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Review Article

RANKL/RANK/OPG Pathway: A Mechanism Involved in Exercise-Induced Bone Remodeling

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Bones as an alive organ consist of about 70% mineral and 30% organic component. About 200 million people are suffering from osteopenia and osteoporosis around the world. There are multiple ways of protecting bone from endogenous and exogenous risk factors. Planned physical activity is another useful way for protecting bone health. It has been investigated that arranged exercise would effectively regulate bone metabolism. Until now, a number of systems have discovered how exercise could help bone health. Previous studies reported different mechanisms of the effect of exercise on bone health by modulation of bone remodeling. However, the regulation of RANKL/RANK/OPG pathway in exercise and physical performance as one of the most important remodeling systems is not considered comprehensive in previous evidence. Therefore, the aim of this review is to clarify exercise influence on bone modeling and remodeling, with a concentration on its role in regulating RANKL/RANK/OPG pathway.

1. Introduction

Bones as an alive organ consist of about 70% mineral and 30% organic material. Calcium and phosphorous crystals, hydroxyapatite, and some ions such as sodium, fluoride, and magnesium are constituents of the mineral part. The organic part contains mostly collagen fiber and, in a lower amount, glycoproteins and proteoglycans [1]. The skeleton has several roles in the body such as protecting internal organs, frame of the body, and safe storage for some vital minerals like calcium. In contrast with what it seems, bones are a vivid tissue which is in turnover all the time [2]. About 200 million people are suffering from osteopenia and osteoporosis around the world; approximately 1 out of 3 women and 1 out of 5 men older than 50 years old have some forms of bone abnormalities [3]. From the population aging, it has been estimated that the prevalence of bone diseases would rise up in the near future. In the United States of America, it is estimated that bone disorders would increase 2.4 times in women and 3.1 times in men until 2050 [4].

Bones have various types of cells including osteoclasts, osteoblasts, osteocytes, and bone lining cells [5, 6]. Osteoblasts are originated from hematopoietic stem cells (HSCs, macrophage lineage of hematopoietic stem cells), and osteoclasts are originated from mesenchymal stem cells (MSCs) via some stages such as osteoprogenitors and preosteoblasts [7]. Fundamentally, bone modeling and remodeling include osteoclasts function in the removal of the bone surface and osteoblasts function on precipitating new matrix in them [8, 9]. This process is responsible for protecting skeleton function and fracture restoring. Any kind of defect in bone turnover coordination would result in bone diseases such as Paget's disease, fibrous dysplasia, osteoarthritis, osteoporosis, and fragility fractures [10–13].

Osteoclasts are the major cells in charge of bone resorption. They are positioned on the surface of bones and form trenches by their function. Activated osteoclasts release proteolytic enzymes which destroy connective tissues in bones. They also secrete some acids that resolve the mineral part of bones [14]. Through the different stages of osteoblasts differentiation, the level of some biomarkers, which are known as osteogenic markers, changes significantly. Among these markers, osteocalcin (OCL), Runx2, alkaline phosphatase, and osterix (Osx) can be named. On the other hand, for modulating monocyte-to-osteoclast differentiation, osteoblasts would release osteoprotegerin (OPG) and receptor activator of NF-kB ligand (RANKL), as well as macrophage colony-stimulating factor (M-SCF) [7, 15, 16]. RANKL/RANK, Wnt/b-catenin, and Jagged1/Notch1 are 3 important pathways modulated by osteoblasts which affect the bone mass density via the regulation of osteoblasts and osteoclasts functions [8]. In the RANKL/RANK/OPG pathway, RANKL binds to RANK as its receptor and eventually leads to osteoclast precursor maturation. Osteoprotegerin is known as a decoy receptor for RANKL which prevents RANKL-RANK binding and the following reactions [17].

