The Pathology and Treatment of Osteoporosis

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Abstract

Osteoporosis is a disease that causes bone fragility and decreased bone mass. Osteocytes and signaling molecules regulate the function of osteoclasts and osteoblasts. The RANK pathway is a key regulator in osteoclastogenesis. When the osteoclastic function is greater than osteoblastic function, bone integrity decreases. Causes of osteoporosis include hormones, medications, and diet. Treatments for osteoporosis are hormonal, medicinal, and lifestyle changes. More research needs to be done to find more effective medications with fewer negative side effects to improve the overall quality of life for patients. Currently, lab trials are being done on potential new medications to treat osteoporosis.

The Pathology and Treatment of Osteoporosis

Over 343 million people worldwide have osteoporosis (Scholz-Ahrens et al., 2007). In the United States alone over $37.4 million was spent in 1997 to treat patients with osteoporosis. Osteoporosis is defined as a bone condition that causes fragility, microarchitectural deterioration, and decreased bone mass (Gür et al., 2001). Due to the loss of bone strength, many people have skeletal fractures (Alshbool, et al., 2014). There are many reasons that a person can develop osteoporosis such as decreased sex hormones, failure to develop strong bones during development, old age, malnutrition, excessive bone resorption, failure of bone reformation, medications, or genetic defects (Alshbool, et al., 2014; Raisz, 2005). An adult's bone mass is determined by their peak bone mass and the rate at which the bone is broken down (Gür et al., 2001). A person's skeletal mass and bone density remain rather constant from the time a person is done growing until 50 years of age (Kanis, 2002). At this point, the integrity of the bone structure begins to deteriorate, which results in bone fragility. Bone resorption is dependent on the number of relative osteoclasts and osteoblasts (Alshbool, et al., 2014). Osteoclasts perform bone resorption while osteoblasts promote bone formation.

The Skeletal System

The skeletal system is used for protection, movement, and mineral storage within the human body. Bones are made up largely of calcium and collagen matrices. The outer layer of bone, the compact bone, is made up of these collagen matrices that interconnect to create a rigid structure. Under the compact bone lies the trabecular bone. This consists of lightweight collagen and calcium structures. Bone marrow is in the middle of the bone, below the trabecular bone; it contains precursors for osteoblasts and osteoclasts. Osteocytes, located within the bony matrix, regulate the function and development of osteoblast and osteoclast precursors in the bone

marrow. Osteocytes are aging cells that come from osteoblasts, and they are embedded in the bony matrix. These cells can dysregulate osteoclastic and osteoblastic activity due to endogenous and exogenous factors. When osteoclastic activity dominates the osteoclast/osteoblast equilibrium, osteoporosis can occur. If osteoclast activity takes precedence over osteoblast activity, the bone mineral density (BMD) decreases. Decreased BMD can lead to fractures from even minor injuries. Postmenopausal women are at the highest risk for fractures due to decreased estrogen levels. Estrogen is a key regulator in maintaining osteoclast/osteoblast equilibrium.

Bone Structure

Bone is made up of hard compact bone on the outside with spongy bone and bone marrow on the inside. Bone marrow is important to produce red and white blood cells (Travlos, 2006). The compact bone is made up of a collagen framework (Weiner & Traub, 1992). Collagen is made up of two α1-chains and one α2-chain. Van Leeuwenhoek was the first person to identify lamellae as the basic unit that makes up bone. Lamellae are made up of parallel mineralized collagen fibrils. Lamellae make up canaliculi that form rings around Haversian canals, which contain nerves and blood vessels. Over 95% of bone is made up of osteocytes while 5% is osteoblasts and 1% is osteoclasts (Brotto & Bonewald, 2016). The lacunae contain the cell bodies; long cytoplasmic processes, also known as “fingers,” extend from the cell bodies in tunnels called canaliculi to interact with other cells within the bony matrix to obtain oxygen and other nutrients (Klein-Nulend et al., 2003). Mature osteocytes are stellate-shaped, thanks to their “fingers.” Hemichannels on the surface of osteocytes align to make gap junctions (Plotkin & Bruzzaniti, 2019). Osteocytes are also the mechanosensory cells that detect stress put upon the bones (Klein-Nulend et al., 2003). Thanks to the cytoplasmic processes, osteocytes can perform direct cell-to-cell coupling. This allows signals to be passed along the entire bone rapidly.

Osteocytes can sense mechanical stress due to interstitial fluid movement. In response to stimuli, osteocytes modify their surroundings by sending out signaling molecules to osteoblasts and osteoclasts.

Bone Marrow Function

Bone marrow makes up about 5% of the total body weight in humans (Travlos, 2006). The bone marrow is found within the central cavity of axial and long bones. This tissue is composed of hematopoietic islands, adipose cells, vascular sinus, and trabecular bone. Bone marrow has stem cells, lymphoid and myeloid precursors that are necessary for proper immune function, barrier cells, and macrophages. Due to having so many important precursors, bone marrow is extremely sensitive to chemical exposure. The bone marrow is also very sensitive to dietary restriction, inflammation, malnutrition, and protein intake. These changes can have detrimental effects on the precursor cells in the bone marrow. The precursor cells create blood cells through hematopoiesis and cells in the immune system through lymphopoiesis. When blood cells mature, they are allowed into the venous sinuses by barrier cells. The blood cells can then enter the bloodstream. However, platelets are made by megakaryocytes and are released into the venous sinus lumen via cytoplasmic processes. For hematopoietic precursor cells in the bone marrow to properly develop they must be “guided” by hematopoietic and lymphopoietic factors. Erythropoietin is made by the kidneys; it causes the production of erythrocytes from hematopoietic precursors. Hematopoiesis first starts with pluripotent stem cells in the bone marrow. The functions of the stem cells are to perform self-renewal and give rise to all hematopoietic cells. These stem cells are then influenced to differentiate into different cell lineages by different growth factors. When B-cells are produced they mature in the bone marrow,

where they are regulated by stromal cells. When T-cells are produced they go to the thymus to mature.

Physiology of Bone

Bones respond to mechanical stress by increasing the rate of remodeling to create stronger bones. Mechanical stress can come in the form of impact, such as running, or through muscle contractions, such as weightlifting. There is a delicate balance in bone remodeling called the “osteoclast/osteoblast equilibrium.” This equilibrium is maintained through complex signaling pathways. Osteocytes regulate osteoclastic and osteoblastic activity through these signaling pathways to maintain the equilibrium. Osteoclasts and osteoblasts can regulate themselves and one another. Genes also play a role in maintaining balance in the bone. All these factors working together allow for the proper rate of osteoclast and osteoblast activity.

Physiology of Healthy Bone

The skeletomuscular system is composed of bones, skeletal muscle, tendons, ligaments, cartilage, joints, and connective tissue (Brotto & Bonewald, 2016). The main functions of the skeletomuscular system are movement and support. During embryonic development, this system begins to form from somites from the mesoderm. The genes that control the organogenesis of the skeletomuscular system during development are closely related.

The skeletomuscular system is remarkable in that the bones can alter their shapes in response to the stress put on them by the muscles. It has been theorized that electrical signals from strong muscle contractions protect osteocytes from death (Brotto & Bonewald, 2016). Bone is a dynamic tissue that responds to anabolic loading and catabolic immobilization. Mechanical loading can occur during exercise. In response to strong muscle contractions that occur during exercise, osteocalcin is released from osteoblasts, osteoclasts, and osteocytes. Osteocalcin is a

