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Molecular bases for the effect of exercise on postmenopausal bone health

Molekulární podstaty efektu cvičení na zdraví kosti po menopauze

Bachelor's thesis

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Declaration

I hereby declare that I compiled this bachelor's thesis on my own and presented all the cited sources of information and literature. This work or any part thereof has not been submitted in any previous application for another or the same academic title.

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Acknowledgments

Abstract

Hormonal changes caused by menopause, most notably the decline in estradiol levels, lead to rapid bone loss, putting a significant percentage of postmenopausal women at serious risk of developing osteoporosis. Postmenopausal osteoporosis influences many aspects of women's health and their overall well-being. Regular physical activity is considered one of the essential means to slow the bone loss process and prevent bone fragility. Many molecules play a role in the reaction of bone to mechanical stress during exercise, both those produced directly by bone cells and those expressed by other tissues, such as myokines and cytokines. The molecular connections between the menopausal changes in bone and the skeletal reaction to exercise are not well-known, especially considering variables like different types of exercise or individual levels of osteoporosis risk. Learning more about these links could help us better understand how exactly exercise influences postmenopausal bones. Moreover, it would be beneficial for creating more effective guidelines for osteoporosis prevention through exercise and developing better prevention and treatment approaches for postmenopausal osteoporosis.

Keywords: menopause, bone metabolism, physical activity, exercise, postmenopausal osteoporosis

Abstrakt

Hormonální změny způsobené menopauzou, zejména pokles hladiny estradiolu, vedou k rychlému úbytku kostní hmoty. Díky tomu je významné procento žen po menopauze ohroženo rozvojem osteoporózy. Postmenopauzální osteoporóza ovlivňuje celkové zdraví a kvalitu života žen. Pravidelná fyzická aktivita je považovaná za jeden ze způsobů, kterými se dá zmírnit proces úbytku kostní hmoty a předejít křehkosti kostí. Mnoho molekul se účastní reakce kostí na mechanickou zátěž při cvičení, přičemž některé z nich jsou produkované přímo kostními buňkami a jiné pocházejí z ostatních tkání, například myokiny nebo cytokiny. Molekulární souvislosti mezi kostními změnami způsobenými menopauzou a reakcí kostí na cvičení nejsou zatím dobře prozkoumány, obzvlášť s ohledem na různé typy cvičení nebo individuální rizika osteoporózy. Lepší porozumnění těmto souvislostem by nám mohlo pomoci lépe pochopit, jak přesně cvičení ovlivňuje postmenopauzální kosti. Tyto informace by mohly rovněž přispět k vytvoření účinnějších programů pro prevenci osteoporózy prostřednictvím cvičení a k rozvoji lepších preventivních a léčebných přístupů týkajících se postmenopauzální osteoporózy.

Klíčová slova: menopauza, kostní metabolismus, fyzická aktivita, cvičení, postmenopauzální osteoporóza

List of Abbreviations:

BMD = bone mineral density

ERα = estrogen receptor α

FSH = follicle-stimulating hormone

GH = growth hormone

IGF = insulin-like growth factor, e.g. IGF-1

IGF-IR = insulin-like growth factor-1 receptor

IL = interleukin, e.g. IL-1, IL-6

LRP = LDL receptor-related protein, e.g. LRP5

mRNA = messenger ribonucleic acid

OPG = osteoprotegerin

OPN = osteopontin

PTH = parathyroid hormone

RANK = receptor activator of nuclear factor kappa-B

RANKL = receptor activator of nuclear factor kappa-B ligand

S-CTX = serum cross-linked C-telopeptides of type I collagen

TNF = tumor necrosis factor, e.g. TNF-α

TNFRSF11B = TNF receptor superfamily member 11b

Wnt = wingless-related integration site

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1. Introduction

Almost every woman will in their lifetime be affected by menopause. Menopause is the end of the female reproductive period and is usually diagnosed after 12 months without a menstrual period (Lumsden, 2016; McKinlay et al., 1992). The average age at which women experience their final menstrual period lies between 50 and 52 years old, but it can be influenced individually by many factors, such as the age of menarche or smoking (McKinlay et al., 1992). It is accompanied by many symptoms, commonly including hot flashes, chills, night sweats, and insomnia (McKinlay et al., 1992). General health and general well-being deteriorate during the menopausal transition (Mishra et al., 2003).

The decline in ovarian function during and after menopause has two main hormonal consequences: a decrease in production and thus circulating levels of estradiol, one of the female sex hormones, estrogens, and an increase in concentration of follicle-stimulating hormone (Santoro et al., 2021). These humoral changes influence a myriad of organs and processes in women's bodies, even outside reproductive health. One of the most significant secondary effects of the decline in ovarian function is the effect on bone metabolism. Menopause has been linked to bone loss, bone fragility, and osteoporosis since the 1940s (Albright et al., 1941), and since then, this connection has been studied profoundly. That said, new ways in which lowered levels of estrogens influence factors of bone metabolism are still being researched and discovered today.

One of the most recommended methods for protecting bones from the effects of hormonal deficiency and preventing osteoporosis during menopause is regular physical activity, most often in the form of weight-bearing exercise. Exercise is considered an effective way to prevent bone fragility and fractures. Various studies have tried to find the best exercise type, intensity, and regimen for bone protection and increasing bone mineral density (BMD) in postmenopausal women, and even though no conclusive result was achieved, all of them agree on the positive effect of regular physical activity on general bone health (Kemmler et al., 2020; Shojaa et al., 2020).

Even though exercise is often advised to postmenopausal women with the goal of protecting their bones against weakening, the molecular mechanisms of the effect of physical activity on bone health after menopause are still in research. Knowledge of these molecular mechanisms and the connections between them would help to explain the different effects of

various exercise types and regimens in preventing osteoporosis and elucidate the interindividual risks stemming from insufficient activity. Many molecules and molecular pathways affecting bone metabolism have been studied as possible effectors of bone remodeling in response to physical activity and mechanical stress. However, the reaction to physical activity and exercise in bone is complex and seems to be different in postmenopausal women compared to other people, as the perimenopausal decline in estrogens impacts some molecular pathways and the expression of some molecules more than others. Research into these differences is needed in order to improve the guidelines for the prevention of postmenopausal osteoporosis. The goal of this work is to describe those molecular factors and processes that link exercise with the improvement of bone health in postmenopausal women.

2. Influence of menopause on bone metabolism

The key factor in bone turnover during and after menopause is estradiol. In women during their reproductive years, it is produced primarily by the ovaries. Thus, its serum concentration significantly decreases during menopause. While normal levels of estradiol for adult premenopausal women are 30 to 500 pg/mL depending on the phase of the menstrual cycle, after menopause they drop to a range from 0 to 30 pg/mL (Strauss & Barbieri, 2019).

In young, healthy women, estrogens suppress bone remodeling and resorption while maintaining bone formation. This is done through several different mechanisms, including an increase of calcitonin and bone morphogenetic protein-2 production, changes in bone sensitivity to parathyroid hormone (PTH), promotion of intestinal calcium absorption, and direct effect on bone cells (Bartl & Frisch, 2009; Nie et al., 2020; Zhou et al., 2003). Estrogens affect all the main bone cell types directly, as osteoblasts, osteocytes, and osteoclasts all express estrogen receptors (Bord et al., 2001; Braidman et al., 2001). Lowering the apoptosis rate of osteocytes and osteoblasts is one of the positive effects of estrogens that have been observed (Kousteni et al., 2001). On the other hand, they promote apoptosis and suppress differentiation in osteoclasts (Khosla et al., 2012). Thus, estrogens play a role in maintaining bone homeostasis and protecting women against bone loss during their reproductive years.

