Irisin effects on bone: systematic review with meta-analysis of preclinical studies and prospects for oral health

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Abstract: Bone quality is an important issue in dentistry. Low bone density may be associated with more severe periodontitis, and may influence implant therapy success. Recent evidence suggests that physical activity can improve alveolar bone quality. Irisin is an exercise-mediated peptide that might be involved in this process. We assessed the effect of exercise and that of intra-peritoneal irisin administration on bone quality in healthy and osteoporosis-induced rodents. This study was registered at PROSPERO (CRD42020184140), and followed PRISMA guidelines. A search by two independent examiners was conducted in five databases and gray literature up to July 2021, without restrictions regarding language or date of publication. Initially, they analyzed retrieved titles and abstracts (n=3,844) based on eligibility criteria. Of this total, 19 studies remained for full-text reading, and 16 proceeded to the data extraction and quality assessment phases. Meta-analyses were conducted (n= 6 studies) to establish the effects of irisin administration on cancellous bone mineral density (BMD). Exercise or irisin administration enhanced bone quality, but the meta-analysis showed that BMD increased only slightly in osteoporotic rodents (BMD: mean difference 0.03 mg/cm3 - 95% CI 0.01-0.05). This indicates that they had no significant benefits on the bones of healthy animals. Implications of key findings evidence the potential of irisin as an agent able to mitigate bone loss caused by osteoporosis, an outcome that could favor dental rehabilitation. More studies investigating the effect of irisin on alveolar bone are needed to elucidate its therapeutic viability and implications.

Keywords: Bone and Bones; Chronic Disease; Exercise; Dentistry; Physiology.

Introduction

Bone quality is an important issue in dentistry. Systemic diseases such as obesity, diabetes *mellitus* and osteoporosis may lead to bone alterations, which are associated mainly with periodontitis and loss of alveolar bone.[1,2] Low bone density could be a risk factor influencing implant success[3] and impairment of tooth support structures[4] during dental treatment. On the other hand, recent studies have shown that physical activity can improve the quality of alveolar bone.[5,6] During exercise, muscle fibers

under contraction release myokines, which exert local and systemic effects.[7] These myokines play an important role as exercise-induced hormones that interact with bone.[8]

Irisin is an exercise-mediated peptide[9] encoded by the fibronectin type III domain-containing protein 5 (FNDC5) gene,[10] which regulates adipocyte and osteocyte metabolism[11] through a specific αV-class of integrin receptors.[12] The effects of irisin on bone seems to increase Atf4 and Runx2 expressions, resulting in an osteogenic effect.[13] Irisin also reduces osteoclast differentiation and pro-inflammatory cytokines, and increases anabolic factors such as β-catenin, which induces osteoblast differentiation.[14] Although some studies have indicated the positive relationship between exercise or recombinant-irisin injections (r-irisin) and bone anabolism,[15–18] there is still no consensus substantiating this effect. Kim et al.[12] reported that r-irisin injections increased sclerostin (Sost) expression in osteocytes, inducing bone resorption. On the other hand, Colaianni et al.[19] found no effects of r-irisin on the bone of healthy rodents, whereas there was a preventive and curative effect on animals submitted to hindlimb osteoporosis. Furthermore, Colaianni and Grano[20] found no effect on trabecular bone, but did observe an increase in cortical bone surface. As can be observed, the resulting consequences of exercise or r-irisin injections on bone are not yet conclusive.

Before irisin can be considered a potential agent for attenuating bone loss, it must be tested to determine whether there is enough evidence of its effects, based on pre-clinical studies. Animal protocols tend to evaluate homogenous samples with standardized conditions of feeding and environment. In addition, the irisin sequence is almost identical across most mammalian species.[17] Irisin seems to have autocrine, paracrine and endocrine effects on oral and bone tissues[21]. Moreover, the evidence of bone stimulation makes it a promising agent for the dental treatment of patients with osteoporosis and other systemic conditions that induce alveolar bone loss. Thus, the aim of this systematic review and meta-analysis was to evaluate the effects of exercise and irisin injections on the bone quality of both healthy and osteoporotic rodents.

Methodology

Registration protocol and study design

This systematic review was registered at PROSPERO under protocol number 184140, and followed PRISMA- (Preferred Reporting Items for Systematic Review and Meta-Analysis) adapted guidelines.[22] The methodology was adapted from Ferreira et al.[23]

Eligibility Criteria, Search Strategy and Data Extraction

Two independent reviewers searched animal studies published up to July 20, 2021, on five online databases (PubMed, Scopus, Web of Science, Embase and Science Direct). The PECO question focused on evaluating bone quality (Outcome) in rodents (Population) submitted to exercise or intra-peritoneal r-irisin administration (Exposure), in comparison with sedentary/placebo groups (Comparison). The search strategy involved the following keyword combinations: "irisin" OR "FNDC5" OR "fibronectin-type III domain-containing 5" AND "bone." We used the filter "animal studies" when possible, with no restrictions on language. The searches were complemented using the OpenGrey database ("gray literature"), and similar terms.

The same two authors (L.J.P. and E.F.A.) conducted all the bibliographic searches, using the Mendeley® (www.mendeley.com) reference manager software to save studies retrieved from all the databases. Articles whose titles and abstracts did not meet the eligibility criteria were excluded, as well as opinion/technical reports, review articles, guidelines, and letters to the editors. Furthermore, articles not quantifying serum irisin levels, or investigating other therapeutic agents in association with irisin were also excluded. Two authors evaluated articles from the selected abstracts, and judged their suitability by reading their full texts independently of each other. Citations from the reference lists of selected articles were searched manually. The authors solved any disagreements in a consensus session.

Data extraction

The selected articles were submitted to data extraction including the following variables: authors, year of publication, study design, animal characteristics (source and sample size), average age, type of bone, type of physical activity, and irisin administration dose, as well as statistical analyses and main outcomes (Table 1). When the lack of information compromised data extraction, or caused risk of bias, an attempt was made to contact the authors by email in up to 4 consecutive weeks. Two independent authors determined the quality classification criteria and the risk of bias.