hormone that increases throughout the body during exercise and has been shown to increase muscle strength. This hormone is deposited into the bony matrix by late osteoblasts and embedded osteocytes during bone formation. Osteoclasts release osteocalcin during bone resorption. Osteocytes are made when osteoblasts become surrounded by the bony matrix (Plotkin & Bruzzaniti, 2019). Once the osteoblasts are trapped, they alter the gene expression pattern, which changes them into osteocytes. Osteoblasts and osteoclasts can communicate through crosstalk via secreted factors and direct contact. There are key differences in the function and characteristics between osteocytes, osteoblasts, and osteoclasts. Osteocytes are the longest living cells in the bone; they can reside in the bony matrix for decades before succumbing to apoptosis or autophagy (Brotto & Bonewald, 2016). Osteoblasts and osteoclasts have an average lifespan of a few days to a few weeks. Osteocytes are also susceptible to the effects of aging; these cells cannot be replaced except during bone remodeling, which makes them senescent cells. Senescent cells are defined as cells that will eventually stop replicating but do not die off. When osteocytes die, minerals fill the lacuna through a process called micropetrosis. During micropetrosis, the osteocytes are turned into "living fossils." Osteocytes can also die when there is a low canicular flow that causes reduced nitrogen oxide (NO) production by osteocytes (Klein-Nuland). Reduced NO production and subsequent apoptosis of the osteocytes attract osteoclasts to the area. The osteoclasts then begin to excavate the area around the osteocyte. However, when there is proper canicular flow, osteocytes release NO, which causes osteoclasts to detach from the bony matrix (Klein-Nuland). NO essentially acts as a local inhibitor of osteoclasts due to its short half-life. This back-and-forth action of the osteoclasts causes a "treadmilling" effect where the osteoclasts detach and reattach to the bony matrix. Endothelial cells also produce NO in response to shear stress; this protects them against

apoptosis. During osteoclast activity, the bony matrix releases insulin-like growth factors (IGFs) that recruit osteoblasts (Plotkin & Bruzzaniti, 2019). IGFs promote osteoblast differentiation and inhibit osteoblastic apoptosis.

Osteoclasts degrade the bony matrix by creating an acidic environment (Plotkin & Bruzzaniti, 2019). First, the osteoclasts attach to the ruffled border of the bony matrix; this is where osteoclast enzymes are secreted. The osteoclasts attach to the ruffled border and then activate the vacuolar ATPase (proton pump). These protons then enter the subcellular lacunar compartment. Proton production is regulated by carbonic anhydrase II. This enzyme initiates the reaction of CO_2 and water to make carbonic acid and protons. Chloride channel CIC 7 (CLCN7) allows chloride ions to enter the subcellular lacunar compartment as well. Osteoclasts secrete enzymes that dissolve bone minerals and collagen and only work in acidic environments.

The bony matrix surrounds the bone marrow that contains pluripotent stem cells, red and white blood cells, platelets, and blood cell progenitors (Rosen & Klibanski, 2009). Stromal cells are also located in the bone marrow; they can become connective tissue in any organ and their development is regulated by hormonal signaling. Some of the stem cells in the bone marrow become osteoblasts; this process is accelerated when a bone is broken or during puberty. The bone-specific transcription factors needed for osteoblast differentiation are RUNX family transcription factor 2 (Runx2), core-binding factor subunit alpha-1 (Cbfa1), and osterix. Without these transcription factors, osteoblasts would be unable to differentiate and there would be no bone remodeling occurring. One study showed that when the Runx2, Cbfa1, and osterix genes were deleted in mice, osteoblasts were unable to differentiate properly, which resulted in a lack of mineralized bone (Plotkin & Bruzzaniti, 2019).

Important minerals needed for bone formation are magnesium, calcium, and zinc (Gür et al., 2001). Magnesium acts as a surrogate for calcium during transport in the mineralization process, and it is an important cofactor for enzymes used in this process. Zinc is important for maintaining a healthy bone mass because it inhibits bone resorption and stimulates bone formation. Calcium makes up most of the hard bony matrix. If there is a deficiency in any of these minerals this could cause a decrease in both BMD and bone mass. Osteoclasts can sense calcium in the bone via calcium-sensing receptors (CaSRs). $Ca2^{+}$ binds to CaSR in high concentrations; this causes apoptosis in osteoclasts. This is due to high concentrations of calcium mimicking the environment of demineralized bone. This is because if the bone has already been broken down, there is no need for osteoclastic function.

Bone Signaling Pathways

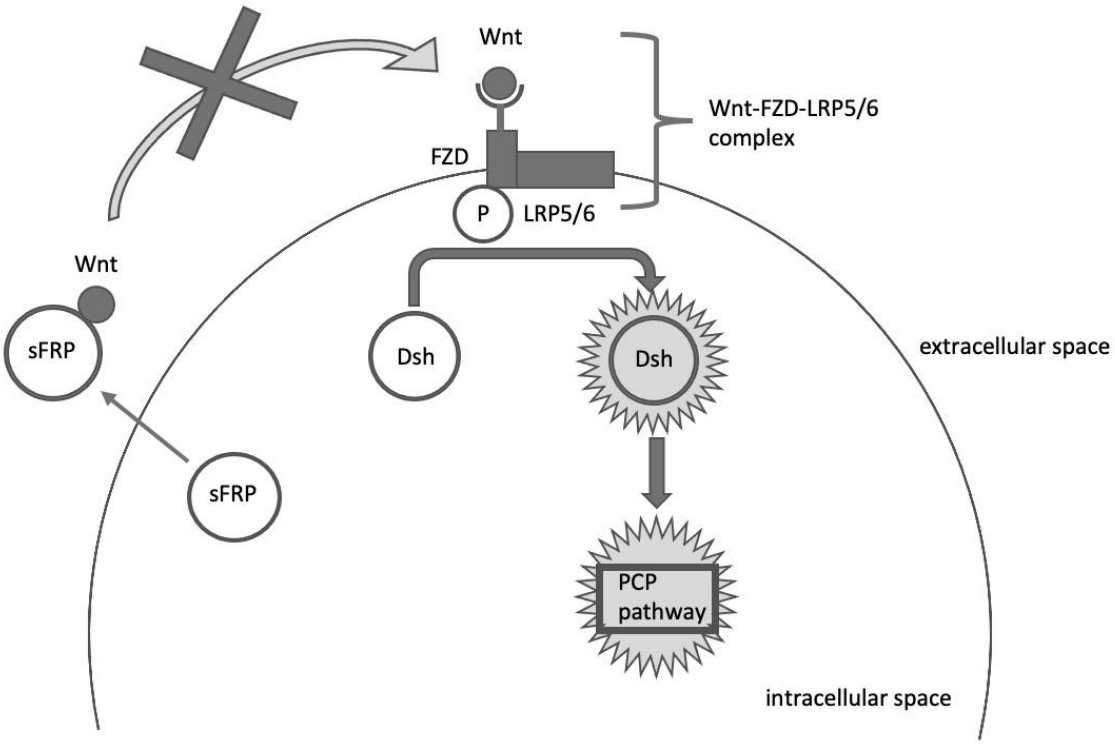
Osteocytes are the main regulators of bone mass through the Wnt/β-catenin pathway (Brotto & Bonewald, 2016). Osteocytes communicate to cells at the surface of the bony matrix. Crosstalk with the prostaglandin pathway triggers the Wnt/β-catenin pathway. Crosstalk occurs when there is mechanical loading; this causes a decrease in negative regulators of bone formation. Osteocytes also regulate homeostasis through phosphate regulating neutral endopeptidase on chromosome X (Phex), dentin matrix protein 1 (Dmp1), and fibroblast growth factor 23 (FGF23). Dmp1 and Phex downregulate FGF23, which causes the kidneys to increase the reabsorption of phosphate to maintain proper levels of BMD. When Dmp1 or Phex is absent, phosphate is excreted by the kidneys; this can lead to osteomalacia (the softening of the bone) or rickets (the softening of bone in children).

Wnts are important for maintaining bone mass (Plotkin & Bruzzaniti, 2019). They are a type of secreted glycoprotein. The Wnt pathway is significant in osteoporosis research because it

promotes osteoblastogenesis and inhibits osteoclastogenesis. There are many osteoporosis therapies being developed that specifically target the Wnt pathway. This pathway starts as Wnts bind to frizzled (FZD) receptors complexed with lipoprotein receptor-related protein 5/6 (LRP5/6). The Wnt-FZD-LRP5/6 complex is then phosphorylated, which makes it active. The complex activates disheveled (Dsh). Dsh then activates the planar cell polarity (PCP) pathway (Figure 1). This pathway controls cell migration and tissue morphogenesis. Secreted frizzled proteins (sFRPs) can bind to Wnt to prevent it from binding to FZD receptors. This means that sFRPs inhibit Wnt signaling. Osteoprogesterin (*OPG*) is one of the most studied Wnt signaling pathway target genes in osteoblastic cells. OPG is needed for osteoclast differentiation. It also counteracts osteoclastogenesis induced by nuclear factor κ-B ligand (RANKL) binding to its receptor. Interestingly, Wnt16 inhibits osteoclastogenesis by actively upregulating OPG levels. When OPG is present, osteoclast apoptosis occurs; this is due to the FasL/Fas pathway being activated, which leads to cell death. Studying the Wnt pathway's interactions with osteoclastic processes will allow researchers to develop more effective therapies to prevent osteoporosis.