There are several risk factors for bone health such as aging [18], estrogen deficiency, inflammation [14], metabolic diseases, improper diets [19], kidney dysfunction [20], side effects of some drugs like glucocorticoids [21], and oxidative stress [22]. There are various ways to protect the skeleton from disease and resorption or at least delay the onset of such disorders. For example, physical activity, healthy diets, and medical intervention can help the prevention of age-related bone loss or osteoporosis [18]. Several medications like bone resorption inhibitors and bone formation stimulators are in a postmenopausal treatment lineup [23]. These include bisphosphonates (e.g., alendronate) [24], strontium ranelate [25], denosumab (RANKL inhibitor) [26], and PTH [27]. A limitation in this kind of treatment is the risks of complications such as fever or muscle pain [28, 29]. Having a proper regimen that is nutrient-dense is one of the major strategies in saving and augmenting bone mass. Vitamin D, calcium, phosphorus, magnesium, zinc, and copper are some examples of necessary nutrients for skeleton health [4, 30, 31].

Planned physical activity is another useful plan for maintaining optimal bone health. It has been suggested that planned exercise would effectively regulate bone metabolism [29, 32]. Some studies have reported that exercise may postpone the beginning of osteoporosis by improving peak bone mass within adolescence [33, 34]. The exact mechanism by which exercise improves bone health is not clear yet. However, it has been accepted that increasing muscle mass and mechanical stress in bones results in boosting osteoblasts activities [35–37]. Previous studies reported different mechanisms covering the effect of exercise on bone health by modulation of bone remodeling. However, the regulation of RANKL/RANK/OPG pathway as one of the most important remodeling systems is not considered comprehensive in previous reports. Therefore, the aim of this review was to clarify exercise influence on bone modeling and remodeling, with a concentration on the role of the RANKL/RANK/OPG pathway.

2. The RANK/RANKL/OPG Pathway

The RANKL/RANK/OPG system is known for its roles in osteoclasts maturation, bone modeling, and bone remodeling. Receptor activator of NF-kB (RANK), receptor activator of NF-kB ligand (RANKL), and osteoprotegerin (OPG) are the main components of this signaling system. Interestingly, taking part in bone hemostasis is not the only effect of the RANKL/RANK/OPG pathway [2].

RANKL (also known as OPGL, ODF, and TRANCE), as a homotrimeric protein, is produced by osteoblasts and some other cells like activated T cells [38–40]. The secreted type of RANKL is a result of proteolytic division or alternative splicing on the membrane form [41]. Matrix metalloproteases (MMP3 or 7) and ADAM (a disintegrin and metalloprotease domain) are responsible for RANKL proteolytic cleavage [42, 43]. RANKL, which is a secretion of preosteoblasts, osteoblasts, osteocytes, and periosteal cells [44–46], make RANK activated, which is expressed by osteoclasts and its precursors [47]. RANKL has assignments for stimulating preosteoclasts' differentiation [48], adherence osteoclasts to bone tissue [49] and their following activation [48, 50], and their maintenance [51]. Preosteoclasts combine together and make a multinuclear cell which is affected by RANKL [8] not clear.

RANKL can be also produced by other organs such as thymus, lymph nodes, lung, and mammary glands, as well as the spleen and bone marrow [40]. RANKL might be released from epithelial cells in the lobules of mammary glands during pregnancy. Based on an animal study, RANKL helps in hyperplasia of these epithelial cells which is necessary for lactation and milk production [52].

RANK is also a homotrimeric transmembrane receptor from the TNF family. Its primary expression is limited to OPCs, dendritic cells, and mature osteoclasts [38]. RANK does not have innate protein kinase activating activity as other TNF family receptors have. All of the TRAFs 2, 5, and 6 bind to RANK but only TRAF 6 is required for bone health [53–56]. Aside from bone cells, RANK would be expressed by some carcinoma cells, such as breast cancer or prostate cancer, and also expressed in the mammary gland [52, 57, 58]. One of the roles of RANK that has received attention is its role in cancer cell proliferation; this makes RANK interesting in future therapy for cancers [57].

In addition to osteoblasts, there are plenty of cells that could express osteoprotegerins, such as the heart, liver, spleen, and kidney. A recent study suggested that B cells are in charge of 64% of bone marrow OPG expression [59]. As a TNF superfamily, OPG plays an anti-osteoclastogenesis role with binding to RANKL [60]. OPG takes part as a decoy receptor for RANKL and inhibiting RANKL-RANK binding through it. In fact, several agents that induce RANKL influence OPG regulation [61, 62]. Recent studies have shown that increases in plasma OPG levels in postmenopausal women lead to bone mass reinforcement [50]. Furthermore, in an experiment conducted by the use of mice, OPG was found to be a protector of large vessels from calcification [49]. Moreover, OPG has been suggested as an inhibitor of atherosclerotic plaque calcification [63].