Risk of Bias (RoB) assessment

We evaluated the risk of bias using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) RoB tool. This instrument contains 10 entries, related to 6 types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases.[24]

Quality criteria assessment

A quality evaluation of the selected studies was made according to the Animal Research: Reporting *In Vivo* Experiments (ARRIVE) guidelines.[25] This instrument contains a predefined score for 20 categories.[25,26] Each criterion is graded, as previously reported.[25–29] The sum of the scores ranged from zero to 36 points. The maximum score for each domain of the questionnaire was also calculated, as described by Javed et al.[27] The Quality Score/Maximum Score ratio was also calculated, and generated three possible range coefficients, where 0.8–1 was "excellent," 0.5–0.8 was "average," and scores below 0.5 were considered "poor."[27]

Statistical analysis

The multiple meta-analyses were performed by using the META package[30] of R statistical software.[31] We decided to include only studies that evaluated bone mineral density (BMD) using micro-computed tomography (μCT) in each forest plot, to avoid methodological heterogeneity in each meta-analysis. We evaluated healthy/sham and osteoporosis-induced animals separately, and included only studies evaluating intermittent irisin injection essays in the meta-analyses (excluding exercise studies). The inverse variance and the DerSimonian–Laird methods were used to estimate the between-study variance ($\tau 2$).

The mean difference (MD) was the effect measurement (i.e., the mean value in exposure groups – irisin administration – minus the mean value in the sedentary/placebo group – without irisin administration – for both healthy and osteoporotic animals. Random effect models were used for all the analyses. In this design, we used the mean value, the standard deviation and the sample size for each study, as reported (or estimated) for both the experimental and the control groups. The publication bias was not evaluated quantitatively by the Egger test or the funnel plot, despite the small number of studies grouped in the funnel plot.[32]

Results

Study selection and characteristics

A search of all the databases identified 3,844 references. After excluding duplicates, and reading the titles and abstracts, fourteen references were selected for full-text appraisal. Three articles were excluded after reading their full text.[33–35] Kawao et al.[33] and Chen et al.[34] did not investigate irisin administration in vivo, and Xin et al.[35] did not use intra-peritoneal injections (Table 2). Ultimately, sixteen articles were eligible for qualitative assessment. Six of these articles reported μCT-based assessment of cancellous BMD, and comprised the meta-analyses (Figure 1).

Results for individual studies

Only four of the 16 selected studies investigated the effects of exercise on bone parameters. One submitted animals to low-intensity swimming, and another, to resistance ladder climbing (both for 8 weeks).[14,15] The third article subjected one group of animals to voluntary exercise in a polycarbonate running wheel for 2 weeks, and evaluated i.p. 3.24 ng of r-irisin daily for two weeks in another group.[16] The fourth evaluated the effects of an 8-week treadmill running protocol.[36] The remaining 75% of the studies (12/16) evaluated irisin administration.[12,13,17–19,37–39] Most doses of r-irisin were 100 μg/kg i.p. once a

Table 1. Data extraction of the selected animal studies.

References	Animal model (age at beginning of study)	Groups (n/group)	Bone evaluation*	Exercise protocol	Irisin administration / evaluation	Statistical analysis#	Results of exercise or irisin administration	Frequency of irisin administration
Colaianni et al., 2015[13]	C57BL/6 Male mice 2 months old	Control group vehicle n = 4-5 r-irisin group n = 4-5	Dynamic histomorphometry μCT X-ray from femur and tibia	Not performed	r-irisin 100μg/kg i.p. Once a week (4 weeks)	Unpaired Student's t test	r-irisin increased radiodensity of femora and tibia; cortical tissue mineral density (C-TMD) and tibial cortical bone surface No effect on trabecular bone r-irisin increased the three-point bending test and force at peak; bone formation rate (BFR) and mineral apposition rate (MFR) r-irisin increased osteoblasts and decreased osteoclasts	Intermittent
Kim et al., 2015[15]	C57BL/6 Male mice 19 months old	Control group n = 6 Resistance exercise group n = 7	Dual energy X-ray absorptiometry (DXA)	Resistance ladder climbing 3 days/week 8 weeks	Serum irisin quantification Irisin quantification in skeletal muscles extracts (ELISA)	Independent t test	Exercise increased serum irisin levels and soleus skeletal muscle extract. No significant difference in Bone mineral contents or Bone mineral density when compared with control group.	Intermittent
Colaianni et al., 2017[19]	C57BL6 Male mice 2 months old	Control groups – not suspended (n= 8): vehicle r-irisin	μCT and Contact radiography Histological analysis RT-PCR Western Blot	Not performed.	Vehicle (physiologic water sterilized by 0.22 μ filtration) r-irisin 100μg/kg i.p. once a week for 4 weeks Preventive protocol: Once a week (4 weeks) during HLS Curative protocol: after 4 weeks of HLS	One-way analysis of variance (ANOVA) with Bonferroni's post hoc test.	Preventive Protocol HLS decreased both cortical and trabecular BMD and trabecular BV/TV of femur r-irisin prevented and recovered (curative protocol) both cortical and trabecular BMD and BV/TV r-irisin treatment did not altered trabecular BMD and BV/TV of femur in animals not submitted to HLS.	Intermittent

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Colaianni et al., 2017[19]		Hindlimb suspended (HLS) preventive groups (n = 8): vehicle r-irisin HLS curative groups (n = 8): vehicle* r-irisin* reload+ vehicle* rest vehicle+ *Treatment after 4 weeks of HLS + not HLS	*Ex vivo* primary cell cultures	Not performed.		One-way analysis of variance (ANOVA) with Bonferroni's post hoc test.		Intermittent
Zhang et al., 2017[16]	C57BL/6J	Control group: empty cages for two weeks (n = 18).	μCT	Voluntary exercise in polycarbonate running wheel	r-irisin 3.24 ng i.p. daily for two weeks.	One-way ANOVA	Two weeks of voluntary wheel-running exercise increased FNDC5 and PGC1α mRNA levels in bone tissue	Continuous
	APN-KO	Exercise group: voluntary wheel running for two weeks (n = 18).	Histology and immunohistochemical staining protocols	2 weeks	Lentiviral FNDC5, Control EGFP, FNDC5 shRNA lentivirus and scramble shRNA lentivirus i.p. (4×10^8 transducing units per mice) daily for four or two weeks		Protein expression of FNDC5 and irisin increased over sixfold in bone tissue after exercise	
	Male mice	r-irisin for two weeks (n =6)	Bone marrow used for western blot and RT-PCR analysis				r-irisin significantly increased μCT bone volume/total volume, trabecular thickness, and cortical thickness compared with the saline-treated group (μCT analyses only for r-irisin groups versus control. Exercise effects not investigated by μCT).	
	5 weeks old	Saline for two weeks (n=6)	ELISA				The lentiviral injections of FNDC5 in APN-KO mice significantly increased FNDC5 mRNA expression in bone	
		Lentiviral FNDC5 for four weeks (n=5).						