Figure 1

Wnt Signaling



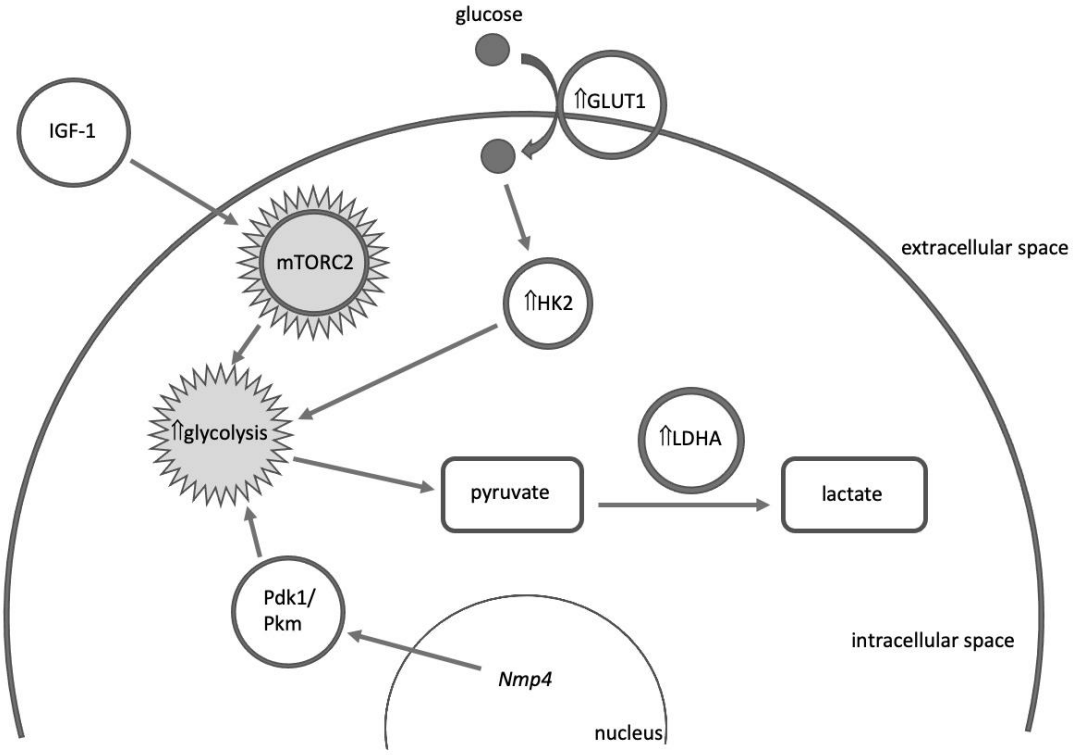
Note. Wnt binds to FZD-LRP5/6 complex. The Wnt-FZD-LRP5/6 complex is then phosphorylated. Dsh is activated by the complex. The activated Dsh then activates the PCP pathway. If sFRPs are secreted, they will bind to Wnt. This will prevent Wnt binding with the FZD-LRP5/6 complex. This is an original figure.

While it is important to understand the signaling pathways involved in regulating osteoclast and osteoblast activity, it is also necessary to know the genetics behind these mechanisms. The *Nmp4* gene controls many important processes in the body (Shao et al., 2019). In osteoblasts, there are over 15,000 *Nmp4* binding sites. *Nmp4* directs the cell toward aerobic glycolysis via Pdk1 and Pkm. Glycolysis is the main way glucose is used in osteoblasts. When PTH acts on the bone, it causes osteoblasts to secrete IGF-1. IGF-1 then activates mTOR

complex 2 (mTORC2), which causes glucose to be redirected to glycolysis rather than the TCA cycle. PTH also downregulates sclerostin in osteocytes, which activates the Wnt pathway. Osteoblasts upregulate glucose transporter 1 (GLUT1) and hexokinase 2 (HK2) to increase glucose use. This causes increased activity in lactate dehydrogenase A (LDHA) (Figure 2) and pyruvate dehydrogenase kinase 1 (PDK1). Pyruvate is then made into lactate rather than acetyl-CoA. This pathway is significant because it causes osteoblastic activity while inhibiting osteoclastic activity. *Nmp4* and PTH therapies are common targets for combatting the effects of osteoporosis.

Figure 2

PTH Effects on Osteoblasts



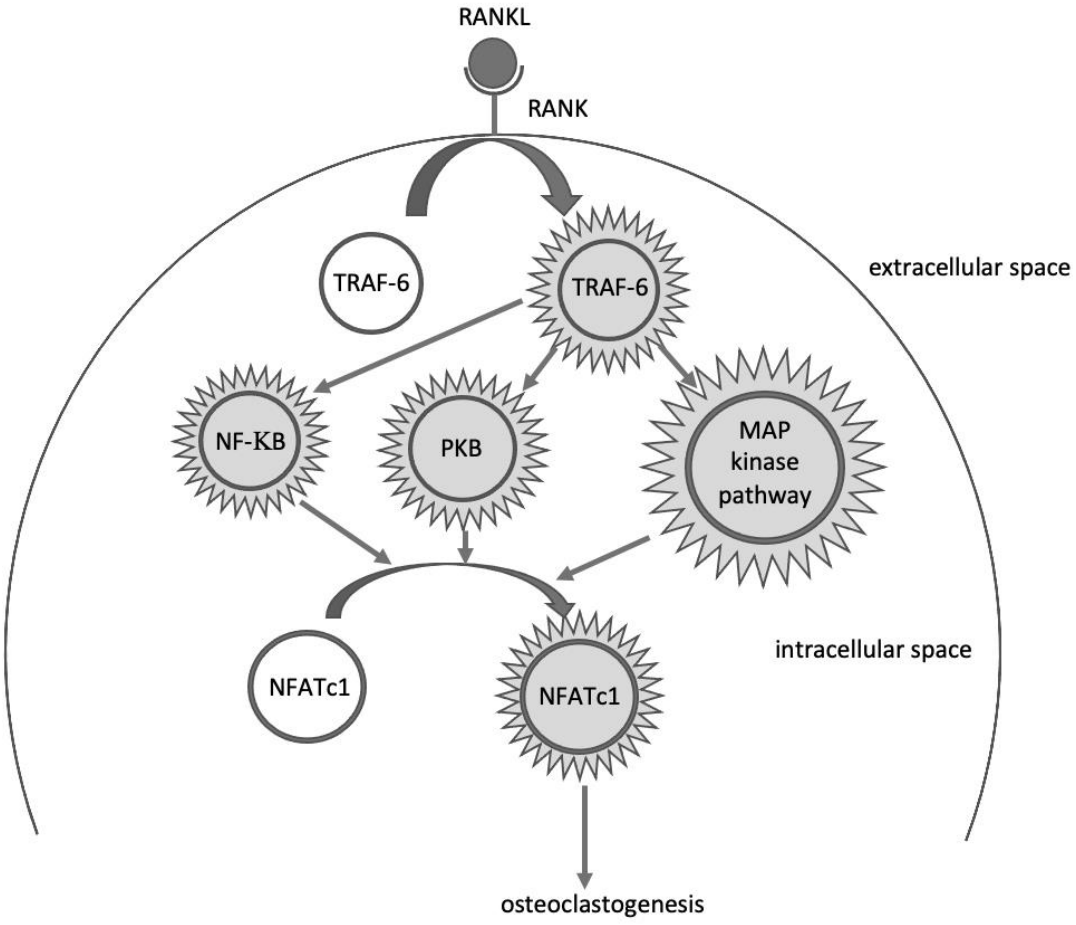
Note. When PTH acts on the bone, osteoclastic activity increases. The rate of glycolysis is increased by *Nmp4* upregulating Pdk1 and Pkm. Pdk1 and Pkm direct the cell towards aerobic

glycolysis. Osteoblasts secrete IGF-1 when acted on by PTH. IGF-1 activated the mTORC2 complex. The activated mTORC2 complex directs glucose towards glycolysis. GLUT1 is also upregulated; this allows the cell to take in more glucose at a faster rate. HK2 is upregulated, allowing glycolysis to occur at a faster rate. The product of glycolysis is pyruvate. LDHA is upregulated to convert pyruvate into lactate. This is an original figure.