The perimenopausal decrease in concentrations of estrogens, especially in the concentration of estradiol, leads to upregulation of both osteoclastogenesis and, surprisingly, also osteoblastogenesis at different rates (Manolagas, 2000) which causes deregulation in bone homeostasis and an increase in bone turnover resulting in bone loss. This can lead to pathologies such as osteopenia or osteoporosis. The bone remodeling rates double at menopause (Recker et al., 2004) and further increase in the following years. Fourteen years after menopause, the remodeling speed gets to a level three times higher than before menopause (Farlay et al., 2019). Estradiol is positively correlated to absolute bone density, and its absence in menopause is accompanied by increased serum levels of bone turnover markers, such as bone-specific alkaline phosphatase, immunoreactive free deoxypyridinoline, urinary cross-linked N-telopeptides of type I collagen, and serum cross-linked C-telopeptides of type I collagen (S-CTX) (Rogers et al., 2002).

Lowered ovarian function also causes an increase in serum levels of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and others, which further contributes to the changes in bone mass and structure (Fischer et al., 2018). These proinflammatory factors are not only

linked to bone loss but to impaired fracture healing and osteogenic differentiation as well (Fischer et al., 2018). Increased follicle-stimulating hormone (FSH) levels also correlate with some of the changes in bone, including changes in BMD during the menopausal transition (Park et al., 2021; Sowers et al., 2006); however, the research on this is limited and the mechanism is not yet well explained. Others found no association between elevated FSH levels and changes in bone, especially in older postmenopausal women (Crandall et al., 2013; Jeong et al., 2022; Wu et al., 2021).

The bone loss rate is at its peak during the first few years after menopause, no matter the age at onset (Ahlborg et al., 2001). During the transmenopause, an interval of one year before and first two years after the last menstrual period, there is a loss of 7,38 % of BMD in lumbar spine and 5,8 % of BMD in femoral neck (Greendale et al., 2012). Then, after the first few years, BMD in postmenopausal women decreases by around $1,9 \pm 0,7$ % every year. (Ahlborg et al., 2003). Many other bone health parameters also change during the menopausal transition, even in otherwise healthy women. Higher heterogeneity of mineralization, decreased micro-hardness, and decreased mineral/organic ratio were all observed in women one year after menopause by Farlay et al. (2019). They have also reported that the latter two qualities lower even more in the following years. Changes in the periosteal diameter are also related to postmenopausal serum levels of estradiol (Ahlborg et al., 2003).

Similar hormonal effects on bone can be observed in young women with amenorrhea or men with age-caused sex steroid deficiency, although in men, the transition is slower and can start even later in life (Riggs et al., 2008). For that reason, testosterone's influence on bone has not been studied as much as the influence of estrogens.

2.1. Bone health in menopause

Estrogen deficiency in postmenopausal women is a major cause of osteoporosis. Postmenopausal osteoporosis is a widespread issue with prevalence ranging from 30 % to 40 % in postmenopausal women, according to several studies from different countries using the World Health Organization criteria (Choi et al., 2021; Imran et al., 2022; Melton, 1995). It is characterized by elevated bone remodeling rates compared to normal postmenopausal levels (Recker et al., 2004; Riggs et al., 2008), and it is accompanied by medical complications like bone fragility and a higher risk of fractures. The bones that are most at risk are those with a large portion of cancellous bone, as they are most affected by the postmenopausal BMD loss.

The vertebral bodies, the femoral neck, the ribs, and the wrists belong to this group. For this reason, the femoral neck and lumbar spine are used as the regions of interest in most bone health studies (Kanis, 1997).

Postmenopausal osteoporosis prevention is an important topic in the medical community. Some of the most critical factors that play a role in maintaining healthy postmenopausal BMD and preventing osteoporosis are regular physical activity and healthy BMD before menopause. Premenopausal BMD might even be a better indicator of postmenopausal BMD than the period of time elapsed since menopause (Ahlborg et al., 2001). In women, the biggest aggregation of bone mass happens in the teenage years, another period in life that follows significant hormonal changes in the body. Up to 90 % of peak adult bone mass is accumulated by age 18 (Baxter-Jones et al., 2011). Regardless of that, physical activity throughout life leads to significantly higher BMD than exercising only before or only after menopause (Kopiczko, 2020). Therefore, regular physical activity is essential both before and after menopause to reach and maintain higher peak bone mass.

The process of going through the menopausal changes can itself be alleviated by regular exercise. Women following the recommended physical activity guidelines have better relative health, increased well-being, and fewer somatic menopausal symptoms compared to other women in the same age group (Mansikkamäki et al., 2015). In another study, higher amount of physical activity was related to a smaller number of severe urogenital symptoms (Dąbrowska-Galas et al., 2019). On the other hand, physically inactive women had an increased probability of anxiety or depressive moods and more severe symptoms (Mansikkamäki et al., 2015). Another symptom accompanying menopause is the loss of skeletal muscle. Recommended levels of exercise are beneficial for maintaining not only bones but also muscle in postmenopausal women (Sipilä et al., 2020).

3. Influence of exercise on postmenopausal bone health

Sufficient levels and frequency of physical activity influence BMD and bone mineralization. Westerlind et al. (1997) observed that even though the rate of bone turnover is predominantly regulated by estrogens and their loss, the general balance of bone formation and bone resorption is affected by levels of prevailing mechanical strain in animal models. In humans, regular physical activity before menopause increases the chance of having higher BMD and mineralization level after menopause (Kopiczko, 2020).

Physical activity affects the skeleton in multiple ways. Firstly, it generates forces which create signals, such as bone strain and fluid shear stress. Osteocytes can detect these mechanical signals thanks to their specific shape with lean processes, which are more sensitive to such signals than the cell body, and with their integrin attachments (Adachi et al., 2009; Wang et al., 2008). They respond to these mechanical signals with production, or suppression, of various locally and systematically active factors. During this mechanotransduction, the bone cells interact with and are influenced by extracellular fluid forces (Hsieh & Turner, 2001).

Secondly, exercise influences the rest of the body and can activate signaling pathways or stimulate the production of molecules that affect bones. Out of the tissues influenced by exercise, muscles are arguably the most important in impacting the bone. Myokine secretion is activated in skeletal muscles as a response to physical activity. Factors such as irisin, fibroblast growth factor 21, insulin-like growth factor (IGF)-1, and vascular endothelial growth factor A are released into the bloodstream (Schnyder & Handschin, 2015), and some of them have proven effects on bone health. On the other hand, regular physical activity lowers circulating levels of certain inflammatory cytokines, such as tumor necrosis factor (TNF)-α or IL-6 which also impact bone (Fischer et al., 2004; Paolucci et al., 2018).

No exercise regimen has been found more beneficial for postmenopausal bones than others, as shown by the many discrepancies in studies published on this topic. Some of them do not observe any significant differences in BMD change caused by different exercise types (Kemmler et al., 2020), while others recommend a specific training type, such as squat maximal strength training (Mosti et al., 2014), power training (Stengel et al., 2005), or combinations of high-impact exercise with high magnitude resistance training (James & Carroll, 2010; Xu et al., 2016). The effect of changing other variables of the exercise regimen was also reported. Shojaa et al. (2020) observed a significant influence of training frequency on BMD changes in both lumbar spine and femoral neck, recommending a lower training frequency of under two sessions

per week over a higher frequency of exercise. However, others report that the effect of physical activity on BMD is dependent on the dose and that the frequency needs to be relatively high to bring significant results in postmenopausal women (Gonzalo-Encabo et al., 2019; Kemmler et al., 2016).