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Zhang et al., 2017[16]	APN-KO	Control EGFP (n=5).				One-way ANOVA		Continuous
	Male mice	FNDC5 shRNA lentivirus + wheel running two weeks (n = 5).						
	5 weeks old	Scramble shRNA lentivirus + wheel running two weeks (n =5).						
Kim et al., 2018[12]	C57BL/6J	9-month-old female mice for OVX experiment and analysis of vertebrae and femurs	Bone histomorphometric analysis	Not performed.	r-irisin 1mg/kg/day i. p. for 6 days.	Two-way ANOVA	FNDC5 null mice had a significantly lower level of RANKL mRNA in whole bones both in males and females, whereas OPG was not altered.	Continuous
		5-month-old female mice used for μCT analysis of femurs and gene expression analysis of tibia	μCT			Unpaired Student's t test	FNDC5 null mice had significantly higher femoral trabecular bone mass and greater connectivity density than wild-type mice	
	FNDC5 KO	8-week-old C57BL/6J wild-type female mice euthanized after 2 weeks of OVX to measure irisin level in plasma	RT-PCR				r-irisin raised the sclerostin mRNA level in osteocyte-enriched bones *in vivo*.	
		8-week-old C57BL/6J wild-type male mice were used for irisin injection experiments	West					
	8 weeks to 9 months old	(n = 4-7 animals per group)	Quantitative Proteomic analysis					
			Ex vivo cell cultures					

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Kim et al., 2018[12]	Male and female mice			Not performed.	r-irisin 1mg/kg/day i. p. for 6 days.			Continuous
	Sprague-Dawley rats	Control -vehicle - group (n = 8).	Static and dynamic histomorphometry of proximal tibia and lumbar vertebrae relative mineralized bone surface, mineral apposition rate, and bone formation rate; and identification of osteoclast and osteoid surfaces				r-irisin decreased osteoclast surface and increased osteoid surface and bone formation rate.	
Narayanan et al., 2018[17]	Male	Vehicle + r-irisin group (n = 8).	Immunohistochemistry of distal femur osteocyte proteins (TNF- α, IL–6, IL-10, IL-4, annexin V, sclerostin, RANKL and OPG).	Not performed.	r- irisin i.p. (18 ng/ml injections) 2 times/ week (3.5 d apart) for 3 weeks.	Two-way ANOVA (2x2 factorial scheme)	IBD caused an increase in osteocytes positive for TNF-α, IL-6, sclerostin and osteoclastogenesis regulators RANKL and OPG. Additionally, osteocyte apoptosis, as measured by annexin V, was elevated in IBD. r-irisin treatment lowered these factors to levels at or lower than vehicle.	Intermittent
		Inflammatory bowel disease (IBD) group (n = 8).						
		Inflammatory bowel disease (IBD) + r-irisin group (n = 8).						
	2-months old							
Kang et al., 2019[14]	prague-Dawley rats.	Control diet group (n = 10).	BMD and microstructure of femur and tibia via μCT.	8 weeks of low-intensity swimming exercise initiated with 45 min/day for the first two weeks and 60 min/day for the last six weeks.	Serum irisin levels were measured using ELISA kits.	Independent t test	8 weeks of swimming exercise improved obesity, BMD, bone microstructure and bone metabolic factors.	Continuous
		High fat diet (HFD) induced osteoporosis (8 weeks) group (n = 10).	Osteocalcin, CTX-1 and irisin levels in the blood			One-way ANOVA	Irisin levels in the blood and the expressions of FNDC5 and PGC-1α in the bone were significantly lower in the HFD group than in the CD group, but higher in the swimming group than in the HFD group.	

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Kang et al., 2019[14]	Male 8 weeks old	High fat diet (HFD) induced osteoporosis (8 weeks) + swimming exercise (further 8 weeks) group (n = 10).	Immunohistochemistry of femur IL-1, β-catenin, PGC-1α and FNDC5 in the tibia and femur	8 weeks of low-intensity swimming exercise initiated with 45 min/day for the first two weeks and 60 min/day for the last six weeks.	Serum irisin levels were measured using ELISA kits.		Swimming exercise reduced HFD-induced IL-1 and increased β-catenin, FNDC5, and PGC-1α in bone.	Continuous
Metzger et al., 2019[36]	Sprague-Dawley rats Male 8 weeks old	Control group (n = 8). Control + irisin (n = 8). Inflammatory bowel disease (IBD) induced by dextran sodium sulfate (n =8). Inflammatory bowel disease (IBD) induced by dextran sodium sulfate + irisin (n = 8).	Volumetric BMD on the proximal tibia and femoral neck and L4 Three-point bend test was conducted at the tibia midshaft and compression test at the femoral neck Dynamic and static histomorphometry and bone immunohistochemistry (TNF-α, IL-6, RANKL, OPG, sclerostin and annexin V) and bone formation rate.	Not performed	r- irisin i.p. (18 ng/ml injections) 2 times/week (3.5 d apart) for 3 weeks.	2 x 2 factorial ANOVA	Animals induced to inflammatory bowel disease showed lower bone mineral density and higher osteocyte pro-inflammatory cytokines. Cancellous vBMD was not different among the four groups r-irisin treatment ameliorated bone inflammatory profile but did not alter bone mineral loss. r-irisin treatment mitigated declines in osteoid surface and restored IBD animals osteoclast surfaces to control levels r-irisin treatment improved bone formation rate without modifying BMD. r-irisin treatment in IBD rats mitigated the increase in TNF-α, IL-6, RANKL, OPG, sclerostin and annexin V	Intermittent

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Metzger et al., 2019[36]				Not performed	r- irisin i.p. (18 ng/ml injections) 2 times/ week (3.5 d apart) for 3 weeks.	2 x 2 factorial ANOVA	r-irisin did not improve bone mechanical properties	Intermittent
Storlino et al., 2020[35]	C57BL6 mice Male mice Experiment 1: 8 weeks old. Experiment 2: 18 months old.	Experiment 1: Control + vehicle group (n = 8). Hindlimb suspended (HLS) + vehicle group (n = 8). HLS + r-irisin group (n = 8). Experiment 2: Old control + vehicle group (n = 8). Old + r-irisin group (n = 8).	Expression of Bcl2 and Bax by qPCR. Total and cleaved Caspase-9 and Caspase-3 expressions in cortical bone by western blotting. Histological analysis of osteocytes survival and empty lacunae of femur cortical bone	Not performed.	100 µg/kg r- irisin i.p. injection once a week for four weeks.	Student t test or ANOVA followed by Tukey's *post hoc* analysis.	r- irisin treatment mitigated apoptotic index by increase of Bcl2/ Bax in both hindlimb and old mice bone. r- irisin prevented disuse-induced reduction of viable osteocytes and increase of empty lacunae, as well as Caspase-9 and Caspase-3 activations.	Intermittent
Iemura et al., 2020[37]	C57BL/6J mice Male (Exp. 1 and 2) 20 weeks old	Experiment 1: Sham group (n = 8). Orchidectomy (ORX) (n = 8). Experiment 2:	Quantitative computed tomography (QCT) of tibia. qPCR analysis of Runx2, Osterix, ALP, osteocalcin, type I collagen (Col-1), RANKL and OPG.	Not performed.	100 µg/kg r- irisin i.p. once a week for eight weeks.	Mann–Whitney U test or Two-way ANOVA followed by Tukey–Kramer test	Animals of androgen deficiency groups showed decreased expression of irisin. r-irisin treatment significantly blunted trabecular BMD and BV/TV reduction in ORX mice. BMD and BV/TV in sham-operated animals were similar with or without r-irisin administration.	Intermittent