The continuous remodeling that occurs in bones is made possible by hematopoietic stem cells and monocyte/macrophage progenitor cell-derived osteoclasts (Dou et al., 2016). Osteoclasts are regulated by receptor activator of nuclear factor kappa-β ligand (RANKL) and macrophage-colony stimulating factor (M-CSF). When RANKL binds to its receptor on the osteoclast, tumor necrosis factor (TNF) receptor-associated factor-6 (TRAF-6) is activated (Figure 3). TRAF-6 then activates nuclear factor kappa B (NF-κB), protein kinase B (PKB), and the mitogen-activated protein (MAP) kinase pathway. These pathways work together to activate the nuclear factor of activated T cells c1 (NFATc1); this is the "master regulator" of osteoclast proliferation, differentiation, and maturation. When the calcium concentration is low, osteoblasts upregulate RANKL; this causes an increase in osteoclast formation (Plotkin & Bruzzaniti, 2019). The RANKL pathway is a key regulator in maintaining the osteoclast/osteoblast equilibrium. If RANKL binding to RANK is inhibited, osteoclastic activity decreases. If RANKL continuously binds to RANK, the bone will be broken down to increase the calcium concentrations. This pathway is another target of novel drugs for osteoporosis.

Figure 3

RANKL Pathway



Note. RANKL binds to RANK; this complex then activates TRAF-6. The activated TRAF-6 then activates NF-κB, PKB, and the MAP kinase pathway. These all activate NFATc1 which promotes osteoclastogenesis and osteoclast activity. This is an original figure.

Estrogen also plays an important role in the maintenance of a healthy bone mass (Raisz, 2005). Estrogen and other sex hormones bind to sex hormone-binding globulin (SHBG) on the plasma membrane of cells in the bone and the body. Estrogen specifically acts on cells in the osteoblastic lineage, but it is theorized that it also influences osteoclasts and lymphocytes. One

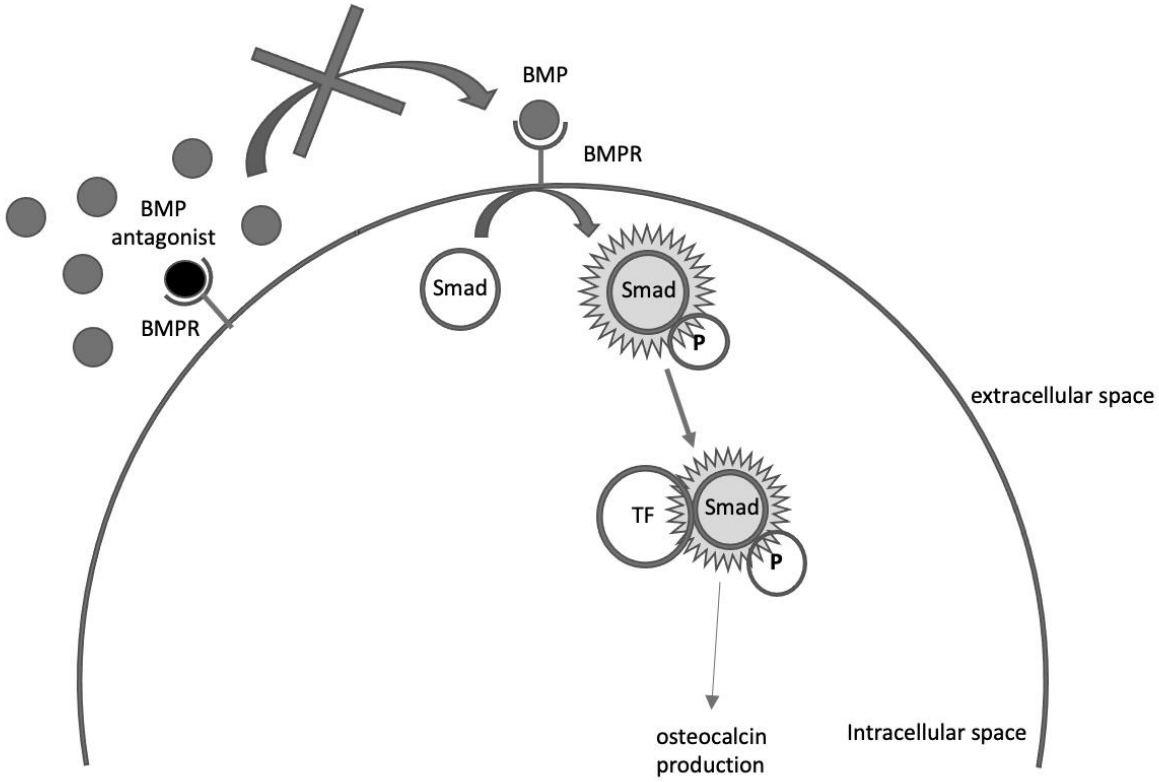
theory suggests that estrogen taken up by cells in the bones helps to suppress ROS. When estrogen levels are low, ROS levels increase; this causes TNF-α osteoblasts to produce RANKL. RANKL then binds to the receptor activator of NF-κB (RANK) on hematopoietic cells. RANKL binding to RANK causes osteoclasts to differentiate. RANK/RANKL interactions can be blocked by osteoblasts when they secrete osteoprotegerin (OPG). When bone resorption factors are secreted, osteoblasts upregulate RANKL expression and downregulate OPG production.

Another regulator of bone remodeling is forkhead box protein O1 (FoxO1) (Dou et al., 2016). FoxO1 is a transcription factor needed for metabolic processes and maintaining homeostasis. When PKB is activated during osteoclastogenesis, it can negatively regulate FoxO1 via phosphorylation. FoxO1 is needed to reduce reactive oxygen species (ROS) by upregulating antioxidant enzymes. Downregulation of FoxO1 will cause a decrease in antioxidant enzyme production, which will result in the accumulation of hydrogen peroxide in the mitochondria. Osteoclastogenesis and bone resorption are regulated by elevated hydrogen peroxide levels.

Bone morphogenic proteins (BMPs) belong to the transforming growth factor β (TGFβ) family (Plotkin & Bruzzaniti, 2019). These growth factors result in ectopic bone formation by promoting osteoblast differentiation. BMPs are precursors that are cleaved to become active. The active BMPs then phosphorylate Smad proteins to activate them. The phosphorylated Smad then binds to transcription factors to stimulate osteocalcin production (Figure 4). BMP activity is regulated by extracellular antagonists and potentiators. Antagonists of BMP work by sequestering BMP in the extracellular space and blocking the receptors for BMP binding. When osteocalcin is not produced, less osteoblastic activity occurs. Potentiators work by binding to BMPs and potentiating their pro-osteoblastic effects.

Figure 4

BMP Signaling



Note. Active BMP binds to the BMP receptors (BMPR). This complex then phosphorylates Smad to activate it. The activated Smad then binds to transcription factors. This causes the production of osteocalcin. BMP antagonists work by binding to the BMPR. This prevents BMP from binding, so no osteocalcin is produced. This is an original figure.

Osteoporosis Diagnostics and Prevalence

It is important for healthcare professionals to have the ability to diagnose osteoporosis in their patients. By diagnosing osteoporosis at its onset, patients can have a higher quality of life due to starting medications and therapies sooner. This will reduce their risk of bone fractures from minor injuries. Diagnostic techniques for detecting osteoporosis early have improved over the past decades; however, there is still a long way to go. Older people are more likely to develop

osteoporosis due to hormonal and lifestyle changes. It is necessary to understand the risk factors for developing osteoporosis to help prevent it later in life.