Some exercise types and regimens may be more effective for postmenopausal women, while others work better for premenopausal women, as observed by Bassey et al. (1998). This could be explained by pre- and postmenopausal bones reacting differently to the same exercise sessions. While the short-term response to mechanical stress is similar in healthy and osteoporotic human bone cells, the long-term response is significantly lower in the osteoporotic cells (Sterck et al., 1998). This could translate to impaired reaction of osteoporotic bones to exercise and therefore a need for higher frequency or intensity of exercise. On the other hand, increasing the intensity of exercise recommended for postmenopausal women is debatable, as their bones are at higher risk of fracture during more strenuous activities. There is not enough research focusing on the differences between the changes in BMD of premenopausal and postmenopausal bones in response to regular physical activity. Further evaluation is needed in order to gain conclusive results.

4. The effects of exercise-induced molecules on postmenopausal bones

As discussed in the previous chapter, exercise affects bones in many various ways, but some are more significant for postmenopausal bones and their metabolism specifically. The expression and secretion of multiple molecules of bone metabolism can be affected by the perimenopausal decline in estradiol, and so can the reaction of bone to mechanical signals itself. This chapter focuses on some of these factors that link exercise to improving postmenopausal bone health.

4.1. Molecular factors produced by bone cells

Bone metabolism is complex with its molecular pathways intertwined and influencing each other. Many molecules produced by bone have various systematic and local effects, including autocrine signaling. Some of the most important molecular pathways contributing to bone homeostasis are arguably the Wingless-related integration site (Wnt)/β-catenin pathway and the receptor activator of nuclear factor kappa-B ligand (RANKL)/receptor activator of nuclear factor kappa-B (RANK) pathway (Al-Bari & Al Mamun, 2020). Their dysregulation can lead to conditions such as osteopenia or osteoporosis. Changes in circulating levels of estrogens influence the production and secretion of many molecules produced by bone, including some that take part in these pathways, such as sclerostin, osteoprotegerin (OPG), and RANKL, and others that impact bone homeostasis independently, such as osteopontin (OPN). These molecules are also directly or indirectly influenced by mechanical stimuli, as shown in Figure 1. Their involvement in bone metabolism in the context of menopausal transition and influence of exercise is described in detail in the following subchapters.
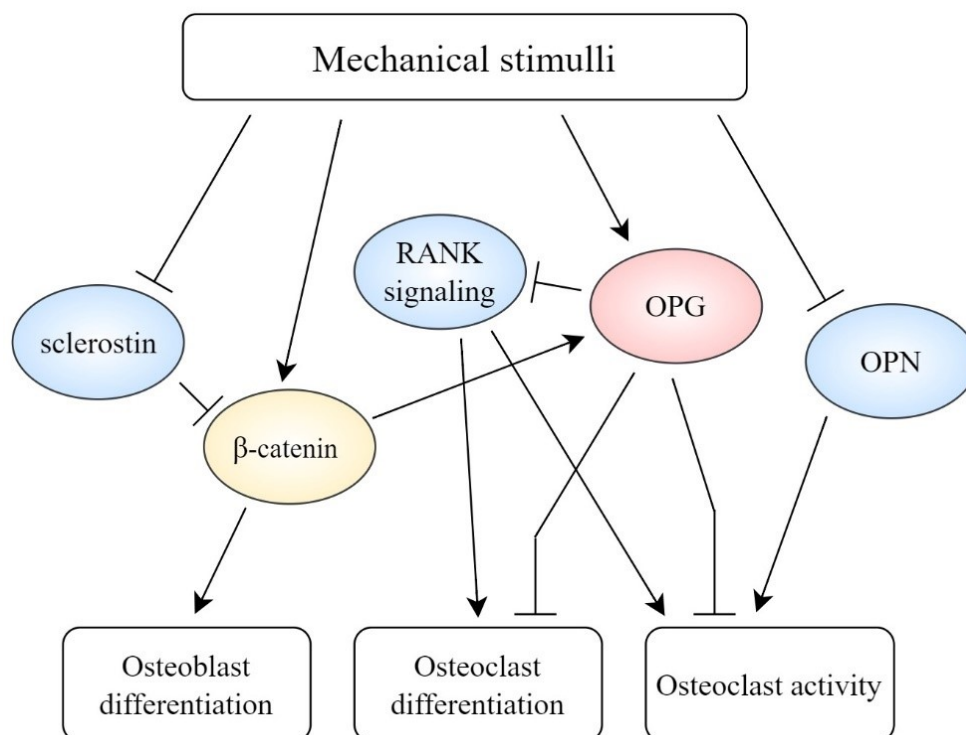
Figure 1. Effect of mechanical stimuli on molecular factors produced by bone cells and their subsequent impact on bone. Molecules that are upregulated after menopause are marked in blue, those that are downregulated after menopause in red, and those not influenced by menopause in yellow. Sharp arrows indicate stimulation; blunt arrows indicate inhibition. RANK – receptor activator of nuclear factor kappa-B; OPG – osteoprotegerin; OPN – osteopontin. Source: author.

4.1.1. Wnt/β-catenin pathway and sclerostin

The Wnt/β-catenin pathway, also called the canonical Wnt pathway, has a wide range of functions, especially during embryonic development (Logan et al., 2004). In adults, one of its roles is the maintenance and regeneration of bone through enhancing osteoblastic differentiation (Chen et al., 2007; Xu et al., 2014). The secretion and binding of Wnts to specific receptors, such as Frizzled, with the LDL receptor-related protein (LRP)-5/6 co-receptors, inhibits the phosphorylation and degradation of β-catenin (Huang et al., 2004; MacDonald et al., 2012). The Wnt signaling thus causes an accumulation of β-catenin in the nucleus (Komiya et al., 2008). There, β-catenin activates specific transcription factors that target, among others, genes necessary for osteoblastic differentiation (Cadigan et al., 2014).

Sclerostin is a key negative regulator of the Wnt/β-catenin pathway and a Wnt antagonist, and as such, it suppresses osteoblastic differentiation (Kubota et al., 2009). It does so by inhibiting the Wnt/β-catenin signaling through binding to LRP5 and LRP6, although

probably not by direct competition for LRP binding between sclerostin and Wnt (Li et al., 2005; Lim et al., 2021). Thus, growing levels of sclerostin cause downregulation of osteoblastic differentiation, seriously impacting the bone. It has been observed that serum sclerostin has a significant negative correlation with BMD in various locations, such as femoral neck and total hip, in postmenopausal women (Zou et al., 2016).

Several studies show that serum sclerostin levels rise with age (Amrein et al., 2012; Mödder et al., 2011; Zou et al., 2016) and go through a significant increase during the menopausal transition (Matsui et al., 2016). After menopause, serum sclerostin levels continue to grow with advancing years (Ardawi et al., 2011). The link between sclerostin and female sex hormones has been studied in further detail. Serum sclerostin levels are negatively correlated to circulating estradiol levels (Matsui et al., 2016), while estrogen treatment in postmenopausal women leads to significantly lowered both serum sclerostin levels and bone sclerostin messenger ribonucleic acid (mRNA) levels (Fujita et al., 2014; Mödder et al., 2011). These results suggest an adverse effect of estradiol on sclerostin expression and concentration and therefore explain the increase in sclerostin after the decline of estradiol levels caused by the menopausal transition. This puts postmenopausal women at a severe risk of decline in osteoblastic differentiation and thus bone formation.

