osteolysis. Osteocytes besides promoting bone resorption indirectly, through increasing RANKL production, can also directly resorb bone during periods of excessive calcium demand, a phenomenon called osteocytic osteolysis [27].

These unfavorable effects of irisin on the bone are difficult to fit in its so-called beneficial role on several metabolic abnormalities. In an attempt to explain the differences between previous studies and this last one, Kim et al. used the PTH paradigm, which is a molecule exerting both anabolic and catabolic effects on the skeleton depending on the intermittent administration or constantly increased levels, respectively. In a similar setting, chronic high irisin levels could promote bone resorption as shown by Kim et al. [23] while constantly decreased or null irisin levels, as in FNDC knockout mice might be beneficial for bone, and furthermore, an intermittent irisin pulse, such as after exercise, might transiently induce bone remodeling starting from the induction of the short-living osteoclasts and then continue through their cross-talk with the longer-living osteoblasts; in the latter case the intermittent induction of bone remodeling may have an overall beneficial skeletal outcome [12,13] which could explain the previously reported positive effects of irisin on bone. If verified, this emerging dual role of irisin in the skeletal system will redefine its therapeutic potential against osteoporosis.

Kim et al. identify for the very first time receptors for irisin in osteocytes and this is a great contribution to scientific community, since the same receptors could be now searched in other tissues regarded as potential target tissues of irisin, *e.g.*, the adipose tissue, the brain and the liver. Furthermore, the identification of irisin receptors may facilitate research on its upstream regulation and its downstream signaling cascades, with potential therapeutic implications. However, purified irisin binds to several integrin complexes, with integrin αV/β5 having the highest affinity. Whether the binding of irisin to integrin receptors is essential for its physiological activity *in vivo*, is under question and needs further investigation. Comparative analysis between anti-irisin and anti-αV/β5 receptor therapies in mouse models could demonstrate whether there is a common response and consequently whether αV/β5 is the main receptor that regulates bone remodeling *in vivo*, or not. On the other hand, integrins *e.g.* αV/β5 display specificity also for other ligands including osteopontin, bone sialoproteins, and vitronectin [28], raising concerns about interference of its pharmacological inhibition with other processes (*i.e.* angiogenesis) leading to possible side effects. Furthermore, similar experimental procedures could be followed in order to identify if irisin has specificity also for other receptors either tissue specific or soluble that could regulate its activity. Even though Kim et al., shed light on the molecular basis of irisin in mouse systems, the existence and functioning of irisin in humans is still debatable, thus further experimentation is needed to confirm or refute the physiological importance of irisin.

By their publication, Kim et al. upended existing literature on the pure beneficial effect of irisin on bone metabolism, thus raising new questions. Are there αV/β5 integrin receptors in other bone cells besides the osteocytes, *e.g.*, in the osteoblasts or osteoclasts? If yes, which is their distinct role? Are there other irisin receptors in the bone, whose binding could result in different responses, thus further explaining the controversy in the literature? Is the number and functionality of irisin receptors in the osteocytes affected in osteoporosis or other metabolic bone diseases and is there any effect of current osteoporosis treatment on these receptors? How would inhibition of irisin or its binding to its receptor affect the skeleton? Would pharmacological inhibition of irisin affect other organs and systems leading to side-effects since its precursor FNDC5 is widely expressed in multiple tissues [29]? On the other hand, it should be defined whether potential irisin inhibitors also target the FNDC5 protein, affecting its functionality and related biological processes. Notably, it is not clear whether FNDC5 acts like a receptor and in this case its cleavage to irisin production could regulate its activity.

Almost seven years after the discovery of irisin [2], the physiological significance of irisin-mediated effects in humans remains enigmatic, since the main functions of irisin have been identified in mice and *in vitro* assays. Whether these effects recapitulate human physiology needs further investigation, particularly as the pattern of expression of irisin in humans is unclear due to an unusual ATA start codon at the human FNDC5 gene that is correlated with low translation efficiency of the human FNDC5 protein [30,31]. A major limitation that should be addressed during the next period is the establishment of reliable and standardized technical assays for the quantification of irisin and FNDC5 in biological samples. Nevertheless, irisin emerges as a promising novel player in metabolism and a potential therapeutic target for metabolic bone diseases, although its exact role on bone metabolism should be further clarified.

Conflicts of interest/financial disclosure statement

None of the authors has any conflict of interest in regard to the present submission.

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