Diagnosis of Osteoporosis

Osteoporosis is diagnosed through the assessment of BMD; if a person's BMD falls more than 2.5 standard deviations below the young adult female reference mean, then they have osteoporosis (Kanis, 2002). If a person's BMD falls 1.0-2.5 standard deviations below the young adult female reference mean, then they have osteopenia. Osteopenia is like osteoporosis in that there is decreased bone mass, density, and mineral content but the bones are not as fragile compared to bones with osteoporosis. Osteopenia can be diagnosed before an osteoporosis diagnosis. Osteopenia can be considered a precursor to osteoporosis due to a lowered BMD. A person's BMD is determined by the about of minerals present divided by the area that is measured and by dual-energy X-ray absorptiometry (DXA). In DXA two X-rays are used at the same time at different frequencies. This allows the radiologist to observe the density of the bone without it being obscured by soft tissues surrounding the bone. Typically, BMD is measured in the hip and vertebrae, as these are the areas that are the most likely to fracture due to bone fragility. In addition to this, DXA can only observe a two-dimensional image of the bone. This means that the true volumetric density is not measured, but rather a relative number. This technique is still reliable due to the vast amounts of data available with to compare the results with. Some studies suggest that ultrasound may be the best technique to observe skeletal fragility due to the limitations of DXA. If a patient has had more than one fracture due to fragile bones, then they are diagnosed with severe osteoporosis. Men and women have a similar BMD, but women are more likely to have osteoporosis due to menopause.

Most postmenopausal women will develop osteoporosis due to low estrogen levels (Kanis, 2002). However, about 15% of young, healthy women have a standard deviation of 1.0 below the young adult female reference mean. Less than 0.5% of young women have osteoporosis with a standard deviation of 2.5 or more. In a study of 260 postmenopausal women over the age of 50, over half of the participants had a low BMD (Siris et al., 2001). Most of those diagnosed with low BMD did not previously have a low BMD diagnosis. This study also found that the BMD was highly predictive of a person's fracture risk. Women that are diagnosed with osteoporosis are twice as likely to fracture a bone (Iskrant & Smith, 1969).

Another diagnostic technique for osteoporosis is to analyze blood samples to determine the relative circulating numbers of osteoporotic numbers (Kanis, 2002). Some of the markers for bone formation and resorption are alkaline phosphatase, hydroxyproline, and pyridinium crosslinks. Urine tests can also be done to determine the amounts of dihydropyrimidine dehydrogenase (DPD), a bone resorption marker, and calcium ($Ca2^{+}$) present in the urine (Wei et al., 2012). High $Ca2^{+}$ presence in the urine indicates a higher rate of bone resorption than bone formation.

Prevalence of Osteoporosis

Musculoskeletal diseases increase with age (Scholz-Ahrens et al., 2007). Over a period of fifteen years, people ages 45-64 experienced a 33% increase in musculoskeletal diseases. Some examples of musculoskeletal diseases are osteoporosis, carpal tunnel syndrome, arthritis, and back pain. Osteoporosis makes up about 20% of musculoskeletal diseases worldwide. As the average life expectancy increases there will be an increase in the prevalence of osteoporosis.

One study showed that 25% to 30% of aging women had bone loss that resulted in a serious condition (Wei et al., 2012). In 1997 the United States spent over $186.9 million treating

musculoskeletal diseases (Scholz-Ahrens et al., 2007). The United States healthcare system treats musculoskeletal diseases in older generations while aiming to prevent them in younger generations. Groups at the highest risk for developing osteoporosis are women and those over the age of 50 (Stevenson et al., 1989). Providing early and preventative treatments for osteoporosis could save the healthcare system millions of dollars per year.

Causes of Osteoporosis

Osteoporosis can be caused by a variety of factors including age, hormone levels, medications, lifestyle, diet, and genetics (Rosen & Klibanski, 2009). Often the cause of osteoporosis is out of the control of the patient. As women age, their estrogen naturally decreases with a sharp drop off after menopause. Estrogen is crucial for regulating bone resorption and formation. Another common cause of decreased BMD is glucocorticoids (Patschan et al., 2001). Glucocorticoids are steroids often given to people with a spinal cord injury or who have a transplant. Glucocorticoids can inhibit osteoblasts from building up the bony matrix, which can result in lower bone density. This can lead to osteoporosis and bone fractures. Dietary deficiencies of zinc, magnesium, vitamin D, and calcium can also lead to a low BMD (Raisz, 2005). If the body does not get enough minerals, it will break down the bony matrix to access the minerals stored there. High levels of adipose tissue around the abdomen have also been correlated to low bone density in premenopausal women (Cohen et al., 2013). Adipocytes produce pro-inflammatory mediators and inhibit osteoblasts, which causes low BMD. Lastly, several genes regulate the activity of osteoblasts and osteoclasts. If there is a mutation present in these genes, this can result in a lowered bone density.

Hormonal Causes of Osteoporosis

Sex hormones play a critical role in maintaining bone mass and BMD (Deng et al., 2017). The primary cause of senile osteoporosis is menopause. Estrogen is an important regulator of osteoblast formation. When estrogen levels drop this causes an increase in bone resorption. Estrogen and androgens inhibit osteoblasts from releasing osteoclast stimulating factors (Patschan et al., 2001). When estrogen and androgen levels drop, this causes an increase in osteoclast activity, which leads to a lower bone density. Testosterone is also an important regulator of bone density (Goetz et al., 2017). Testosterone levels are naturally higher in men due to testosterone production by the testicles. Some of the testosterone produced is aromatized by aromatase to produce estrogen. Testosterone and estrogen directly inhibit osteoclasts. Lower numbers of osteoclasts lead to lower bone resorption, which in turn causes less bone loss. Estrogen also causes osteoclasts' apoptosis and stimulates osteoblasts to construct the bony matrix. Interestingly, one study showed that female to male transgender individuals that took testosterone for hormonal therapy before the age of 25 had a reduced peak bone mass (Goetz et al., 2017). This means that these individuals are at a higher risk for developing osteopenia and osteoporosis later in life.

An estrogen deficiency in men and women can increase the cortical vascular porosity in bone (Sharma et al., 2018). An increase in cortical vascular porosity can decrease bone strength and predispose a person to osteoporosis. In addition to this, mechanotransduction is hindered, preventing osteocytes from properly regulating osteoblast and osteoclast function. When there is not enough estrogen, the vascular pores increase in size rather than in number. This causes a serious compromise in the integrity of the bone. One study showed that there is a strong

correlation between low BMD, low serum copper levels, and low dietary $Ca2^{+}$ intake in postmenopausal women (Gür et al., 2001).

Another cause of low bone density in men and women is hypogonadism (Kanis, 2002). In women, hypogonadism can be caused by a primary condition or by gynecological disorders, amenorrhea, hyperprolactinemia, premature menopause, or a chronic illness. In men, hypogonadism can be caused by hyperprolactinemia, castration, chronic illness, or Klinefelter's syndrome.

Often osteoporosis is associated with old age and women who have gone through menopause. However, young women with eating disorders oftentimes have hormonal imbalances. Severe undernutrition, such as in anorexia nervosa, can lead to gonadotropic hypogonadism (Rosen & Klibanski, 2009). This then leads to an estrogen deficiency, which causes osteoporosis. Young women that are anorexic can become amenorrheic, so their estrogen levels drop. When these women gain weight, their ovaries return to functioning normally, so estrogen levels increase. This leads to an increased bone mass and density that reverses the apparent osteoporosis. Peptide YY (PYY), anorexigenic hormone, and ghrelin are hormones that contribute to the bone loss observed in women with anorexia. During a fasted state, ghrelin is secreted and peaks right before a meal. People that are anorexic have lower levels of ghrelin, which results in less hunger. High ghrelin levels have been correlated to a higher body mass index (BMI) and bone density. When ghrelin is administered it increases osteoblastic activity. Obesity can cause low levels of PYY, which lowers bone density. One study found that out of 130 premenopausal women who experienced anorexia, over 92% met the criteria for osteopenia and 38% met the criteria for osteoporosis (Rosen & Klibanski, 2009). Children that develop

anorexia will achieve a lower peak bone mass than children that do not have anorexia. This can predispose them to osteopenia and osteoporosis.