The impact of estrogens on other components of the Wnt/β-catenin pathway has also been observed. However, it seems to be more complicated since multiple different components of the pathway are influenced by either estrogens or their receptor, estrogen receptor α (ERα). ERα is involved in the regulation of β-catenin and osteocyte reaction to mechanical loading (Armstrong et al., 2007; Liedert et al., 2020), while estrogens themselves cause the activation of β-catenin and can change the sensitivity of bone cells to the response to mechanical loading (Liedert et al., 2020). Further research is needed to determine if this information could be useful in improving bone health in postmenopausal women.

While the decline in estradiol causes upregulation of sclerostin, mechanical loading of bones causes a decline in sclerostin expression in murine bones (Robling et al., 2007; Tu et al., 2012). This can be observed as both the loading-dependent decrease in osteocyte expression of sclerostin and a lowered amount of sclerostin in the canalicular network (Robling et al., 2007). Lara-Castillo et al. (2015) reported a significant decrease in sclerostin positive osteocytes 24 hours after *in vivo* mechanical loading, which only returned to baseline 48-72 hours post-load. In addition to that, decreased expression of Dkk1, another Wnt/β-catenin pathway antagonist, was also observed.

Sclerostin downregulation is an integral part of the mechanotransduction cascade as it allows Wnt signaling in the parts of bones where osteogenesis is structurally needed. The increase in osteogenesis is dose-responsive to the loading (Tu et al., 2012).

Exercise has been observed to lower resting serum sclerostin levels in osteoporotic postmenopausal women after interventions as short as 12 weeks (Janik et al., 2018). The same effect was observed in male patients with osteopenia after a 12-month exercise program, where the decrease in serum sclerostin was around 7 % (Hinton et al., 2017), suggesting a universal effect of regular physical activity on decreasing sclerostin levels. Interestingly, Hinton et al. (2017) followed two different modes of exercise but did not find any significant difference between the effects of resistance training and jump training. In addition, sclerostin has been indicated as one of the factors in bone loss caused by mechanical unloading as happens, for example, in bed-bound patients. In response to mechanical unloading, sclerostin expression increases and downregulates the Wnt/β-catenin pathway causing a decline in bone formation, while this effect is prevented in sclerostin-deficient mice (Lin et al., 2009).

Mechanical loading has been observed in several studies to cause an increase in the concentration of β-catenin after loading sessions *in vitro* and *in vivo* (Armstrong et al., 2007; Jackson et al., 2021; Lara-Castillo et al., 2015). However, the reaction to mechanical loading and the related changes in the Wnt/β-catenin pathway are significantly lower in ovariectomized mice compared to control group as a result of lowered levels of estrogens which have been shown to alter the ability to respond to mechanical load in osteocytes (Jackson et al., 2021; Liedert et al., 2020). This suggests that higher-intensity physical activity might be necessary to induce this reaction.

Dependency of the mechanotransduction on estrogens could be an impediment to the exercise-induced downregulation of sclerostin. Holguin et al. (2016) observed significant age-related impairment of sclerostin reaction to mechanical loading which led to lower loading-induced bone formation in older mice. However, this impairment has not yet been observed or researched in humans. Therefore, sclerostin could be one of the key molecules to target in the prevention of postmenopausal osteoporosis, as regular physical activity has been shown to decrease serum sclerostin levels and thus counteract the negative impact of the menopausal transition.

4.1.2. Osteoprotegerin

OPG is a negative regulator of the RANKL/RANK signaling pathway. It functions as a decoy receptor, binding RANKL molecules and thus not allowing them to get to their receptor, RANK (Boyce & Xing, 2008). As such, it has a positive effect on bone strength through lowered bone resorption (Cawley et al., 2020) and it has been shown to increase bone density *in vivo* (Simonet et al., 1997). In cancellous bone, the primary source of OPG are mature osteoblasts, while in cortical bone, several different cell types contribute (Cawley et al., 2020).

RANKL/RANK pathway has many functions in different tissues. In bone, it provides signaling for osteoclast differentiation, activation, and survival, leading to normal bone remodeling (Boyce & Xing, 2008). RANK is a cytokine receptor from the tumor necrosis factor receptor superfamily that is expressed by both osteoclast precursors and mature osteoclasts (Dougall et al., 1999; Nakagawa et al., 1998). Its ligand, RANKL, is in bone mainly expressed by osteoblastic stromal cells and osteocytes (Xiong et al., 2015). After RANKL binds to RANK, several other adapter molecules, such as TNF receptor-associated factors or growth factor receptor-bound protein 2, bind to RANK's cytoplasmic domain and continue the signalization inside the developing osteoclasts (Kim et al., 1999; Wada et al., 2005).

The RANKL signaling is essential for proper development and resorptive function of osteoclasts (Armstrong et al., 2002; Li et al., 2000), while OPG modulates its response to maintain bone homeostasis and has been shown to reduce bone resorption caused by osteoclasts (Tong et al., 2019). This suggests OPG could play a role in protecting bone against the elevated rate of bone resorption during menopause and maintaining a healthy BMD. On top of that, several genetic polymorphisms in TNF receptor superfamily member 11b (*TNFRSF11B;* gene encoding OPG), such as rs6993813 and rs3134069, are associated with differences in BMD in postmenopausal women, mostly at femoral neck, but also other skeletal sites like lumbar spine and total hip (Choi et al., 2005; Peng et al., 2018; Shang et al., 2013; Takács et al., 2010). For example, G/G genotype of the 245T/G polymorphism correlates with BMD at several sites in postmenopausal women (Yamada et al., 2003). It could thus be used as a predictive marker for lower BMD. Others, such as rs3102735, could be used as predictors for the risk of osteoporotic fractures (Langdahl et al., 2002).

OPG expression in bone is highly influenced by circulating estradiol levels in addition to a variety of other hormones, cytokines, and the Wnt/β-catenin pathway (Boyce & Xing, 2008) as the stabilization and accumulation of β-catenin leads to an increase in OPG expression

in bone cells (Glass et al., 2005). Estrogens stimulate the production of OPG by binding to estrogen receptors of osteoblastic cells, and thus contributing to the suppression of osteoclast formation (Michael et al., 2005). This indicates that the perimenopausal decline in circulating levels of estrogens could cause an increase in osteoclast differentiation and activity by decreasing OPG production. Similar effect has been observed by Li et al. (2015) in ovariectomized mice, where *Tnfrsf11b* mRNA expression in bone decreased significantly following the drop in estradiol levels after ovariectomy, while RANKL expression increased. Thus, the RANKL/OPG ratio increased. Such an increase usually leads to osteoclastogenesis and promotes bone resorption (Boyce & Xing, 2008). Simonet et al. (1997) reported that recombinant murine OPG can protect rats against bone loss related to ovariectomy.