3. RANKL/RANK/OPG Pathway and Bone Metabolism

Before the discovery of the RANKL/RANK/OPG signaling pathway in the 1990s, it was suggested that some agents expressed by osteoblasts are responsible for osteoclasts activation. But it was unexpected that these agents have been members of the TNF superfamily and they could have more functions than the bone turnover in the body [2]. Obviously, it is osteoblasts' task to recruit osteoclasts for bone resorption sites. Also, osteoblasts could regulate bone resorption by secreting OPG and RANKL. In fact, RANKL embedded from osteoclasts binds to its receptor (RANK) on the surface of OCPs and increases osteoclasts differentiation and mature osteoclasts. OPG could bind to RANKL and inhibits osteoclasts differentiation which means upregulation of OPG/RANKL ratio preventing osteoclastogenesis [64, 65]. Similar to other TNF family receptors, RANK does not have any innate protein kinase activities to regulate the signaling pathway. TRAF 6 is the only essential TRAF, among all TRAFs, that bind to RANK for regulating OCPs and osteoclasts activities. To support this claim, several studies have reported that a deficiency in TRAF 6 results in the development of osteoporosis [8, 53–55].

Probably the conclusive determinant in bone resorption is the RANKL/OPG ratio. Most of the time, both RANKL upregulation and OPG downregulation lead to bone loss [66]. There are several endogenous factors that affect the control of the RANKL/RANK/OPG system including some cytokines (TNF-a, IL-1, IL-6, IL-4, IL-11, and IL-17), hormones (vitamin D, estrogen, and glucocorticoids), and mesenchymal transcription factors [13, 67]. OPG is regulated by not only cytokines, hormones, and growth factors but also by Wnt/b-catenin [8, 68–70]. For osteoclast precursors conversion to mature osteoclasts, c-Fos is needed which is an activated transcription factor for RANKL [2, 71]. sRANKL is the soluble form of RANKL which appears in plasma. Elimination of RANKL and RANK in animal studies shows a major effect in inhibiting bone mass loss and osteoporosis [72]. Based on clinical observations, enhancing OPG concentrations in plasma leads to bone mass density augmentation in postmenopausal women [73].

In so many skeleton and nonskeletal disorders, alterations in RANKL and OPG proteins and their mRNA are observable [66]. Enhancement in ROS (reactive oxygen species) production through the function of NADPH oxidase enzymes controls osteoclastogenesis via regulating RANKL expression [14, 74]. Also, proinflammatory cytokines which increase in inflammatory conditions lead to the overexpression of RANKL by T cells that correlate with lower bone mass density (BMD) [56, 75]. In some pathological conditions like postmenopausal osteoporosis or arthritis rheumatoid which influenced hormones and cytokines level, bone resorption would significantly increase. These types of diseases would increase bone remodeling mostly through RANKL and M-CSF expression enhancement [2, 9].

Juvenile Paget disease is diagnosed via osteopenia, fractures, fast remodeling in woven bones, and development of bone deformities. In two Paget disease cases, depletion in OPG has been reported [38, 76]. Idiopathic hyperphosphatasia is also an autosomal osteopathic medicine and alterations in OPG level have a vital role in this disease. In this regard, OPG inactivation has been observed in some clinical trials [38, 69]. RANK signaling pathway is involved in a giant cell tumor of bone (GCTB) which is a rare and painful cancer. This cascade leads to excessive bone resorption and metastasis in these patients [77]. In rheumatoid arthritis, the inflammation advent results in overexpression in RANKL and subsequently bone-weakening [78].

3.1. Exercise and Bone Health. Exercise or planned physical activity is supposed to contribute to maintaining optimal health and healthy body weight [79, 80]. Exercise could indicate a "rejuvenating effect" and possibly prevent age-related skeleton disorders and bone resorption [18, 81]. Exercise has several advantages in protecting body health particularly bone modeling and remodeling [80]. The ability for bones to adjust with mechanical force and stress has been observed in the late 19th century [82].