Medicinal Causes of Osteoporosis

The most common medications that cause osteoporosis are glucocorticoids (Patschan et al., 2001; Kanis, 2002; Rosen & Klibanski, 2009). Glucocorticoids are often prescribed when there is a spinal cord injury, a transplant, or an immune disorder that requires the immune system to be impaired (Patschan et al., 2001). When a person starts taking glucocorticoids, bone loss and fat gain are rapid (Kanis, 2002; Rosen & Klibanski, 2009). Glucocorticoids reduce bone mass by causing stem cells in the bone marrow to switch over to adipocyte lineages (Rosen & Klibanski, 2009). This causes the integrity of the bones to decrease and increases the likeliness of a fracture. The vertebrae are the most susceptible to bone loss while a person is taking glucocorticoids (Kanis, 2002). One way to avoid bone loss is to opt for inhaled glucocorticoids instead of oral glucocorticoids. Patients that have had two or more bone fractures have a 12-fold fracture risk while taking this medication. Ca^{2+} levels are also elevated in the urine when a patient takes glucocorticoids (Patschan et al., 2001). High urinary Ca^{2+} levels are observed due to the increased osteoclastic activity and decreased Ca^{2+} reabsorption in the kidneys. Glucocorticoids also cause a decrease in calcium uptake in the intestines due to the diminished synthesis of the calcium-binding proteins. Glucocorticoids inhibit osteoblastogenesis and osteoclastogenesis and reduce osteoblastic lifespan. Another study found that patients taking glucocorticoids had reduced alkaline phosphatase levels circulating in their blood (Scholz-Ahrens et al., 2007). There was an overall decrease in BMD, bone mass, Ca^{2+} absorption, vitamin D, and parathyroid hormone levels in people that took glucocorticoids long term.

Lifestyle Causes of Osteoporosis

High levels of adipose tissue in the abdomen have been correlated with lower BMD and increased rates of osteoporosis. One study showed that in premenopausal women, those in the highest tercile for trunk fat had the lowest bone quality (Cohen et al., 2013). Previous studies have suggested that increased adipose tissue may help protect bones from fracture (Reid). However, this study showed that the contrary is true; premenopausal women with lower levels of trunk fat had superior bone quality and the lowest risk of fractures (Cohen et al., 2013). Older sources may have suggested these outdated beliefs due to elderly women losing weight and breaking bones easily. People that are anorexic are also more likely to have osteoporosis. These hypotheses were suggested at a time when the scientific community did not have a good understanding of the pathophysiology of osteoporosis.

A recent study found that obesity rates will increase to 18% in men and 21% in women by 2025 (Agovino et al., 2019). It is not a fluke that obesity and osteoporosis have risen over the past three decades (Rosen & Klibanski, 2009). Proinflammatory cytokines are produced by visceral abdominal fat (VAT) (Cohen et al., 2013). It was found that premenopausal women with higher levels of VAT had lower marker levels for bone resorption, which suggests that high levels of VAT slow down the bone remodeling process. Fat content and bone remodeling are regulated by the hypothalamus via the sympathetic nervous system (Rosen & Klibanski, 2009). While obesity significantly lowers BMD, anorexia can also have a negative impact on bones. Eating less food means that a person cannot obtain all the essential nutrients that they need. Not only will this impact the cells' ability to function, but it will also prevent the osteoblasts from creating a structurally sound bony matrix. Many people that are anorexic have osteopenia or

osteoporosis. Having an extremely high or low BMI can cause severe musculoskeletal problems that may become irreversible.

Genetic Causes of Osteoporosis

Studies done on mice have shown that when the runt-related transcription factor 2 (*Runx2*) gene is deleted there is a decrease in bone mass density (Raisz, 2005). Runx2 is needed for the maintenance of healthy bones. The absence of osterix, a downstream factor involved in the Runx2 pathway, can also cause a decrease in bone mass. Runx2 and osterix are necessary for osteoblast differentiation. On the contrary, when Runx2 is overexpressed, there is also a decrease in bone mass. This is due to a decrease in bone mass LDL receptor-related protein 5 (*LRP5*) gene expression, which is needed for Wnt ligand signal transduction. The Wnt pathway determines the fate of the cell. If *LRP5* is mutated, this can lead to an increase in bone density and a decrease in fractures. Mutations to LRP5 can also lead to overgrowth of the skeleton, which can cause other abnormalities. In rodents with LRP5 deletion, there were much higher rates of osteoporosis.

Pathophysiology of Osteoporosis

Under normal conditions, the bone can regulate its breakdown and synthesis. When the osteoclast/osteoblast equilibrium is shifted towards osteoclastic function, inferior bone structure occurs. Pathways that encourage osteoclastogenesis and osteoclast function are activated during osteoporosis, while osteoblastogenesis and osteoblast function are inhibited.

Signaling in Osteoporosis

Patients with a fracture history are more likely than not to have lower bone formation and poorer bone quality compared to those without a history of fractures (Cohen et al., 2013). While osteoporosis is often associated with aging, some individuals are predisposed to it due to genetics, lifestyle, or medications. It is well documented that people with high amounts of

abdominal fat have decreased bone mass and BMD. Having excessive abdominal fat also increases the amount of fat in the bone marrow. Since the bone marrow is where osteoblast and adipocyte precursors are located, this alters their maturation process. The growth hormone/insulin-like growth factor-I (GH/IGF-I) axis and peroxisomal proliferated-activated receptors (PPARs) control whether these mesenchymal precursors differentiate into adipocytes or osteoblasts. Decreased growth hormone (GH) secretion can lead to obesity and an increase in adipocyte production. This means that fewer osteoblasts are produced, thus leading to lower bone mass and BMD. This can lead to osteopenia and eventually osteoporosis.

Sufficient mineral levels must be available in the body to have strong bones. Magnesium deficiency has been linked to increased rates of osteoporosis. One study showed that women with osteoporosis had significantly lower magnesium levels in their blood when compared to a healthy control group (Gür et al., 2001). Low magnesium levels can be caused by a poor diet, type II diabetes, kidney problems, and digestive issues. When magnesium levels drop, osteocalcin synthesis drops, too. Recent studies have found that over 80% of magnesium is lost during food processing (Cazzola et al., 2020). This means that a large percentage of people don't get enough magnesium in their diet to support healthy bones. This leads to an overall decrease in bone mass and BMD.

Medications can lead to osteoporosis through several different mechanisms. Glucocorticoids directly inhibit osteoblasts, osteoclasts, and osteocytes (Patschan et al., 2001). Parathyroid hormone-related peptide is needed for osteoblasts to commit to the osteoblastic lineage and for their maturation (Plotkin & Bruzzaniti, 2019). When a patient takes glucocorticoids for extended periods, the effects of parathyroid hormone (PTH) in the bones are much greater (Patschan et al., 2001). This can become a problem called secondary

hyperparathyroidism. When PTH acts on the bones, it causes them to break down to release calcium. This causes decreased bone mass and BMD. Glucocorticoids also act as an antagonist for gonad function; this leads to a decrease in the production of sexual hormones such as estrogen and testosterone. These drugs also decrease the kidneys' ability to reabsorb calcium while filtering the blood and the ability of the intestines to absorb calcium. When calcium levels are low, bone mass and BMD decrease as well. Lastly, glucocorticoids also decrease osteocyte numbers. Osteocytes are necessary for maintaining a healthy BMD and bone mass. They sense damage to the bone and release signals to the osteoblasts and osteoclasts to complete bone remodeling. When this process is decreased, bone damage cannot be repaired as efficiently. This can lead to osteoporosis. Another issue with decreased osteocyte numbers is the increase in empty lacunae (Raisz, 2005). When osteocyte numbers decrease, they cannot simply fill in the space where they once were. With excessive osteocyte death, the bones become extremely weak, which can lead to fractures and slow healing.

Osteoblasts and osteoclasts operate in a balanced system in a healthy individual. When this balance is thrown off, the bone mass can decrease (Raisz, 2005). If osteoclasts are more active than osteoblasts, then the bone will be continuously broken down faster than it can be built back up. When this happens, the trabecular bone can be completely broken down. This prevents osteoblasts from having a structure to build on, so they are unable to reconstruct the bone. However, high rates of osteoclast activity are not always associated with decreased bone mass. Even though the osteoclast activity is high during puberty, there is still a net balance in favor of osteoblastic activity, so the bone is built back up at a faster rate.

If osteoblasts inadequately form from their precursors, osteoporosis can occur (Plotkin & Bruzzaniti, 2019). Factors that can cause improper osteoblast development are high levels of

stress, poor diet, or a genetic mutation. When osteoblasts cannot fully mature, they are unable to properly rebuild the bony matrix after osteoclasts have broken it down. Immature osteoblasts create irregular patterns of bone that are not mineralized; this can lead to bone fragility.