However, these results might not apply entirely to postmenopausal women and the risk of osteoporosis, as the data on OPG levels in humans are very conflicting. For example, Azizieh et al. (2019) reported lower serum levels of OPG in both osteopenic and osteoporotic women, as could be expected, but others have observed significantly higher serum levels of OPG in postmenopausal women with osteoporosis compared to healthy women in the same age group (Veshnavei, 2022; Wahhab et al., 2020). One possible explanation of the unexpected latter result could be that an increase in OPG concentration is a protective mechanism that osteoporotic bones induce to help regulate the rapid bone resorption and bone loss (Rogers et al., 2002). To add to the range of results, some studies show no correlation between OPG serum levels and osteoporosis at all (Liu et al., 2005; Ueland et al., 2007). This further complicates the effort to assess the association between OPG and postmenopausal osteoporosis in women.

Although the precise relationship between serum OPG levels and postmenopausal osteoporosis remains unclear, an increase in OPG concentration and a decrease in the RANKL/OPG ratio have been suggested as possible ways of protection against bone loss and osteopenia. In ovariectomized mice, treatment with recombinant human OPG reversed osteopenia and significantly increased BMD and bone volume (Kostenuik et al., 2004). Similar effects were reported by Shimizu-Ishiura et al. (2002) who observed a reduction in bone loss via impairment of the osteoclast resorption activity after administering OPG to ovariectomized mice.

An increase in OPG concentration may be accomplished by mechanical stress on bones. Kim et al. (2006) observed that shear stress on murine bone marrow stromal cells leads to a decrease in the RANKL/OPG ratio. They reported that the OPG levels grow, and RANKL levels decrease with increased load duration. Other studies confirmed the effect of *in vitro*

mechanical stimulation on RANKL (Galea et al., 2020), but results on the influence of exercise *in vivo* are not consistent (Bergström et al., 2012; Marques et al., 2011; Pichler et al., 2013). The OPG response however is similar in exercise-induced bone loading and in *in vitro* experiments. Circulating OPG levels in women who exercise regularly are significantly higher than in sedentary women (West et al., 2009), and one year of regular activity significantly increases mean serum levels of OPG in postmenopausal women (Bergström et al., 2012). Even exercise interventions as short as eight weeks can cause an increase in OPG levels (Hur et al., 2018). On the other hand, Kim et al., 2019 observed no changes in OPG expression after an exercise intervention of 12 weeks. It could be argued that studies of 8-12 weeks are too short to yield any significant results or that other differences in the exercise protocol, such as exercise type or intensity, could cause the different results. Seeing as Hur et al. (2018) have focused solely on resistance exercise, while both Bergström et al. (2012) and Kim et al. (2019) used a combination of resistance and endurance exercise, this could mean that resistance exercise interventions are more effective in bringing results after shorter periods.

Even though the influence of the menopausal transition on OPG is debatable, the increase in serum levels of OPG post-exercise is generally viewed as a positive influence on bone health no matter the serum OPG levels pre-exercise, as it downregulates osteoclastogenesis and bone resorption, processes known to be upregulated after menopause (Manolagas, 2000). This suggests that OPG is a potential player in the protection against osteoporosis.

4.1.3 Osteopontin

Osteopontin is a glycosylated phosphoprotein found in the extracellular matrix of bone tissue (Icer et al., 2018) expressed by all the main bone cell types (Luukkonen et al., 2019; Merry et al., 1993; Terai et al., 1999).

Although OPN has various functions, it is often associated with bone destruction (Icer et al., 2018). It aids in binding of osteoclasts onto bone surface through its interactions with integrins and other molecules, such as the vitronectin receptor, present on osteoclast plasma membrane (Reinholt et al., 1990; Ross et al., 1993). However, it has many other functions: it modulates osteoclastic activity (Chellaiah & Hruska, 2003; Walker et al., 2010), and it can stimulate osteoblast proliferation and matrix mineralization (Zhang et al., 2020).

Several studies observed higher serum levels of OPN in postmenopausal women compared to premenopausal groups (Chang et al., 2010; Cho et al., 2013; Reza et al., 2016). Serum OPN levels negatively correlate with BMD at lumbar spine and total hip in postmenopausal women (Cho et al., 2013; Vancea et al., 2021). Moreover, serum concentration of OPN is increased in postmenopausal patients with osteoporosis compared to healthy postmenopausal women (Al-Nejjar et al., 2015; Fodor et al., 2013). This evidence indicates that OPN plays a significant role in postmenopausal osteoporosis. According to Vancea et al. (2021), serum OPN levels might even be used as a biomarker for an early diagnosis of postmenopausal osteoporosis. Such conclusion can be supported by findings of Yoshitake et al. (1999) who reported that OPN-deficient mice are resistant to ovariectomy-induced bone resorption and osteoporosis. In this study, a significant reduction of bone volume was observed after ovariectomy in wild-type mice but not OPN-deficient mice.

The effect of exercise on the OPN levels in postmenopausal women has yet to be thoroughly researched. Still, it has been shown that serum OPN levels negatively correlate with the amount and intensity of regular physical activity, being more than 25 % lower in very active studied patients compared to inactive patients with coronary artery disease (Sponder et al., 2016). Recent studies show that OPN levels decrease significantly in response to high-intensity interval exercise in overweight or obese women and men (Raman et al., 2023). This decrease lasts for roughly a day, as serum OPN levels returned to pre-exercise levels 25 hours after exercise (Raman et al., 2023), but more data is needed to support this notion. Exercise programs as short as eight weeks, resulting in fat loss, have been shown to cause a decline in serum OPN levels, even though OPN concentration is not correlated to body fat (You et al., 2013). With these results, it is expected that exercise will have some positive effect on circulating OPN levels in postmenopausal women as well.

More research in this area is needed to reach meaningful conclusions, but the results we have today show that osteopontin could be one of the molecules playing a role in the positive effect exercise has on postmenopausal bone health.

4.2. Molecular factors produced by other tissues

Bone metabolism and bone turnover are not only influenced by physical activity directly but also through endocrine signaling. Different molecules, such as cytokines, growth factors, and microRNAs, are secreted by many tissues, especially skeletal muscle and the adipose tissue, in response to exercise and influence bone as well as many other parts of the body. Arguably, the most important in signaling to bone, with regards to exercise, are myokines, molecules secreted by skeletal muscle, such as myostatin, IGF-1, or irisin (Lombardi et al., 2016). Expression of some of these molecules is dysregulated in postmenopausal women, and thus their serum concentrations can be elevated or decreased.

