University of Mosul

College of Veterinary Medicine

Effect of Atorvastatin and Rosuvastatin on Some Biomarkers of Osteoporosis Induced by Ovariectomy in Rats

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M.Sc. / Thesis

Veterinary Medicine / Veterinary Biochemistry

Supervised by

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2022 A.D.   **1444 A.H.**

Effect of Atorvastatin and Rosuvastatin on Some Biomarkers of Osteoporosis Induced by Ovariectomy in Rats

A Thesis Submitted
by

Sumaya Sultan Salih

To
The Council of College of Veterinary Medicine
University of Mosul
in
Partial Fulfillment of the Requirement
for the Degree of Master of Science
in
Veterinary Medicine / Veterinary Biochemistry

Supervised by
Assistant Professor
Dr. Elham Mohammad AL-Khashab

2022 A.D.   **1443 A.H.**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ﴾

صدق الله العظيم

إقرار المشرف

أشهد بأن إعداد هذه الرسالة الموسومة " **تأثير الأتورفاستاتين والروزفاستاتين في بعض المؤشرات الحيوية لهشاشة العظام المحدثة عن طريق استئصال المبايض في الجرذان** " جرى تحت إشرافي في جامعة الموصل/كلية الطب البيطري ،وهي جزء من متطلبات شهادة الماجستير في اختصاص الطب البيطري / الكيمياء الحياتية البيطرية.

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التاريخ :   / / ٢٠٢٢

إقرار المقوم اللغوي

أشهد بأن هذه الرسالة الموسومة " **تأثير الأتورفاستاتين والروزفاستاتين في بعض المؤشرات الحيوية لهشاشة العظام المحدثة عن طريق استئصال المبايض في الجرذان** " قد تمت مراجعتها من الناحية اللغوية وتصحيح ما ورد فيها من أخطاء لغوية وتعبيرية وبذلك أصبحت الرسالة مؤهلة للمناقشة بقدر تعلق الأمر بسلامة الأسلوب وصحة التعبير.

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إقرار رئيس لجنة الدراسات العليا

بناءً على التوصيات المقدمة من قبل المشرف والمقوم اللغوي ورئيس فرع الفسلجة والكيمياء الحياتية والادوية ، أرشح هذه الرسالة للمناقشة.

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التاريخ :   / / ٢٠٢٢

قرار لجنة المناقشة

نشهد بأننا أعضاء لجنة التقويم والمناقشة قد اطلعنا على هذه الرسالة وناقشنا الطالبة **(سمية سلطان صالح)** في محتوياتها وفي ماله علاقة بها بتاريخ / /٢٠٢٢ ووجدناها جديرة بنيل شهادة الماجستير في اختصاص الكيمياء الحياتية البيطرية.

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قرار مجلس الكلية

اجتمع مجلس كلية الطب البيطري بجلسته المنعقدة في / /٢٠٢٢ وقرر منحها شهادة الماجستير في الكيمياء الحياتية البيطرية.

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<tr><td>أ.د. ظافر محمد عزيز</td><td>أ.د. رعد عبدالغني بشير السنجري</td></tr>
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Acknowledgement

Abstract

This study was designed to explore the effect of two types of statins Atorvastatin (lipophilic) and Rosuvastatin (hydrophilic) on some biomarkers of bone formation, alkaline phosphatase (ALP), dihydroxycholecalciferol (1,25 $(OH)_2$ D3, osteocalcin (OC), calcium (Ca) and phosphorus (P), and bone resorption N-telopeptide of type 1 collagen (NTx) in serum of adult female ovariectomized rats. In addition to determine calcium (Ca), phosphorus (P) and magnesium (Mg) content in femur bone ash of female ovariectomized rats. Also, histological examination was performed in left femur bones of these rats. Forty female rats (2.5-3months) age, weighing (200-220 g) were housed at controlled conditions of temperature (22-25°C), (12h light-12h dark) cycle in the animals house of the College of Veterinary Medicine / University of Mosul. The animals were divided into four equal groups that include; group 1: sham group and given distilled water, group 2: ovariectomized (ovx) group as a model of osteoporosis and given distilled water, group 3: ovx group treated orally with 20 mg/kg body weight daily of atorvastatin and group 4: ovx group treated orally with 20 mg/kg body weight daily of Rosuvastatin. All groups were treated for 60 days. After 30 and 60 day of treatment, blood samples were collected from all groups and separated serum for biochemical analysis, right femur bones were excised and ashed to estimate calcium, phosphorus and magnesium percentage, and left femur bones were excised for histological examination.