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Iemura et al., 2020[37]	Female (Exp. 3) 7 weeks old	Control/sham group (n = 7). Control/ORX group (n = 7). r-irisin/Sham group (n = 7). r-Irisin/ORX group (n = 7). Experiment 3 Sham surgery (n=8). Ovariectomy (OVX) group (n = 8).		Not performed.	100 μg/kg r- irisin i.p. once a week for eight weeks.	Mann–Whitney U test or Two-way ANOVA followed by Tukey–Kramer test		Intermittent
Xu et al., 2020[18]	SPF Sprague-Dawley rats Female No information regarding age	Control group (n =15). Ovariectomized (OVX) group (n = 15). OVX + r-irisin group (n = 15).	μCT of vertebral bone; osteocalcin, TNF-α, and IL-6 (ELISA assay); serum alkaline phosphatase (ALP) qPCR for Bcl-2, Caspase-3, Runx2, OC, Nrf2 and NLRP3	Not performed.	1 mmol/l r-irisin (no information regarding origin, administration via or frequency).	Not clearly defined.	ALP significantly increased in OVX group and declined in r-irisin group. Levels of TNF-α and IL-6 reduced in OVX animals while OC increased. r-irisin minimized these alterations. r-irisin group increased Tb.Th, Tb.N and BMD while Tb.Sp declined compared with that in the OVX group r-irisin group increased mRNA expression levels of Runx2, OC, Bcl-2 and Nrf2, while caspase-3 and NLRP3 displayed the opposite trends	No information regarding frequency
He et al., 2020[43]	C57BL/6 mice	Sham-operated group (n = 10)	μCT of subchondral bone, trabecular bone below the growth plate, and cortical bone in the tibia.	Not performed.	100 μg/kg of r-irisin i.p. weekly for four weeks.	Unpaired Student t-tests (two-tailed) or one-way ANOVA followed by Newman-Keuls multiple comparison tests.	Irisin reduced the expression of matrix metalloproteinase (MMP)-13 in cartilage and caspase 3 in the subchondral bone. Bone volume fraction, trabecular number, connection density, and the structure model index was improved by irisin administration.	Intermittent

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He et al., 2020[43]	Male 3 months old	Control (Anterior cruciate ligament transection – ACLT- operated) group (n =10) ACLT-operated + r-irisin group (n = 10)	Bone histomorphometric analysis	Not performed.	100 µg/kg of r-irisin i.p. weekly for four weeks.	Unpaired Student t-tests (two-tailed) or one-way ANOVA followed by Newman-Keuls multiple comparison tests.	Irisin increased subchondral bone remodeling, proliferation of osteocytes, and attenuated osteoarthritis progression.	Intermittent
Luo et al., 2020[41]	C57BL/6 mice Female 10 weeks old	Sham + saline group (n = 12) OVX + saline group (n = 12) OVX + r-irisin group (n = 12)	µCT of cortical and trabecular bone in the femur. Strength evaluation of tibia. Bone formation and resorption parameters of distal femurs.	Not performed.	100 µg/kg of r-irisin i.p. twice a week for five weeks.	One-way ANOVA, followed by Tukey's test.	Irisin increased bone mineral density, bone volume ratio, connection density, and trabecular parameters. In OVX mice, irisin increased bone stiffness, number of osteoblasts on the trabecular surface and reduction of osteoclasts numbers. r-irisin-treated OVX mice presented a higher osteocalcin level and a lower tartrate-resistant acid phosphatase serum concentration.	Intermittent
Metzger et al., 2020[42]	Sprague-Dawley rats Male	Control group (n =6) Hindlimb unloaded (HU) group (n = 6)	Volumetric bone mineral density (accessed by peripheral quantitative computed tomography) of the proximal tibia metaphysis, femoral neck, and proximal humerus. Dynamic and static cancellous histomorphometry of the proximal tibia metaphysis, femoral neck, fourth lumbar vertebrae, and proximal humerus.	Not performed.	18 ng/ml injections of r-irisin i.p. three times per week for four weeks.	One-way ANOVA, followed by Tukey HSD *post hoc* test.	Irisin treatment increased bone formation rate, and lowered osteoclast surfaces and osteocyte TNF-α, IL-17, RANKL, and sclerostin in the unloaded hindlimb.	Intermittent

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Metzger et al., 2020[42]	8 weeks old	Hindlimb unloaded + r-irisin group (n = 6)	Immunohistochemistry for cathepsin-K-covered bone surfaces.	Not performed.	18 ng/ml injections of r-irisin i.p. three times per week for four weeks.	One-way ANOVA, followed by Tukey HSD *post hoc* test.	Irisin treatment increased bone formation rate, and lowered osteoclast surfaces and osteocyte TNF-α, IL-17, RANKL, and sclerostin in the unloaded hindlimb.	Intermittent
Morgan et al., 2021[40]	Wistar rats Female No information regarding age	Control group (n = 10) Sham group (n = 10) OVX + vehicle group (n = 10) OVX + irisin group (n = 10)	Calcium content in right femur (detected by an atomic absorption spectrophotometer). Inorganic phosphate content (detected by a spectrophotometer according to Plummer's method). Number of osteocytes, osteoclasts, and resorbed bone cavities of bone tissue, evaluated by histological examination.	Not performed.	100 µg/kg/week of irisin for four weeks (no information regarding via).	One-way ANOVA, followed by Tukey LSD *post hoc* test.	Irisin administration increased dry and ash weight of the femur, as well as concentration of calcium and phosphorus in bone ash. OVX + irisin animals presented smooth periosteal and endosteal surfaces with few subperiosteal resorbed bone cavities, numerous osteocytes, and few osteoclasts Irisin treatment reduced serum levels of osteocalcin, bone alkaline phosphatase, tartrate-resistant acid phosphatase, calcium, phosphorus and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).	Intermittent
Zhao et al., 2021[36]	C57BL/6 mice	Sham group (n = 10)	µCT of cortical and trabecular bone in the right femur.	8 weeks of treadmill running protocol, starting with speed gradually increasing (from 8 meters/min to 13 meters/min for 30 minutes in the first week) followed by 5 days/week sections of 45min at a speed of 13 meters/min and with a slope of −9° in the other weeks.	FNDC5 expression in tibia accessed by Western Blot and RT-PCR techniques.	One-way ANOVA, followed by Bonferroni *post hoc* test.	Exercise promoted mRNA expression of FNDC5 (irisin precursor), Akt and b-catenin, and enhanced serum irisin levels compared to OVX group.	Continuous