Mechanical load is one of the most important agents for bone mass density enhancement. The Mechanostat theory, which is first mentioned by Frost, expresses that bones have their own innate biological system to induce bone formation, in response to mechanical forces. This system includes bone cells, major osteocytes which are impressed by mechanical strain, transmitting it to the osteoclasts and osteoblasts and resulting in regulation of the skeleton homeostasis [37, 83, 84]. It has been accepted that mechanical forces help promote bone mass and strength. Interestingly, the skeleton could discriminate between internal force and strain-driven [85]. Osteoblasts, osteoclasts, and other bone cells are influenced by various endogen and exogen factors like cytokines. Proinflammatory and anti-inflammatory cytokines have major roles in skeleton modeling and remodeling [39]. Studies have shown that joint disorders like arthritis could make asymmetry pro- and anti-inflammatory cytokines, leading to bone loss [39, 86]. Exercise might increase anti-inflammatory cytokines and cause improvement in inflammatory cytokines [87, 88]. Also, mechanical load as an exercise regulates collagen synthesis during bone formation [89]. Muscle tension is transferred to the bones and leads to provoking osteoblasts proliferation [90]. In contrast, the lake of exercise, weightlessness, or bedridden would reduce osteoblasts activity and increase osteoclasts function [91].

Exercise is categorized into 6 classes: static weight-bearing exercises such as single-leg standing, high-impact weight-bearing exercises like running or dancing, low-impact weight-bearing exercises such as Tai Chi, high-impact non-weight-bearing exercises, low-impact non-weight-bearing exercises like swimming, and combination exercises [37, 92]. Investigations have indicated that regular physical activity with long duration and moderate intensity would decrease bone resorption and increase bone mass in both

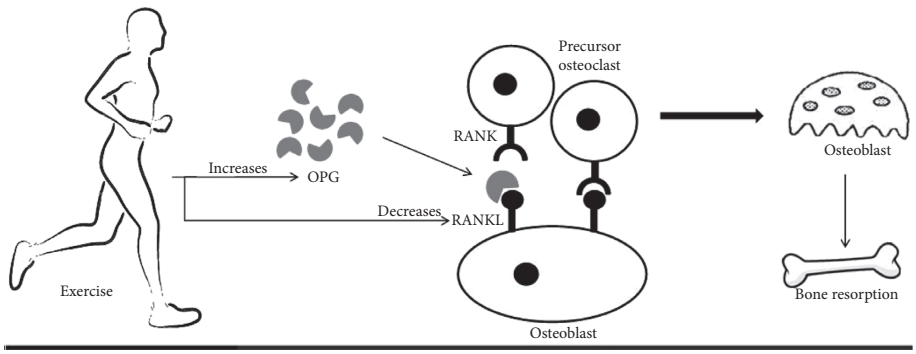

Figure 1: Interaction of exercise and RANKL/RANK/OPG biomolecular pathway. OPG: osteoprotegerin; RANK: receptor activator of nuclear factor κB; RANKL: receptor activator of NF-kB ligand.

healthy and pathologic subjects [80]. Bone health would improve through weight-bearing exercises and helps bone density in growth, promoting bone health in aging [93–95]. In a 12-month clinical trial, postmenopausal inactive women with high doses of exercise had a greater bone mass density compared to women with moderate-dose exercise. This effect remained for almost a year after finishing study [29]. Intensive weight-bearing exercise may propagate P1NP, BAP, OPG, phosphate, and PTH levels [65, 96].

Exercise brings a cycle of reactions in the hypothalamus-hypophysis-adrenal line or hypothalamus-hypophysis-gonad line. These reactions stimulate some hormone expressions which help MSC differentiation to osteoblasts, including growth hormone, PTH, PGE2, and thyroid hormones [65, 97–100]. Sclerostin, a major role in bone formation, is a protein expressed by osteocytes. In fact, sclerostin supports bone mass by prohibiting the Wnt/B-catenin pathway. Wnt is a signaling pathway that proliferates osteoprogenitor and minimizes mature osteoblasts apoptosis. Exercise and the followed mechanical load lead to the reduction in bone sclerostin synthesis. Afterward, osteoblastic bone formation increases and osteoclastic bone loss decreases [37, 101]. There is some strong evidence that demonstrated exercise decline mRNA levels of markers from bone resorption like TRAP, cathepsin-K, and calcitonin receptors [102]. Moreover, participating in exercise could increase some osteogenic markers like OCL, Runx2, Osx, BAP, BMP2, and collagen type 1 in osteoblasts [15, 103–105]. It has been demonstrated that BAP and OCL, which are bone formation markers, are upregulated and TRAP (tartrate-resistant acid phosphatase), which is a bone resorption marker, is downregulated within an 8-week exercise plan in women [65, 103].