Gene Activity in Osteoporosis

Several genes can lead to osteoporosis if they are mutated or dysregulated. One of these genes is methyltransferase-like protein 21C (*METTL21C*) (Brotto & Bonewald, 2016). *METTL21C* creates a protein with protein-lysine N-methyltransferase activity that methylates valosin-containing protein (VCP) chaperones. When *METTL21C* is downregulated, there is less differentiation of myotubes in muscle cells. This leads to cellular atrophy and the loss of muscle tissue. Muscular activity is crucial for proper bone mass and BMD. If there is decreased muscle, then there is less mechanical loading on the bones. This leads to less bone remodeling, which causes osteoporosis. Other genes that are important for bone health are collagen type 1 alpha 1 chain (*COL1A1*) and collagen type 1 alpha 2 chain (*COL1A2*). *COL1A1* and *COL1A2* produce collagen, which is needed for strong bones and muscles. If there is a mutation in these genes, this can lead to impaired muscle function and bone structure. The claudin-18 (*Cldn-18*) gene regulates the rate of bone resorption (Alshbool et al., 2014). When *Cldn-18* is mutated or disrupted, bone resorption and osteoclast activity increase. This leads to an imbalance in the osteoblast/osteoclast equilibrium that causes bones to become weaker. One study showed that mice with Dickkopf Wnt signaling pathway inhibitor 1 (*Dkk1*) gene deletion had extremely high bone mass (Plotkin & Bruzzaniti, 2019). *Dkk1* regulates osteoblast activity, so without this gene, osteoblasts can construct bone without being inhibited. However, when *Dkk1* is overexpressed, osteoblast numbers and activity are much lower. This again can lead to an imbalance in the osteoblast/osteoclast equilibrium that can lead to osteoporosis. Single nucleotide polymorphisms

(SNPs) to *Hox* genes that cause Wnt16 to become dysfunctional result in lower bone mass and BMD. Individuals with this mutation suffer from increased rates of osteoporosis and bone fractures.

Osteoporosis Treatments

Many potential therapies are being developed to treat osteoporosis each year. Current treatments come in the form of hormones, drugs, and lifestyle changes. Oftentimes using a combination of these therapies will yield the best results when combatting osteoporosis. Estrogen is a key regulator when it comes to osteoblast and osteoclast function, so it is one of the main hormone therapies used today. Bisphosphonates are one of the most widely prescribed drugs for osteoporosis; however, they can have negative side effects. Currently, many drugs are being developed and tested to provide better results for people with osteoporosis. Lastly, increasing the intensity of exercise can help improve BMD. By offering their patients a wide range of treatment options, healthcare providers can greatly improve the quality of life for people with osteoporosis.

Hormonal Treatments of Osteoporosis

Amylin is a hormone made by β-cells in the pancreas (Cornish et al., 1998). This hormone is cosecreted with insulin, which is secreted when a meal is eaten. Amylin regulates glucose metabolism in the body. Interestingly, amylin also acts on osteoclasts by inhibiting them. One study showed that a group of mice treated with amylin had a 30-100% increase in bone formation and a 70% decrease in bone resorption after only five days (Cornish et al., 1998). This hormone essentially "uncouples" osteoblast and osteoclast activity and shifts the osteoblast/osteoclast equilibrium. Amylin could be a potentially effective therapy for osteoporosis as it acts on bone without having negative effects on other tissues. This hormone also increases the amount of calcium reabsorbed by the kidneys. Calcitonin gene-related peptide

(CGRP) is structurally like amylin. In a study, it was found that the administration of CGRP to ovariectomized rats prevented bone loss (Cornish et al., 1998). CGRP increased the number of osteoblasts greatly. However, studies have shown that amylin is a better treatment option when compared to CGRP, as it is more potent and blocks more osteoclast activity. There are negative side effects to amylin therapy, including increased fat mass, insulin resistance in muscle and liver cells, hyperamylinemia, and hyperinsulinemia. Amylin does not make adipocytes immune to insulin, so hyperinsulinemia causes lipogenesis, hence, the increased fat mass.

Another promising hormone therapy is calcitonin. Calcitonin decreases the amount of calcium while increasing the amounts of copper, magnesium, and zinc in the body (Gür et al., 2001). One study showed that administering calcitonin and calcium proved effective in increasing bone mass (Gür et al., 2001). Magnesium and zinc are essential for building strong bones. Copper, magnesium, and zinc levels are increased due to calcitonin's effect on the renal tubules, which causes the kidneys to increase the amounts of these elements reabsorbed. Calcitonin also decreases the rate of bone resorption, which leads to a higher BMD.

Estrogen is one of the main hormone therapies that is focused on for postmenopausal osteoporosis (Raisz, 2005). One study showed that one-fourth of a dose of estrogen decreased bone resorption while increasing bone mass in older women. The effectiveness of such a low dose of estrogen could be due to the heightened sensitivity of the skeleton that comes with age. Low levels of estrogen therapy could be effective for older men that have osteoporosis caused by low estrogen and androgen levels. It has been found that hormone replacement therapy (HRT) is more effective than calcium and vitamin D supplements alone (Delmas, 2002). Osteoporotic women with intact uteri should be given estrogen and progestogen to reduce the risk of endometrial cancer. Estrogen increases the risk of cardiovascular disease and breast cancer

(Raisz, 2005). It is recommended that selective estrogen replacement modulators (SERMs) be administered to prevent cancer (Delmas, 2002). SERMs can ensure that only the bones are affected by the estrogen. Tamoxifen is a type of SERM that is commonly used to treat osteoporosis. It is an estrogen antagonist in breast tissue and an agonist in cholesterol metabolism, the endometrium, and the bone. However, tamoxifen increases the risk of endometrial cancer.

Cross-sex hormone therapy is commonly used by transgender people (Goetz et al., 2017). If a male at birth transitions to a female, then she will take estrogen and progesterone to develop the secondary sex characteristics of a female. The same is true for a female at birth transitioning to a male; he would take testosterone to develop the secondary sex characteristics of a male. This second scenario can be problematic as it can lead to lower bone mass and BMD. Transgender men should take testosterone and estradiol to maintain bone integrity. This will make up for the estrogen deficiency that can lead to osteoporosis. One study showed that estrogen is critical for early-life bone formation. Transgender males that transition before peak bone mass is reached at 25 years of age risk having a seriously compromised skeletal system due to an estrogen deficiency. This can be combatted by administering low doses of estrogen with testosterone to make up for the estrogen deficiency while maintaining the secondary sex characteristics of a male. Females that transition to males after 25 years of age do not need to take estrogen due to peak bone mass already being reached. Despite testosterone being aromatized to estrogen, this was not enough to prevent an estrogen deficiency. This is due to males being able to aromatize testosterone at twice the rate that females can. Even if a transgender male is taking testosterone, this will not automatically double the rate of testosterone aromatization. In men with an aromatase deficiency or mutation, it was shown that estrogen increased bone health (Goetz et al.,

2017). Testosterone and estrogen inhibit osteoclast formation, and estrogen initiates apoptosis in osteoclasts. Estrogen also stimulates osteoblasts, which cause an increase in bone formation.

Another hormone therapy for osteoporosis is PTH (Harlow et al., 2017). Osteoporosis can be caused by immobilization due to a severe injury to the spinal cord. One study had three groups of mice: a mobile control group, a group with a spinal cord injury (SCI), and a group with an SCI that was given PTH (Harlow et al., 2017). Both groups with SCIs had immobilized hindlegs; this caused bone mass and BMD loss. This study found that mice that were given PTH had much higher levels of bone formation compared to the SCI group that was not given PTH. Even though osteoclast function was occurring at a faster rate in the group with an SCI that was administered PTH, osteoblast activity was also occurring at a faster rate. An overall increase in bone remodeling activity that maintains the osteoblast/osteoclast equilibrium will lead to higher quality bone being formed. PTH (Teriparatide) and PTHrP (Aboloparatide) are osteoanabolic treatments for osteoporosis (Shao et al., 2019). PTH works by stimulating bone remodeling, which increases bone mass and quality. While PTH is an effective therapy, the FDA has placed a two-year limit on it due to its potency declining after that. More research needs to be done to find other drugs or hormones that can be combined with PTH to increase efficacy. Another way to improve PTH therapy is to find a way to directly target only the bone.