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				After 3 weeks of surgical operation, Exercise + cyclo RGDyk group mice were treated twice weekly with 2.5 mg/kg cyclo RGDyk.				
Zhao et al., 2021[36]	Female	OVX (n = 10)	Alkaline phosphatase activity accessed by staining of proximal left tibiae.		FNDC5 expression in tibia accessed by Western Blot and RT-PCR techniques.	One-way ANOVA, followed by Bonferroni *post hoc* test.	Exercise increased cortical and trabecular volumetric bone mineral density (vBMD) as well as trabecular bone volume fraction (BV/TV), thickness (Tb.Th), numbers (Tb.N) and separation (Tb.Sp) compared to OVX group.	Continuous
	3 months old	OVX + exercise group (n = 10)	Expression of fibronectin type III domain-containing protein 5 (FNDC5) in right tibia accessed by Western Blot and RT-PCR techniques.					
		Exercise + cyclo RGDyk (anti-irisin receptor agents) group (n = 10)						

*Description of studies design and analysis attained only to in vivo experiments. #Statistical analysis as described in each manuscript. Some studies did not investigate the effects of r-irisin in all animal groups. μCT: micro computerized tomography; SPF: specific pathogen free.

week for four weeks[13,19,37,40] or eight weeks;[39] or else twice a week for five weeks.[41] However, Kim et al.[12] administered 1 mg/kg/day of r-irisin i.p. for 6 consecutive days; while Narayanan et al.[17] and Metzger et al.[38] administered 18 ng/ml twice a week for 3 weeks; Metzger et al.,[42] 18 ng/ml injections of r-irisin i.p. three times a week for four weeks; and Xu et al.[18] , 1 mmol/l for an undetermined period.

Table 2. Articles excluded and reasons for exclusion (n = 3).

Reference	Reason for exclusion
Kawao et al., 2018[33]	Study does not evaluate irisin administration *in vivo*
Chen et al., 2020[34]	Study does not evaluate irisin administration *in vivo*
Xin et al., 2020[35]	Study does not evaluate intra-peritoneal injections.

Exercise increased serum irisin[15], as well as FNDC5 and PGC1α mRNA levels in bone tissue.[14,16,36] When investigating healthy rodents, no significant difference was found in bone mineral content or BMD.[15,36] However, when osteoporosis was induced by a high-fat-diet (HFD), irisin administration resulted in BMD improvement.[14]

Administration of r-irisin caused no effects on the trabecular bone of healthy mice,[13,19] but increased cortical tissue mineral density (C-TMD) and tibial cortical bone surface.[13] When applying hindlimb suspension (HLS), r-irisin recovered both cortical and trabecular BMD,[19] mitigated the apoptotic index with an increase in Bcl2/Bax, prevented an increase in empty lacunae and in Caspase-9 and Caspase-3 activations[37], increased the bone formation rate, and lowered osteoclast surfaces, osteocyte TNF-α, IL-17, RANKL, and Sost in the unloaded hindlimb.[42] When