It has been shown that exercise promotes bone health through RANKL/RANK/OPG pathway regulation too [65].

3.2. Favorable Effects of Exercise on Bone Health by RANKL/RANK/OPG Regulation. Figure 1 presents the details of the positive role of exercise in bone remodeling. Osteoblasts and osteoclasts are responsible for bone formation and bone resorption, respectively. So the effects of exercise on these 2 types of cells would help understand the association between exercise, bone modeling, and bone remodeling [88]. Exercise is responsible for the suppression in osteoclastogenesis and bone remodeling, which is mediated through the OPG/RANKL pathway released by osteoblasts, osteocytes, and MSCs [65].

There are multiple animal studies investigating the effect of chronic exercises on the pathway. In a study conducted using rats with CKD, the expression of RANKL and osteocalcin increased after endurance treadmill exercise [106]. Another study demonstrated the effects of exercise on glucocorticoid-induced osteoporotic that was investigated in rats. The results of this study confirmed that RANKL and RANKL-induced bone loss would be inhibited by vibration and treadmill training [107]. It was suggested that the treadmill and vibration stimulation exercise leads to a decrease in RANKL expression and an increase in OPG expression in the glucocorticoid-induced osteoporotic rats [107]. OPG and RANKL were meaningfully increased in response to 5-minute physical activity. In this study, which was performed on prednisolone-induced osteoporotic rats cells, treadmill and vibration platform training were used as examples of physical training. The results of the group treated with treadmill and vibration stimulation training indicated a subsequent decrease in RANKL and an increase in OPG levels [107]. Some limited animal studies reported beneficial effects of acute exercise on the pathway. Decreased RANKL levels and increased OPG levels have been observed in an experiment using acute training murine MC3T3-E1 osteoblasts [108]. In a chronic exercise study which was conducted in rats, an enhancement in the OPG/RANKL ratio was shown because of a decrease in RANKL expression [104]. On the other hand, an in vitro study has illustrated that mechanical strain could lead to abundance in OPG levels and decreases in M-CSF levels without alterations in RANKL levels in human osteoblasts [109]. Rubin et al. suggested that mechanical load could cause a reduction in RANKL, resulting in strong protection of bone loss and osteoclast proliferation [110].

Several previous studies reported the influence of acute exercises on the RANKL/RANK/OPG pathway. Scott et al. reported that acute endurance exercise causes an increase in levels of BAP and OPG in healthy men [96]. High-intensive acute exercise would enhance OPG and RANKL instantly. Also, this study has shown that 5-minute exercise increases IL-1a, IL-1B, IL-6, and TNF-a and 1-hour exercise brought

Table 1: General characteristics of the studies investigating the effects of exercises on RANKL/RANK/OPG regulation. ALP: alkaline phosphatase; BAP: bone alkaline phosphatase, OPG: osteoprotegerin; RANK: receptor activator of nuclear factor κB; RANKL: receptor activator of NF-kB ligand.