Medicinal Treatments of Osteoporosis

NO inhibits osteoclast activity by increasing OPG production (Raisz, 2005). People that take activators of the NO pathways have higher BMDs than those that do not take NO pathway activators. Some studies have shown that people that take non-steroidal anti-inflammatory drugs (NSAIDs) have slightly higher BMD than those that do not take NSAIDs. Vitamin D supplements can combat low vitamin D levels (Delmas, 2002). Low vitamin D levels can inhibit

bone remodeling, which leads to lowered bone mass. Raloxifene is a SERM that decreases the risk of bone fractures, but it does not decrease the risk of breast cancer. Further research will need to be done to combat this (Raisz, 2005). Calcium and vitamin D supplements have been shown to increase bone mass when combined with physical activity. Exercise increases one's peak bone mass while shifting the osteoblast/osteoclast equilibrium toward bone formation.

Bisphosphonates have also been shown to be an effective treatment for osteoporosis (Raisz, 2005). These drugs are stable pyrophosphate analogs that have a phosphorus to carbon to phosphorus bond (Delmas, 2002). The properties and efficacy of bisphosphonates are determined by the side chain off the carbon. Bisphosphonates have a high affinity to the bone's surface. Once bound to the bone, bisphosphonates are taken in by osteoclasts; this leads to apoptosis (Raisz, 2005). However, bisphosphonates can be harmful to some women due to their severe side effects (Tella & Gallagher, 2014). Bisphosphonates can cause acid reflux and ulcers if taken orally. When administered intravenously, some people had a bad reaction to bisphosphonates. These reactions include fevers, myalgias, and arthralgias.

Another osteoclast inhibitor is osteoprotegerin (OPG) (Tella & Gallagher, 2014). OPG is made naturally by osteocytes to inhibit bone resorption. It is an antagonist of RANKL, which is needed to initiate bone resorption. Administering additional OPG blocks RANKL, but this was not a practical treatment (Tella & Gallagher, 2014). The body developed antibodies against OPG, which targeted it for destruction. However, a monoclonal antibody against RANKL was made, and it blocked osteoclast activity (Tella & Gallagher, 2014). This drug, denosumab, efficiently increases bone mass and BMD in osteoporotic patients. The same principle was applied to osteoblast activity. Sclerostin is an inhibitor of the Wnt pathway that results in osteoblast activity. Monoclonal antibodies against sclerostin have improved bone density.

Several studies have provided evidence that soy isoflavone supplements increase bone mass and BMD (Wei et al., 2012). While there was not an increase in alkaline phosphatase, a bone formation biomarker, there was a decrease in deoxypyridinoline (DPD), a bone resorption biomarker. DPD is crosslinked type 1 collagen present in the urine. When osteoclasts are active, they break down the collagen in bones. This is then absorbed into the bloodstream and filtered out in the urine. Performing a urinalysis is an easy way to determine the rate of osteoclastic function. People that took soy isoflavone supplements experienced a BMD increase of 54% and a 23% decrease of DPD in their urine. Soy isoflavones have a similar structure to estrogen, which increases BMD.

Another medication that can reverse the effects of osteoporosis is zileuton. In a study, rats that were ovariectomized had downregulated Sema3a and upregulated Sema4d. Sema3a promotes bone formation while Sema4d promotes bone resorption (Saul et al., 2018). Zileuton works by inhibiting bone resorption while increasing the amount of cortical bone, trabecular bone, callus bone, and volume of bone made. However, the bone density was not changed. Interestingly, zileuton has shown that it can restore memory in patients with Alzheimer's.

Zoledronic acid is another anti-osteoporotic drug that inhibits bone resorption (Black et al., 2007). One study showed that after administration with zoledronic acid the risk of fracturing a bone was reduced by 70%. This study was also done with a group taking bisphosphonates and a group taking a placebo. The group that took the bisphosphonates intravenously had a 45% decrease in fracture risk. The control placebo group had no increase or decrease in fracture risk. While zoledronic acid was shown to be the most effective at decreasing the fracture risk, there were more cases of atrial fibrillation compared to the control group.

Genetic Treatments of Osteoporosis

Histone deacetylases (HDACs) are a target for osteoporosis therapy (Dou et al., 2016). HDACs initiate apoptosis in osteoclast precursors and promote osteoblast differentiation. Even though they increase bone mass, long-term use can lead to a decreased BMD.

Nmp4 controls many of the processes in osteoblasts (Shao et al., 2019). One study showed that in mice with an *Nmp4* knockout their bone density and quality increased. This was due to higher levels of COL1a1 mRNA present in *Nmp4* knockout mice osteoblasts. Collagen makes up over 90% of bone so its mRNA needs to be high to have better bone structure. This study suggests that losing the *Nmp4* gene would cause the ratio of collagenous to noncollagenous proteins to shift towards collagenous proteins. However, when osteoblasts perform this process, they make woven bone (Shao et al., 2019). A new study has shown that woven bone is not as strong as lamellar bone, despite forming faster.

Lifestyle Treatments of Osteoporosis

One study showed that osteoporotic patients that stood on a vibrating platform that emitted low magnitude sound waves had significantly increased bone mass (Rosen & Klibanski, 2009). These patients only had to stand on the platform for ten minutes per day for two years. Another study suggested that losing excess fat could help improve bone health (Cohen et al., 2013). It is important to prevent excessive fatty tissue from accumulating especially in developmental years before peak bone mass is reached. In addition to this, eating a diet that is high in calcium can correct calcium deficits caused by mutations (Alshbool, et al., 2014). One study found that Cldn-18 knockout mice that were fed high calcium diets had less bone resorption than the control group that was fed a normal diet. Most older women do not get enough calcium in their diets (Tella & Gallagher, 2014). These women must obtain adequate amounts of calcium to prevent worsening bone fragility. One study showed that older women

that were calcium deficient who were given adequate calcium decreased their fracture risk by 80%. Patients must also obtain adequate amounts of protein and vitamin D3 in their diets. Participating in regular, weight-bearing exercise, not smoking, avoiding consuming more than three units of alcohol per day, and implementing fall prevention strategies are all ways to prevent increasing the risk of bone fracture.

Physical activity increases mechanical loading on the muscles and bones (Borer, 2005). This causes an increase in bone remodeling, which increases overall bone strength and quality. Exercise also increases a person's balance, which can reduce the risk of a serious fall. Many people with osteoporosis have severe falls in older age. This can lead to a severe fracture of the hip or spinal cord. Muscle mass can also help protect bones in the event of a fall. One study showed that consistent exercise for girls started before puberty and carried on through adolescence increased bone volume and BMD. Intense exercise early in life increases peak bone mass (Delmas, 2002). If a woman is postmenopausal, moderate to intense exercise can still be used to treat osteoporosis (Borer, 2005). The key attributes of the exercises that increase bone mass the most are activities that put an unusual loading pattern on the bones and are dynamic. Sufficient nutrients and high calcium and vitamin D3 intake will yield an increase in bone mass when paired with exercise.

Future Treatments of Osteoporosis

One medication called adrenomedullin has a similar effect on osteoblasts to amylin (Cornish et al., 1998). Adrenomedullin is a drug used for vasodilation. Fragments of this peptide can be made to affect osteoblasts without causing vasodilation. More research needs to be done to determine the efficacy and safety of this potential treatment. Another therapy to explore is the control of *Nmp4* expression in osteoblasts (Shao et al., 2019). While there has been conflicting

evidence, more research needs to be done to replicate the results and figure out the mechanisms behind this gene. Lastly, selective osteoclast inhibitors are being developed (Raisz, 2005). They work by blocking proton transport and cathepsin K activity in osteoclasts. Antagonists to αvβ3 integrins are being developed, too. αvβ3 integrins are used by osteoclasts for motility and adherence.

Conclusion

Osteoporosis is a disease that decreases the integrity of the skeletal system. It's important to prevent osteoporosis through diet and exercise at a young age. If preventative measures against osteoporosis are taken, the effects of decreased hormone levels that come with age can be lessened. Currently, research is being done to create better treatments for osteoporosis. In the future, there may even be genetic-based cures for osteoporosis. Creating more effective treatments for this disease will prevent millions of dollars from being spent on it per year.

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