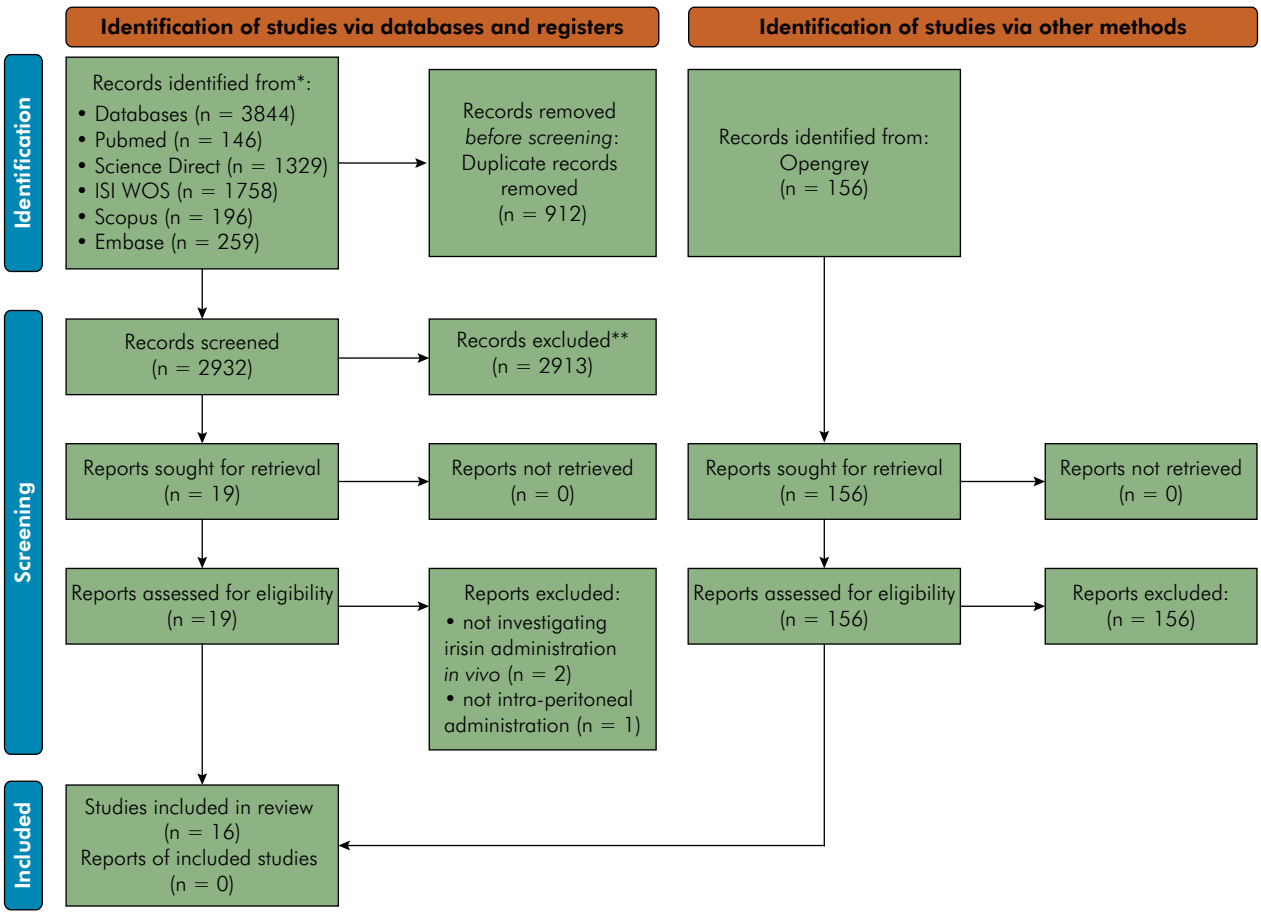

Figure 1. Flow diagram of the screened articles adapted from the PRISMA statement.

osteoporosis was induced by inflammatory bowel disease (IBD), r-irisin decreased the osteoclast surface, and increased the osteoid surface and the bone formation rate[17], in addition to mitigating the increase in TNF-α, IL-6, RANKL, OPG, Sost and annexin V.[38] After Orchidectomy/Ovariectomy (ORX/OVX), r-irisin treatment significantly prevented trabecular BMD, and bone volume/total volume (BV/TV) reduction[39,41], increased Tb.Th, Tb.N, and reduced Tb.Sp.[18] He et al.[43] reported that irisin treatment caused an increase in bone volume fraction, in trabecular number and connection density, and an improvement in the structure model index, besides reducing serum levels of osteocalcin, bone alkaline phosphatase, TRAP, calcium and phosphorus.[40]

Conversely, the results found by Kim et al.[12] were the opposite of all the other studies, namely r-irisin injections (daily for 6 days) increased the Sost mRNA level in 8-week-old wild-type C57BL/6J mice. Moreover, FNDC5 null mice presented significantly lower levels of RANKL mRNA in bones, whereas the OPG was not altered (Table 1).

Bias risk assessment and quality criteria assessment

Data extraction (Table 1) and bias risk assessment (Table 3) indicated low risk of bias for most studies in "selective outcome reporting" (100%) and "baseline characteristics" (93.8%). The "sequence generation" was considered adequate for 43.8% of the studies. On the other hand, there was a high risk of bias for almost all the studies in both the "allocation concealment" and "blinding of participants and personnel" domains. Most studies did not provide sufficient information (or left it unclear) regarding the "random outcome assessment," or presented "incomplete outcome data" (Table 3).

The total score obtained using the ARRIVE guidelines ranged from 19 to 34 points (mean score 27.87 ± 4.51), from a maximum of 36. Nine categories scored "excellent" (between 0.8-1.0), and nine categories were classified as "average" (between 0.5-0.8). Only two categories were classified as "poor" (below 0.5), namely allocation and results baseline data. (Table 4).

Table 3. Assessment of risk of bias in included studies.

Study	A	B	C	D	E	F	G	H	I	J
Colaianni et al., 2015[13]	-	-	-	-	-	-	-	?	+	?
Kim et al., 2015[15]	+	+	-	?	-	?	-	?	+	?
Colaianni et al., 2017[19]	+	+	-	-	-	?	-	?	+	+
Zhang et al., 2017[16]	+	+	-	+	-	?	-	?	+	?
Kim et al., 2018[12]	-	+	-	-	-	?	-	+	+	?
Narayanan et al., 2018[17]	+	+	-	?	-	?	?	?	+	?
Kang et al., 2019[14]	+	+	-	-	-	?	-	?	+	+
Metzger et al., 2019[36]	-	+	-	-	-	?	+	+	+	?
Storlino et al., 2020[35]	+	+	+	?	+	?	?	?	+	+
Iemura et al., 2020[37]	-	+	-	-	-	?	+	+	+	?
Xu et al., 2020[18]	-	+	-	-	-	-	-	-	+	-
He et al., 2020[43]	-	+	-	?	?	?	+	?	+	?
Luo et al., 2020[41]	-	+	-	?	-	?	-	?	+	?
Metzger et al., 2020[42]	-	+	-	?	-	?	-	?	+	?
Morgan et al., 2021[40]	-	+	-	-	-	-	-	-	+	?
Zhao et al., 2021[36]	+	+	-	?	-	?	-	?	+	?

A: sequence generation; B: baseline characteristics; C: allocation concealment; D: random housing; E: blinding of participants and personnel; F: random outcome assessment; G: blinding of outcome assessment; H: incomplete outcome data; I: selective outcome reporting; J: other bias; +: Yes (Low risk of bias); ?: unclear; -: no (high risk of bias).

Table 4. Scores of quality assessment according to ARRIVE guidelines of the studies including animal models.

Study	ARRIVE items																				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	Total
Colaianni et al., 2015[13]	1	1	2	1	2	0	1	1	0	1	0	2	2	0	1	2	1	1	2	2	23
Kim et al., 2015[15]	1	2	2	1	2	2	1	2	2	1	1	2	2	1	2	2	2	1	2	2	33
Colaianni et al., 2017[19]	1	1	2	1	2	2	1	2	1	1	1	2	2	1	1	2	1	1	2	2	29
Zhang et al., 2017[16]	1	1	2	1	2	1	1	2	2	1	1	2	2	1	2	2	1	2	2	2	31
Kim et al., 2018[12]	1	1	2	1	2	1	1	2	1	1	1	2	2	0	1	2	1	1	2	2	27
Narayanan et al., 2018[17]	1	1	2	1	2	2	2	2	2	2	1	2	2	1	1	2	2	2	2	2	34
Kang et al., 2019[14]	1	2	2	1	2	1	1	2	2	1	1	2	2	0	1	2	1	1	2	2	29
Metzger et al., 2019[36]	1	2	2	1	2	1	1	1	2	1	0	2	2	0	2	2	2	2	2	2	30
Storlino et al., 2020[35]	1	1	2	1	2	2	2	2	2	2	1	2	2	0	2	2	1	1	2	2	32
Iemura et al., 2020[37]	1	1	2	1	2	1	1	1	1	1	0	2	2	0	1	2	2	1	2	2	26
Xu et al., 2020[18]	1	1	1	1	2	0	0	1	0	1	0	2	1	0	1	2	1	1	2	1	19
He et al., 2020	1	2	2	1	2	1	1	2	1	1	0	2	2	0	1	2	1	1	2	2	27
Luo et al., 2020	1	1	2	1	2	1	1	1	1	1	0	2	2	0	2	2	1	1	2	2	26
Metzger et al., 2020	1	2	2	1	2	1	1	2	2	2	0	2	2	1	1	2	2	2	2	2	32
Morgan et al., 2021	1	1	2	1	2	1	1	0	0	1	0	2	2	0	0	2	1	0	2	0	19
Zhao et al., 2021	1	2	2	1	2	2	1	2	1	1	0	2	2	0	2	2	1	1	2	2	29
Category score (quality obtained)	16	22	31	16	32	19	17	25	20	19	7	32	31	5	21	32	21	19	32	29	446
Maximum score expected (quality expected)	16	32	32	16	32	32	32	32	32	32	16	32	32	16	32	32	32	32	32	32	576
Ratio quality score/ maximum score	1.00	0.68	0.97	1.00	1.00	0.59	0.53	0.78	0.62	0.59	0.43	1.00	0.96	0.31	0.66	1.00	0.65	0.59	1.00	0.90	0.77