The results showed a significant elevation in serum ALP, OC, NTx, calcium and phosphorus and body weight with a significant reduction in 1,25 $(OH)_2$ D3 in ovx group compared to the sham group, but treatment with 20 mg/kg atorvastatin for 60 days caused a significant reduction in

ALP, and NTx with a non-significant elevation of $1,25(OH)_2D3$. However, treatment with 20 mg/kg rosuvastatin caused a significant reduction in ALP, NTx, OC and calcium with a significant elevation in $1,25(OH)_2D3$ compared to the ovx group.

As well as, the results revealed a significant reduction in bone ash weight, percentage of Ca, P and Mg content in bone ash of the ovx group compared to the sham group, but 20 mg/kg of rosuvastatin treatment led to a significant elevation in the bone ash weight, percentage of Ca and P in bone ash in comparison with the ovx rats. Treatment with 20 mg/kg of atorvastatin elevated Ca and P content in bone ash compared with ovx group.

Histological results showed a low density, thin trabecular bone, a few blood vessels, a high numbers of osteoclasts, with low numbers of osteoblasts in ovx group compared with the sham group, but treated ovx rats with atorvastatin have an increase in the thickness of trabecular bone and medium developed osteogenic tissue, also medium number of osteoblasts and osteoclasts compared with ovx group. Treatment of the ovx rats with rosuvastatin caused a significant increase in trabecular bone thickness, well developed osteogenic tissue, high numbers osteoblasts and low numbers osteoclasts compared with ovx group.

From the results of the current study, we concluded that treatment with atorvastatin and rosuvastatin prevent bone resorption and enhance bone formation in ovx female rats. In addition, rosuvastatin has a better effect on bone metabolism than atorvastatin.

Contents

List of Abbreviations

Abbreviations	Full name
1,25(OH)$_2$D3	dihydroxycholecalciferol
ALP	Alkaline phosphatase
BMD	Bone mineral density
BMP-2	Bone morphogenetic protein-2
CPC	Cresol phtalein complex
CTx	C-terminal telopeptide of type 1 collagen
DHVD3	1,25 Dihydroxy vitamin D3
EDTA	Ethylene Diamine-Tetra Acetate
ELISA	Enzyme Linked ImmunoSorbent Assay
FGF-23	Fibroblast growth factor 23
HDL	High density lipoprotein
HMG-CoA	3-Hydroxy 3-Methylglutaryl Coenzyme-A
IL-6	Interleikin-6
LDL	Low density lipoprotein
LDL-c	Low density lipoprotein- cholesterol
NTx	N-telopeptide of type 1 collagen
OC	Osteocalcin
OD	Optical density
OP	Osteoporosis
OPG	Osteoprotegerin
ovx	Ovariectomized
P1CP	Procollagen type1 C-terminal propeptide
P1NP	Procollagen type1 N-terminal propeptide
ppi	Inorganic pyrophosphate
PTH	Parathyroid hormone
RANK	Receptor activator of nuclear factor-kappa B
RANKL	Receptor activator of nuclear factor-KB ligand
TRAP	Tartrate-resistant acid phosphatase

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Chapter One
Introduction

Statins are a class of medicines that lower cholesterol levels in the blood by reducing its biosynthesis in the liver, by inhibiting the enzyme called 3-Hydroxy 3-Methylglutaryl CoA (HMG-CoA) reductase, which is responsible for the synthesis of cholesterol. Scientifically, statins are referred to as HMG-CoA reductase inhibitors. Statins are the most frequently drug that used in the treatment of hyperlipidemia and atherosclerosis (Pinal-Fernandez *et al*., 2018; Chamani *et al*., 2021). They prevent cholesterol synthesis in hepatic cells, leading to increase in low-density lipoprotein (LDL) receptors, and enhanced the uptake and clearance of atherogenic LDL-cholesterol from the blood stream (Toth and Banach, 2019).

There are two types of statins, hydrophilic statins, and lipophilic statins, based on their ability to dissolve in water or in lipid containing media. Lipophilic statins (as atorvastatin, fluvastatin, lovastatin, simvastatin and pitastatin) can easily enter the cells, whereas hydrophilic statins (as rosuvastatin and pravastatin) present greater hepatoselectivity (Climent *et al*., 2021).