Study name, year	Exercise type	Treatment time	Species/population/ condition	Significant outcome
Scott et al. 2011 [96]	Acute, weight-bearing endurance exercise	8 days	Healthy men	OPG↑, BAP↑
Kish et al. 2015 [121]	Plyometric exercise	5 minutes, 1 hour, and finally 24 hours after exercise	Healthy boys and men	OPG↑, ALP↑
Bergström et al. 2011 [115]	Physical training (fast walking + aerobic training)	1 year	Postmenopausal women	OPG↑, RANKL↔, sclerostin↔
Rubin et al. 2000 [110]	Mechanical strain by a flexcell bioflex instrument	3 days	Murine bone stromal cells	RANKL↓
Notomi et al. 2014 [104]	Resistance training	8 weeks	Male Sprague Dawley rats	RANKL↓, OPG↔, OPG/ RANKL↑
Mezil et al. 2015 [88]	High-intensity low-impact exercise	5 minutes after exercise 1 hour after exercise 24 hours after exercise	Male university students	ALP↑, OPG↑, RANKL↑ ALP↑ ALP↑
Troib et al. 2016 [106]	Endurance treadmill exercise	4 weeks	Young and growth-retarded chronic kidney disease rats	RANKL↑, Osteocalcin↑
Pichler et al. 2013 [107]	Treadmill and vibration stimulation training	NS	Osteoporosis rats	OPG↑, RANKL↓
Esen 2009 [116]	High-intensity walking ($n = 14$)	10 weeks	Middle-aged men	OPG↔, sRANKL↓
Esen 2009 [116]	Moderate-intensity walking ($n = 13$)	10 weeks	Middle-aged men	OPG↔, sRANKL↔
Ziegler et al. 2005 [111]	Endurance running distance of 42.195 km	The first 30 minutes of finishing the run	Long-distance runners	sRANKL↓, OPG↑
Ziegler et al. 2005 [111]	Endurance running shorter distance of 15.8 km	The first 30 minutes of finishing the run	Long-distance runners	sRANKL↓, OPG↔
Tang et al. 2006 [108]	Cyclic tensile strain using a flexercell strain unit with 6%, 12% or 18% elongation	24 hours	Murine MC3T3-E1 osteoblasts	OPG↑, OPG mRNA expression↑, sRANKL↓, RANKL mRNA expression↓ (magnitude-dependent)
Kim et al. 2019 [113]	Combined exercise	12 weeks	Healthy college females	OPG↔, RANKL↔, RANKL/ RANK/OPG signaling mRNA expression↔
Saunders et al. 2006 [109]	Small-scale loading machine that imparts via bending	3 hours	Osteoblastic MG-63 cells	OPG↑, RANKL↔, OPG/ RANK ratio↑
Kim et al. 2017 [112]	Acute exercise of high (80% VO2max) intensity	Immediately after and then recovery 60 minutes after exercise	Osteopenia elderly women	OPG↑, RANKL↔
Kim et al. 2017 [112]	Acute exercise of low (40% VO2max) intensity	Immediately after and then recovery 60 minutes after exercise	Osteopenia elderly women	OPG↔, RANKL↔
Marques et al. 2013 [117]	Resistance exercise accompanied by weight-bearing exercise	32 weeks	Healthy older adults	RANKL↔, OPG↔, OPG/ RANKL ratio↔
Marques et al. 2011 [118]	Resistance exercise (RE)	8 months	Older women	RANKL↔, OPG↔, OPG/ RANKL ratio↔
Marques et al. 2011 [118]	Aerobic exercise (AE)	8 months	Older women	RANKL↔, OPG↔, OPG/ RANKL ratio↔

Table 1: Continued.

Study name, year	Exercise type	Treatment time	Species/population/ condition	Significant outcome
Kim et al. 2018 [114]	Acute exercise of high (80% VO2max) intensity	Immediately after and then recovery 90 minutes after exercise	Healthy college females	RANKL↔, OPG↔, RANKL/ RANK/OPG pathway mRNA expression↔
Kim et al. 2018 [114]	Acute exercise of moderate (60% VO2max) intensity	Immediately after and then recovery 90 minutes after exercise	Healthy college females	RANKL↔, OPG↔, RANKL/ RANK/OPG pathway mRNA expression↔
Klentrou et al. 2018 [119]	Rest and following plyometric exercise (5 min, 1 h, and 24 h)	24 hours	Boys and girls (10 years old in average)	Girls: OPG↑, RANKL↓ Boys: OPG↑, RANKL↑
Dekker et al. 2017 [120]	1 resting and 3 after exercise (5 min, 1 h, and 24 h)	24 hours	Premenarcheal and postmenarcheal girls	RANKL↓OPG↔ OPG/RANKL↑
Maïmoun et al. 2011 [123]	Training 12–30 h/week) professional athlete (compared with free-time physical activity ≤ 3 h/week (nonathlete)	—	Girls (age 10–17.2 years)	OPG↔RANKL↑
Maïmoun et al. 2013 [122]	Participants are divided into 4 groups: high-impact exercise, medium-impact exercise, no-impact exercise, and leisure physical activity	—	Girls from 10.7 to 18.0 years old	OPG↔RANKL↑