A: title; B: abstract; C: introduction-background; D: introduction-objectives; E: methods-ethical statement; F: study design; G: experimental procedure; H: experimental animals; I: housing and husbandry; J: sample size; K: allocation; L: experimental outcomes; M: statistics; N: results baseline data; O: number analyzed; P: outcome, and estimation; Q: adverse events; R: discussion-interpretation/scientific implications; S: general applicability/relevance; T: funding; Total: represents total score obtained by each manuscript out of a maximum of 36 points.

Quantitative analysis of the studies (meta-analyses)

Six of the 16 articles included in the systematic review presented BMD data and were included in the meta-analyses and forest plots (Figure 2). There was moderate to high heterogeneity among the studies (I^2 = 31% for non-osteoporotic, and 88% for osteoporotic animals). Random effect models were preferred.

The BMD for healthy/sham animals receiving irisin, in comparison with animals receiving placebo, indicated a random effect (MD) of zero (95% CI −0.01; 0.01), whereas the random effect (MD) for BMD in osteoporosis-induced animals receiving irisin was 0.03 mg/cm^3 (95% CI 0.01-0.05), in comparison with animals receiving placebo (with a right dislocated diamond without crossing the midline) (Figure 2).

Discussion

The findings of the present study indicated that exercise and r-irisin administration brought about significant positive effects on bone tissues. The meta-analysis showed increased BMD in osteoporosis-induced rodents (but not normal animals) after intermittent irisin injection. These results are important to dentistry, since oral signs and symptoms associated with osteoporosis can cause physical and psychological stress.[44]

The models used to induce bone loss varied among the studies. In these models, bone loss could be linked to systemic and/or local inflammation. An HFD[14] elevates fat accumulation and pro-inflammatory cytokines (TNF-α, IL-1, and IL-6), and, in turn, induces osteoclast differentiation and activity by regulating the receptor activator of NF-κB (RANK) and RANK ligand (RANKL) pathways.[45,46] Ovariectomy[18,39] induces

Study	Experimental		Control		Mean Difference	MD	95%CI	Weight
	Total Mean	SD	Total Mean	SD				
OSTEOPOROSIS = Normal								
Colaianni et al., 2015	6	0.14 0.0100	6	0.13 0.0100		0.01	[0.00; 0.02]	9.9%
Colaianni et al., 2017	7	0.09 0.0070	7	0.08 0.0050		0.00	[-0.01; 0.01]	10.4%
Metzger et al., 2019	8	0.15 0.0450	8	0.19 0.0600		-0.04	[-0.09; 0.01]	4.0%
Metzger et al., 2019	8	0.77 0.0440	8	0.79 0.0330		-0.02	[-0.06; 0.02]	5.6%
Iemura et al., 2020	7	0.43 0.0200	7	0.43 0.0200		0.00	[-0.02; 0.02]	8.4%
Random effects model	36			36		0.00	[-0.01; 0.01]	38.4%
Heterogeneity: I^2 = 31%, τ^2 = <0.0001, p = 0.21								
OSTEOPOROSIS = Osteoporosis								
Colaianni et al., 2017	7	0.07 0.0100	7	0.05 0.0090		0.02	[0.01; 0.03]	10.3%
Metzger et al., 2019	8	0.70 0.0070	8	0.68 0.0620		0.02	[-0.05; 0.09]	2.5%
Metzger et al., 2019	8	0.13 0.0450	8	0.14 0.0250		-0.01	[-0.04; 0.02]	7.6%
Iemura et al., 2020	7	0.39 0.0440	7	0.31 0.0100		0.08	[0.06; 0.10]	9.1%
Luo et al., 2020	12	0.72 0.0200	12	0.67 0.0400		0.04	[0.02; 0.07]	7.2%
Metzger et al., 2020	6	0.14 0.0450	6	0.13 0.0130		0.02	[-0.01; 0.04]	8.3%
Metzger et al., 2020	6	0.50 0.0440	6	0.47 0.0230		0.02	[0.00; 0.05]	7.4%
Metzger et al., 2020	6	0.21 0.0200	6	0.20 0.0160		0.01	[-0.01; 0.02]	9.2%
Random effects model	60			60		0.03	[0.01; 0.05]	61.6%
Heterogeneity: I^2 = 88%, τ^2 = 0.0007, p < 0.01								
Random effects model	96			96		0.01	[0.00; 0.03]	100.0%

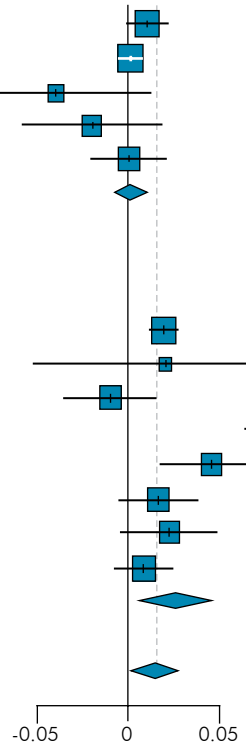

Figure 2. Forest plot and meta-analysis of bone mineral density (BMD) in healthy/sham and osteoporosis-induced animals receiving intermittent irisin injections.