Estrogen depletion after menopause also enhances the production of cytokines in peripheral blood mononuclear cells and thus increases their concentrations in sera of postmenopausal women (Kim et al., 2012). To this day, the best-studied exercise-influenced cytokines are IL-6 and irisin (Domin et al., 2021), both of which are influenced by menopause and impact the bone. However, many others, including TNF-α, IL-1, and various other interleukins, have also been linked to menopause (Pacifici et al., 1991; Pfeilschifter et al., 2002) as well as postmenopausal osteoporosis (Zheng et al., 1997). Thus, they could play a role in postmenopausal bone health and reaction to exercise, just like some of the myokines. Figure 2 summarizes those molecules affected by both menopausal transition and mechanical stimuli. The precise mechanisms are described closely in the following subchapters.
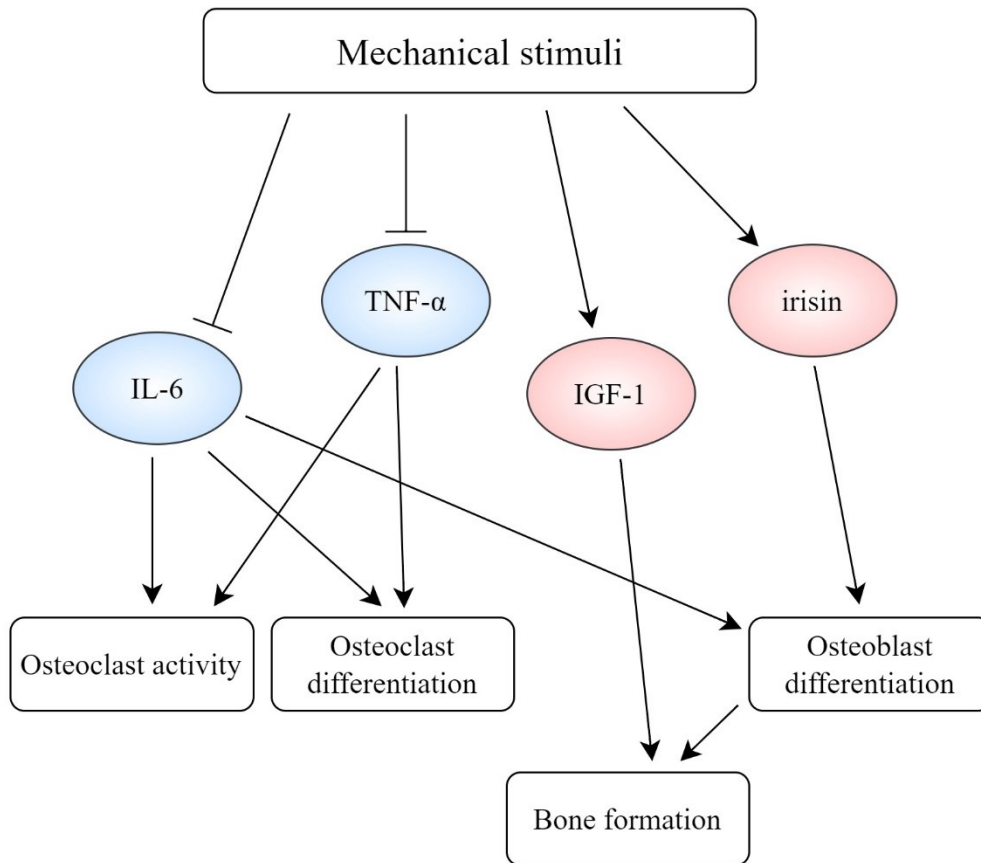
Figure 2. Effect of mechanical stimuli on molecular factors produced by other tissues and their function in bone. Molecules that are upregulated after menopause are marked in blue, and those that are downregulated after menopause in red. Sharp arrows indicate stimulation; blunt arrows indicate inhibition. IL-6 – interleukin-6; TNF-α – tumor necrosis factor-α; IGF-1 – insulin-like growth factor 1. Source: author.

4.2.1 Interleukin-6

IL-6 is a proinflammatory cytokine often linked to osteoporosis (Favalli, 2020; Harmer et al., 2019). It has various roles in bone metabolism, as it activates signal transducer and activator of transcription 3, a transcription factor that plays a part in the regulation of both osteoblast and osteoclast differentiation (Itoh et al., 2006). Exogenous IL-6 with its soluble receptor promotes osteogenic differentiation (Xie et al., 2018). Moreover, IL-6, alongside other cytokines, can substitute RANKL in a non-canonical RANKL-independent osteoclast formation and promote osteoclast function (Feng et al., 2019).

Dysregulation in IL-6 signaling leads to abnormalities in bone metabolism (Xie et al., 2018). Chronic IL-6 overexpression leads to defects in skeletal development and impaired bone growth as a result of increased osteoclast activity, higher osteoclast surface, and uncoupling of the activities between osteoblasts and osteoclasts (De Benedetti et al., 2006). For that reason,

IL-6 production negatively correlates with lumbar BMD (Zheng et al., 1997). On the other hand, IL-6 deficient mice have a normal amount of bone, only higher turnover rates (Poli et al., 1994).

IL6 gene polymorphisms have been studied in the context of the skeleton, BMD, and osteoporosis predispositions. A correlation between the rs1800796 polymorphism and the risk of osteoporosis has been observed (Chen & Li, 2020), while genotype influence on the impact of exercise has been suggested regarding the rs1800795 promoter polymorphism (Dhamrait et al., 2003). Multiple other studies have reported a significant effect of the *IL6* genotype on BMD at various sites (Murray et al., 1997; Ni et al., 2014; Tsukamoto et al., 1999). As an example, C/C genotype of the -174G/C polymorphism is associated with both higher BMD values and a reduced risk of osteoporosis compared to G/G or G/C genotype (Ni et al., 2014), as is the C/C genotype of the -634C/G polymorphism (Li et al., 2008). *IL6* gene polymorphisms are also linked to markers of bone resorption, such as S-CTX (Ferrari et al., 2003). This data could be beneficial in further evaluation of the role of IL-6 in osteoporosis risk.

Postmenopausal women can be characterized by higher circulating levels of IL-6 than premenopausal women (Kim et al., 2012; Latjindung et al., 2020; Rachoń et al., 2002). Non-stimulated peripheral blood mononuclear cells originating from postmenopausal women release higher amounts of IL-6 *in vitro* than cells from premenopausal women, as observed by Rachoń et al. (2002). On top of that, they have also observed spontaneous endogenous activity of the *IL6* gene *in vivo* which is almost non-existent in premenopausal women. This spontaneous IL-6 production was decreased by estradiol, supporting the notion that it is influenced by the menopausal reduction of levels of estrogens. Estradiol also suppresses *in vitro* IL-6 production by bone marrow stromal cells and osteoblastic cells (Jilka et al., 1992). The role of IL-6 in postmenopausal bone loss is further supported by the fact that in IL-6 deficient mice, there is no ovariectomy-induced bone loss similar to wild-type ovariectomized mice (Poli et al., 1994).

Several studies have observed a direct link between circulating IL-6 concentration and postmenopausal osteoporosis, as the production of IL-6 by blood cells is higher in osteoporotic postmenopausal women compared to healthy controls (Favalli, 2020; Zheng et al., 1997) and even compared to premenopausal osteoporotic women (Jeedigunta et al., 2020). It has even been suggested as a possible predictive biomarker for osteoporotic fractures in postmenopausal women (Wang et al., 2022), but more follow-up studies are needed for conclusive results on this topic.

The effect of sex hormone depletion and exercise on IL-6 has been studied in mice. After ovariectomy, the concentration of IL-6 significantly increases in murine tibia (Li et al., 2014) and serum (Abdelfattah Abulfadle et al., 2023). After exercise intervention, both *Il6* mRNA and protein levels in murine bone decline and stay lower than in the ovariectomized mice without exercise (Li et al., 2014).

Physical activity in humans leads to acute IL-6 production and secretion in the skeletal muscle thanks to the contractile activity (Keller et al., 2001) and in general causes an acute response in pro- and anti-inflammatory cytokines (Sun et al., 2004). The magnitude of the responses, including the IL-6 response, depends on the duration and intensity of exercise (Fischer, 2002). However, with regular training the body adapts to physical activity. One of these adaptions is arguably a decreased basal production and plasma levels of IL-6, as the levels of IL-6 are lower in people who exercise regularly and further decrease with increasing amounts of exercise (Colbert et al., 2004). Especially aerobic exercise has been reported to reduce IL-6 serum concentration (Khalafi et al., 2021). Aerobic exercise-induced expression of *IL6* mRNA in skeletal muscle is significantly reduced by 10 weeks of training, even though its expression in resting muscle does not change in such a short time (Fischer et al., 2004). In another study, a 12-week intervention was enough to lower resting serum IL-6 levels in young, healthy women (Kim et al., 2019).