them back to the basic levels [88]. The findings of another study suggested that endurance running made a reduction in sRANKL and an increase in OPG concentrations. The intensity in these results depends on the distance and duration of the path [111]. After a performance period with 80% VO2max and 40% VO2max intensity, OPG levels in serum have increased just under high-intensity exercise conditions in elderly women [112]. Based on a clinical trial done by Mezil et al., the low-impact high-intensity exercise would increase OPG, RANKL, and ALP levels in male university students [88]. The authors noted that, in the absence of adequate exercise, RANKL/RANK/OPG pathway effects would be minimal [113]. Acute training exercise with 60% or 80% VO2max intensity could not change serum RANKL and OPG levels, nor RANKL/RANK/OPG mRNA expression in college women [114].

In addition to acute exercise, the chronic exercises exert similar effects on RANKL/RANK/OPG pathway. In an investigation, long-term and intensive chronic exercise causes upregulation of OPG expression in postmenopausal women as compared to that in sedentary cases [115]. An article reported that after a 10-week high-impact walking plan, it significantly diminished RANKL levels without significant changes in OPG levels in middle-aged men [116]. Some findings suggested that RANKL and OPG levels and their expressions do not necessarily change by exercise. For example, a 32-week resistance exercise accompanied by weight-bearing exercise had no effect on RANKL and OPG levels and their ratio [117]. Likewise, in older women, after 8 months of resistance exercise or aerobic exercise, no significant changes were observed in RANKL and OPG levels [118]. After 12 weeks of combined exercise, Kim et al. found no significant changes in serum OPG and RANKL concentrations, nor in RANKL/RANK/OPG signaling mRNA expression [113].

Along with animal and human adult studies, there are several trails that include children and adolescents. These studies mostly focused on acute exercises. In one of these studies, the osteokines responses to rest and plyometric exercises in children have evaluated. Boys and girls with 10 years old on average were included in this study and the amounts of RANKL and OPG were measured before and after exercise (5 min, 1 hour, and 24 hours). In the pre-exercise analysis, it turned out that boys have higher levels of RANKL which indicated the discrimination in bone turnover between the two genders through the growth time. Girls showed a reduction in RANKL through exercise and it has kept reducing more when they continued to exercise till 24 h. OPG is enhanced by exercise; this enhancement is higher in boys specifically in 5 min and 1-hour exercise against girls which indicated the increment only on 24 h level of exercise [119]. The other survey has measured the plyometric exercise (high-impact) effects on bones in young females and the outcomes expressed a reduction in RANKL levels after 5-minute exercise. It stayed lower than the basic level (pre-exercise) until the end of 24 h exercise. However, OPG did not change at significant levels [120]. The finding of an investigation demonstrated that one session of plyometric exercise could increase both OPG and ALP (alkaline phosphatase) in boys and young men [121]. Another study investigated the bone mass impacts of exercise on adolescent girls. Participants were divided into 4 groups: high-impact exercise, medium-impact exercise, no-impact exercise, and leisure physical activity. The results represent no significant variation in OPG levels among groups; we have a slight reduction in OPG through growing just in a high-impact group. Also, RANKL levels increased along with age except in no-impact exercise (swimmers) which made a reduction in RANKL [122]. The comparison between the professional young female trainers who exercise 12–30 hours per week and nonathlete girls who do unplanned physical activity less than 3 hours in a week has shown that RANKL increases simultaneously with aging in both groups. No significant

change was reported in them [123]. Table 1 displays the characteristics of the studies investigating the effects of different kinds of exercises on the RANKL/RANK/OPG pathway.

Despite strong evidence for the impact of mechanical loads on the RANKL/RANK/OPG signaling system, our knowledge is still limited on how this pathway may contribute to optimal bone health.

4. Conclusion

Based on the different studies that we reviewed, antithesis results have appeared. In most of the studies, exercise and physical activities promote bone health by increasing OPG and decreasing RANKL levels. However, there are several investigations that reported no change in OPG and RANKL levels after exercise. Interestingly, most of the experiments that we investigated have been carried out with high-intensity exercise. According to these studies, the actual effect of exercise on the RANKL/RANK/OPG system needs more investigations. Regardless, the positive impact of exercises on bone health and the overall well-being is undeniable.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of the present manuscript.

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