estrogen deficiency, which enhances the production of interleukin IL-1, IL-6, IL-7, TNF-α and the granulocyte macrophage colony-stimulating factor (GMCSF) by immune cells, leading to osteoclastogenesis and bone resorption.[47] Mechanical unloading[19,37] induces osteocyte apoptosis and the release of intracellular molecules.[48] These molecules (such as high mobility group box 1 - HMGB1, purine metabolites, heat-shock proteins, and uric acid)[47,49] induce the recruitment and activation of macrophages, with consequent secretion of TNF-α, IL-6 and IL-1, initiating inflammatory bone loss.[47,48,50] This mechanism involves upregulation of the RANKL/OPG ratio, hence interfering with Wnt/β-catenin signaling, and increasing Sost production.[37] Inflammatory bowel disease (IBD)[17,38] initiated in the gut results in increased osteocytes positive for TNF-α, IL-6, RANKL and Sost.[51]

The anti-osteoporotic mechanism of irisin seems to involve not only an increase in the number and activity of osteoblasts,[16] but also the suppression of Sost.[13] Sost is upregulated by the inflammatory cytokine TNF-α, which is associated with an increase in RANKL, leading to increased osteoclastic activity.[17,52] The more pronounced effects of irisin on osteoporosis-induced animals might be related to the anti-inflammatory activity of irisin, since muscle-specific PGC-1α knockout animals present upregulation of local muscle inflammatory genes. Moreover, inflammatory diseases have low levels of serum irisin.[53]

Although the great majority of retrieved studies indicated no or only mild positive effects of irisin on bone, Kim et al.[12] found contrasting results, in which irisin increased Sost expression in osteocytes. The explanation for these discrepancies was attributed to differences in the therapeutic scheme of irisin injections. Supposedly, the positive effects of irisin on bone depend on intermittent treatment (reported in all the studies included in the meta-analyses), whereas continuous treatment induces bone resorption[12]. Only the study by Kim et al.[12] evaluated the effect of continuous irisin administration on bone using μCT, hence precluding any comparison. Indeed, a more recent study by Storlino et al.[37] found that Sost mRNA was severely downregulated only upon intermittently administrated irisin, even though other key genes expressed by MLO-Y4 cells were modulated by irisin treatment, administered either continuously or by intermittent short pulses.

Most studies researched intermittent r-irisin administration, while only four investigated exercise models. Kim et al.[15] showed that progressive resistance training did not alter bone quality, including bone mineral content (BMC) and BMD. On the other hand, Zhang et al.[16] found that voluntary exercise increased irisin production and osteogenesis in mice. In the latter study, mice ran an average of five thousand meters a day, whereas the most frequent exercise protocols for rodents call for a one-hour session, 3 to 5 days a week. Increased levels of irisin from physical exercise may vary depending on training intensity and duration. Myokine delivery depends on the intensity and duration of the exercises.[54] Investigations into the effects of different types of exercises and other variables, such as intensity and frequency, are important to gain a better understanding of how irisin works in bone remodeling. The comparison among studies was hindered by their heterogeneity of bone parameters, irisin quantification and exercise protocols.

It is important to consider that we selected only studies using μCT. Moreover, we also conducted subgroup meta-analyses with and without osteoporosis in studies evaluating intermittent irisin injections, but excluded exercise and continuous irisin administration studies in the meta-analyses. Even after controlling all these aspects, we observed that osteoporosis-induced BMD meta-analyses showed high heterogeneity. Heterogeneity over 60% is very common in a meta-analysis that uses animal studies. Rather than abort the meta-analysis design, we felt that the random effect model would be more suitable, because it fits the variation in animal studies better.[55,56]

In evaluating the quality criteria accessed using the ARRIVE guidelines,[25] we observed that the categories of "experimental procedure," "sample size," and "results baseline data" received the lowest ratings. Previous research evaluating the quality of interventional animal studies in rheumatology using the ARRIVE guidelines reported that none of the 41 studies that were investigated reported sample size calculation, or details regarding the animal allocation method, randomization or assessor blinding.[60] In the present study, only one article clearly reported the sample size

calculation.[17] This is a very significant shortcoming, since studies with an inadequate sample size could provide false-negative results, thus leaving potential findings undetected. We believe that the lack of some information may have resulted from restrictions placed on the word count (e.g., abstracts). However, several important journals are adopting the ARRIVE guidelines to improve the reporting quality of publications.[60]

In the present study, we used the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) RoB tool[24] to evaluate the quality of the retrieved animal studies. Our results were similar to those found by previous systematic reviews of pre-clinical studies regarding the risk of bias.[57] Bias due to inadequate information about randomization and blinding is frequent in animal experiments.[58] Attention to these items is crucial to avoid subjective outcome measurements, and to reduce implementation or measurement bias.[59]

Limitations of the present research protocol relate to the lack of information contained in several studies, regarding such factors as randomization, sample size calculation and blinding. However, the overall scores of the ARRIVE guidelines indicated almost 88% adherence (considered as average or excellent - Table 3). Nevertheless, investigations probing the effects of different types of exercises (swimming, ladder climbing or running wheel; voluntary or forced activity), divergent dosages of r-irisin (and frequency), and different sources of osteoporosis induction hindered making adequate comparisons. Worthy of note, the irisin effect maintained the same overall direction in the majority of studies, as indicated in the meta-analyses.

Previous research conducted by our group has shown the positive effects of exercise on alveolar bone quality. Physical practice attenuated the bone loss and epithelial attachment loss levels of rats with ligature-induced periodontal disease. Animals with periodontal disease (PD), submitted to training, presented lower TNF-α expression in periodontal tissues, whereas IL-10 was higher. The TNF-α/IL-10 ratio was also lower in PD-affected animals that exercised, compared with sedentary ones.[5] Moreover, a systematic review using human observational studies indicated that physical activity was directly associated with a lower occurrence of periodontitis.[23] Likewise, aerobic and resistance training reduced orthodontic tooth movement, enhanced the quality of maxillary bone, and increased BMD, trabecular BV, and the BV/TV ratio.[6] In the last cited study, the FNDC5 gene expression of the maxillary bone subject to orthodontic tooth movement was negatively affected. This suggests that the local synthesis and release of pro-inflammatory metabolites during tooth movement[61,62] might downregulate irisin activity.[63] Irisin was recently discovered in 2012. Since then, its physiological role has been under ongoing investigation. Understanding how irisin functions may be key to comprehending many diseases and their development.[64]

Conclusions

Based on the present findings, exercise and/or irisin injections have induced significant bone quality improvements in osteoporotic rodents, in contrast to their non-significant effects on healthy ones. Implications of key findings evidence the potential of irisin as an agent able to mitigate bone loss caused by osteoporosis, an outcome that could favor dental rehabilitation. More studies investigating the effects of irisin on alveolar bone are needed to elucidate its therapeutic viability and implications.

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