The composition of the observed groups could play a role in these findings, as earlier age of menopause is correlated with higher serum IL-6 and higher bone loss in femoral bone within the first ten postmenopausal years (Scheidt-Nave et al., 2001). Overall, these findings suggest that exercise could be used to lower circulating IL-6 levels that naturally increase with age and after menopause.

4.2.2 Tumor necrosis factor-α

TNF-α is another proinflammatory cytokine found in chronically increased serum levels in postmenopausal women (Conceição et al., 2012). It contributes to the dysregulation of osteoclast activity that occurs in postmenopausal bones. It has been suggested that TNF-α plays a role in an alternative osteoclast differentiation route via interaction with downstream elements of the RANKL signaling pathway (Yamashita et al., 2007). *In vitro*, hematopoietic precursors from RANK deficient mice become osteoclasts when stimulated with TNF-α in the presence of some cofactors, such as transforming growth factor-β (Kim et al., 2005), and TNF-α can promote osteoclastogenesis even in nuclear factor kappa-light-chain-enhancer of activated B

cells deficient mice, even though only in a lower amount than RANKL can (Yamashita et al., 2007). However, to induce osteoclast differentiation in the osteoclast precursors, TNF-α needs RANKL as a cofactor (Lam et al., 2000).

Polymorphisms in the *TNF* gene, such as rs1800629, have also been linked to BMD and different risks of fracture, but there has been no significant conclusion on whether any of them could be used as independent predictors of BMD (Futura et al., 2004; Moffett et al., 2005; Wennberg et al., 2002). However, the rs1800629 polymorphism could arguably be used to determine the relative risk of hip fracture in older women (Moffett et al., 2005).

The osteoclastogenic function of TNF-α arguably plays a role in postmenopausal osteoporosis. Serum TNF-α levels are elevated in osteoporotic postmenopausal women compared to healthy postmenopausal controls (Jeedigunta et al., 2020; Zheng et al., 1997) as well as compared to premenopausal osteoporotic women (Jeedigunta et al., 2020). Circulating TNF-α levels rise significantly in women who undergo oophorectomy and reach the highest levels about eight weeks after surgery, but they decrease again if the women start estrogen therapy (Pacifici et al., 1991). Ovariectomy and subsequent estradiol treatment have the same effects in mice (Abdelfattah Abulfadle et al., 2023). This points to a direct influence of estrogens on circulating TNF-α concentration which would explain the chronic elevation after menopause.

Exercise has an anti-inflammatory effect and lowers circulating TNF-α levels, similarly to IL-6 levels. There is a significant decrease in TNF-α after six-week exercise training in ovariectomized mice (Abdelfattah Abulfadle et al., 2023). Exercise training has also been observed to reduce TNF-α levels long-term with a moderate effect size (Khalafi et al., 2021; Petersen & Pedersen, 2005); the best results were obtained by a combination of aerobic and resistance training (Khalafi et al., 2021).

4.2.3 Irisin

Irisin is an adipomyokine released among other tissues by skeletal muscles upon physical activity with several mainly metabolic functions in muscles and adipose tissue (Arhire et al., 2019). In the skeleton, it has the potential to promote the differentiation of bone marrow stromal cells into osteoblasts and thus has an anabolic effect on bones (Colaianni et al., 2014; Zhang et al., 2017). It has been observed that osteoblast number and trabecular and cortical bone thickness increase after intraperitoneal irisin administration (Zhang et al., 2017). These

findings match the fact that serum irisin levels positively correlate with total body BMD (Liu et al., 2021; Roomi et al., 2021).

There is not enough data on changes in the production or secretion of irisin during and after menopause, but serum irisin levels in humans have an inverse association with age (Zhang et al., 2022a), which means older women have lower serum concentrations than younger women. On the other hand, serum irisin levels in mice after ovariectomy increase which could correlate with the change in sex hormones but also with an increase in body weight and adipose tissue content after the surgery (Zügel et al., 2016).

While the impact of menopause on irisin levels is not clear, positive effects of irisin on bone have been observed in postmenopausal women. Lower circulating irisin concentration is associated with a higher risk of osteoporosis and related hip fractures in postmenopausal women (Liu et al., 2021). Studies on mice agree with its positive impact on bone as well. Irisin treatment in ovariectomized mice leads to the return of bone turnover biomarkers and characteristics, such as dry weight or mineral content, to levels comparable to control groups (Morgan et al., 2021). In conclusion, the upregulation of irisin would most likely positively impact postmenopausal bone health.

Acute exercise significantly increases irisin production in muscles and thus its serum levels (Fox et al., 2018; Löffler et al., 2015; Nygaard et al., 2015). Moreover, in women, the concentration rises more than in men, even though the resting levels are comparable in both sexes (Zügel et al., 2016). This suggests that irisin could help protect bones in women, both before and after menopause.

The irisin response seems to be affected by different types of exercise. Tsuchiya et al. (2015) observed a significant increase in plasma irisin levels after resistance exercise, higher compared to both endurance exercise and a combined trial. Huh et al. (2015) also reported resistance exercise to be more effective than endurance exercise at both moderate and high intensity. Moreover, aerobic exercise has only a short-term effect on serum irisin levels which is similar 90 minutes post-exercise compared to pre-exercise levels, even in prolonged exercise sessions (Kraemer et al., 2014). Irisin response to exercise has been reported to be generally short-term by Huh et al. (2015), as the change in serum levels is the highest immediately post-exercise; however, they only focused on male subjects, and thus more research is needed to draw conclusions on the longevity of the irisin response in women.

Contrary to the previously mentioned results, Nygaard et al. (2015) did not observe any differences between endurance and strength exercise concerning irisin levels. The difference in results could be explained by variable intervention design, exercise duration, and intensity. To maximize irisin production and its anabolic impact on bone, the intervention should include resistance exercise in at least 1-hour long sessions. It should also be high-intensity exercise, as post-exercise circulating irisin levels correspond to exercise intensity (Huh et al., 2014).

4.2.4 Insulin-like growth factor 1

IGF-1 is a polypeptide that significantly impacts bone mineral accrual in adolescence (Breen et al., 2011) and has an anabolic effect in adults (Yakar et al., 2018). It is produced by almost all tissues in the body (Yakar et al., 2010), including osteoblasts in the skeleton (McCarthy et al., 1991). Its expression is induced by growth hormone (GH), and it functions as a mediator for GH function in bone (Daughaday et al., 1972). IGF-1 plays a crucial role in secondary ossification and trabecular bone formation (Wang et al., 2015), and type I collagen synthesis in bone (Thomas et al., 1999). The anabolic effect of IGF-1 on bone is enacted by its modulation of the impact of PTH on bone cells and by its signaling in mechanosensing pathways (Tahimic et al., 2013).

IGF-1 is necessary for proper bone development. Wang et al. (2015) observed that IGF-1 receptor (IGF-IR) knockout mice develop dwarfism characterized by smaller body size and decreased femur and tibia length. These knockouts also have a reduced number of osteoblasts in the inner layer of the perichondrium and the proliferating zone of the growth plate, which indicates a role of IGF-IR in osteoblast proliferation and differentiation. In general, these mice had decreased trabecular bone volume and cortical thickness compared to controls.

In humans, IGF-1 concentration in bone matrix correlates positively with bone volume (Seck et al., 1998) and BMD, even with age taken into account (Liu et al., 2008; Zhang et al., 2022b). In older women over 72 years of age, circulating IGF-1 levels positively correlated with BMD at all measured sites, such as femoral neck, radius, or lumbar spine, even though the same correlation was not observed in men (Langlois et al., 1998). The reason for this is unclear, but it could be a result of hormonal differences between the sexes. Sugimoto et al. (1997) observed the same correlation between serum IGF-1 levels and BMD at all measured sites in postmenopausal women.

Serum levels of IGF-1 significantly decrease during the first year after menopause and stay stable during later years (Nasu et al., 1997; Poehlman et al., 1997), in addition to decreasing over the whole life span from early adulthood (Liu et al., 2008; Seck et al., 1998). Thanks to its concentration starting to lower early in life, it has been suggested that IGF-1 could function as an early marker for osteoporosis in both postmenopausal and premenopausal women (Liu et al., 2008). The perimenopausal decrease in IGF-1 corresponds to the period of peak bone loss in the first years of and after menopause.

The concentration in serum is even lower in osteoporotic postmenopausal women compared to healthy postmenopausal group (Zhang et al., 2022b), and serum levels of IGF-1 could arguably be used as clinical predictors of spine fracture risk (Sugimoto et al., 1997). Moreover, specific *IGF1* polymorphisms, such as rs35767, have been associated with the risk of osteoporosis in postmenopausal women (Gao et al., 2018). Wei et al. (2015) have reported an association of the rs35767 and rs972936 polymorphisms with BMD at some sites in patients with osteoporosis but not with the risk of osteoporosis itself, while Yun-Kai et al. (2014) concluded that T alleles of the rs35767 polymorphism could be linked to higher increased risk of osteoporosis, as well as lower BMD at multiple measured sites, such as vertebrae and total hip. More focus on these associations could be beneficial in evaluating these risk factors and determining if they could be used for preventative or prognostic purposes.

One of the ways to increase circulating IGF-1 concentration is physical activity. IGF-1 expression increases in response to acute exercise, even after sessions as short as 10 minutes (Schwarz et al., 1996), but long-term interventions can lead to chronic elevation of serum IGF-1 accompanied by its positive effects on bone (Borst et al., 2001; Hinton et al., 2017). Hinton et al. (2017) observed a 26 % increase in serum IGF-1 levels after a 12-month exercise intervention, but no significant differences between different types of exercise (resistance or aerobic). The change in circulating IGF-1 is observable after a period as short as 12 weeks with aerobic exercise (Praksch et al., 2019). Borst et al. (2001) observed a similar increase after 12 weeks and 25 weeks of resistance training, while Milliken et al. (2003) have not observed any increase after 12 weeks of weight-bearing and resistance exercise. This could mean that aerobic exercise is a more reliable method for increasing the serum concentration of IGF-1 or that 12 week-interventions are too short to assess the effects of exercise accurately. Higher intensity of exercise provokes a bigger acute increase in IGF-1 than lower intensity when measured right after the exercise session (Schwarz et al., 1996), but there was no reported difference in circulating IGF-1 levels between different intensities of exercise interventions in the long-term

(Borst et al., 2001). This means that intensity does not matter as much as the exercise type in targeting IGF-1 production.

5. Conclusion

Regular exercise is considered to be one of the most effective ways to prevent the decline in bone quality and lower the risk of osteoporosis during aging and after menopause. Numerous molecular factors and pathways are influenced by the estradiol decline during the menopausal transition and many others participate in mechanotransduction and the bone reaction to physical activity. Understanding the links between these two processes is beneficial in designing and improving the guidelines for physical activity recommended to postmenopausal women.

Sclerostin, OPG, OPN, IL-6, TNF-α, irisin, and IGF-1 are all molecules that link exercise to postmenopausal bone health, as shown in Table 1. OPG, irisin, and IGF-1 positively affect bones and BMD, either by signaling to increase bone formation or lowering the osteoclastogenesis rate. The decline in these functions, as the concentrations of these molecules decrease during menopause, negatively affects bone health in postmenopausal women. One way to increase their levels is regular physical activity. On the other hand, sclerostin, OPN, IL-6, and TNF-α impact BMD negatively in several ways. Their upregulation during menopause contributes to the rapid loss of bone that accompanies the menopausal transition, but this effect could be balanced by exercise which has been shown to cause long-term decrease in bone or circulating levels of these molecules.

Table 1. Summary of the mentioned molecules, their functions in bone, and their association with the menopausal transition and exercise. Source: author.

Molecule	Impact on bone	Levels after menopause	Effect of long-term exercise	Exercise type preference
Sclerostin	↓ osteoblast differentiation	elevated	downregulation	no preference
Osteoprotegerin	↓ osteoclast differentiation and activity	reduced	upregulation	resistance exercise
Osteopontin	↑ osteoclast activity	elevated	downregulation	no preference
Interleukin-6	uncoupling of osteoblast and osteoclast activity	elevated	downregulation	aerobic exercise
Tumor necrosis factor-α	↑ osteoclast differentiation and activity	elevated	downregulation	combination of aerobic and resistance exercise
Irisin	↑ osteoblast differentiation	reduced	upregulation	resistance exercise
Insulin-like growth factor 1	↑ bone formation	reduced	upregulation	aerobic exercise

There is no perfect exercise regimen seeing as different exercise types can influence some components of bone metabolism more than others. Each of the mentioned molecules responds best to slightly different types, intensities, and frequencies of exercise. OPG and irisin respond better to resistance exercise, while endurance exercise has a bigger effect on IL-6 and IGF-1. This suggests that if we are trying to target as many elements of postmenopausal bone health as possible, a combined protocol of both resistance and endurance exercise would be the most effective.

Interindividual differences, such as gene polymorphisms, should be considered when crafting exercise plans for specific individuals. If a person has an allele or genotype linked to lower BMD, targeting the affected molecule could be more beneficial. On the other hand, if the polymorphism has been shown to lower the mechanotransduction effectivity or the response to physical activity, as has been shown in some molecular factors like IL-6, focusing on other molecules could be more effective in creating a better-suited exercise plan. However, to determine these interactions between gene polymorphisms and bone response to exercise, more research is needed focusing on each of the specific molecules and on the role of genetic predispositions on mechanotransduction in general. Gene-environment interactions are complex, and it is unsure how big of a role genetic predispositions play in the general population with normal amounts of physical activity. However, they could provide further insights into designing individual guidelines dependent on a particular genotype.

The molecules and pathways described in this work represent the most direct currently available evidence for connection between menopausal bone and positive effects of exercise on the molecular level. Still, it needs to be emphasized that other factors might be at play, as the response to exercise in the human body is very complex. Future research might identify other molecules and pathways responsible for the effect of physical activity on bone. Subsequently, this relationship might be further studied in the context of postmenopausal bones. Regular physical activity has a vital role in the prevention of postmenopausal bone loss and bone fragility; moreover, it can reverse some of the negative impacts that menopause has on bones. Better understanding of the connections between the effect of menopause and mechanotransduction in bone may hold therapeutic potential for postmenopausal osteoporosis.

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