The first goal beyond the discovering the HMG-CoA reductase inhibitors was the fact, that this enzyme is the rate-limiting enzyme in the pathway of mevalonate, that is responsible for the production of isoprenoids particularly cholesterol. Since mevalonate pathway is responsible for the production of another important biomolecules which have role in osteoclasts activation, some studies had proposed, that statins may have an inhibiting role for bone resorption, through inhibiting osteoclasts function (Morse *et at*., 2018; Chamani *et al*., 2021).

Several studies have been suggested that statins not effective only in the reduction of high level of cholesterol, reduce the risk of atherosclerosis and acute myocardial infraction, but they also treat other diseases such as osteoporosis (Shah, *et al*., 2015).

Osteoporosis is a common skeletal disease, characterized by low bone density and deterioration the microarchitectural of bone tissue (Ris te li *et al*., 2015). Ovariectomized rats considered a good model for osteoporotic women after menopause (Johnston and ward, 2015). The aim of this study is to investigate the effect of two types of statins Atorvastatin (lipophilic) and Rosuvastatin (hydrophilic) on bone formation and bone resorption in the ovx rats, through the following estimations:

1. Induction of osteoporosis in adult female rats by ovariectomy.
2. Estimation of some bone biomarkers in serum.
3. Estimation of some minerals in the bone ash of the femur.
4. Histological study of bone tissue of the femur.

Chapter Two

Review of Literature

2-1: Statins

Statins are the most frequently drug groups that used in the treatment of hyperlipidemia (Toth and Banach, 2019). They are potent medicines in the lowering levels of lipids and prevent the cardiovascular disease (Murller *et al*., 2021). All statins have an effect by decreasing cholesterol synthesis (Tonelli *et al*., 2011).

Statins are structural analogs of 3- hydroxy-3-methylglutaryl-CoA (HMG-CoA), and competitively inhibit the 3- hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (E.C.1.1.1.34), which is responsible for the first step in cholesterol biosynthesis in hepatic cells (Cruz *et al*., 2020; Orces *et al.,* 2020) Statins reduce the risk of atherosclerosis and acute ischemic stork (Chamani *et al*., 2021).

Statins effectively inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, blocking the biosynthesis of isoprenoid lipids and causing inhibition of protein prenylation (Chamani *et al*., 2021). Prenylated signaling molecules are essential for osteoclast function. Several studies proposed that statins may have anabolic effect on bone and stimulate osteoblast differentiation (Hughes *et al*., 2007; Ruan *et al*., 2012; Chamani *et al*., 2021).

Several studies have found that statins may act against osteoporosis and bone loss or may be inducing bone loss and fractures (An *et al*., 2017; Larsson *et al*., 2019).

The differences among statins came from the ring that attached to active moiety, the ring is partially reduced naphthalene as in (lovastatin, pravastatin and simvastatin), or the ring is pyrrole as in atorvastatin, or the ring is an indole as in fluvastatin, or the ring is a pyrimidine as in rosuvastatin, or the ring is a pyridine as in cerivastatin, or the ring is a quinolone as in pitavastatin(Fong, 2014). The solubility and the pharmacological properties of statins depend on the substituents that found on each ring. The hydrophilicity of rosuvastatin and pravastatin arise from polar substituents plus the active site, while the lipophilicity of atorvastatin, lovastatin, fluvastatin, pitavastatin and simvastatin come from addition of nonpolar parts (Schachter, 2005; Fong, 2014; Climent, *et al.*, 2021).

The lipophilic statins can passively diffuse and enter the cell membrane, this property decrease the hepatoslectivity because they can diffuse into other tissue, in contrast, hydrophilic statins need carrier-mediated to uptake in the liver. The lipophilic statins are cleared by the oxidative biotransformation process, however the hydrophilic statins are eliminated unchanged (Ward *et al.*, 2019; Climent *et al.*, 2021).

Statins have activity in reducing LDL (low density lipoprotein) that transport cholesterol and has a role in the atherosclerosis development and cardiovascular disease (Alenghat and Davis, 2019; Sheridan *et al.*, 2022).

2-1-1: Discovering of statins

Molecules called citrinin and compactin (mevastatin) produced by *Penicillium citrinum* were discovered in 1970, have inhibiting effect to the key enzyme in cholesterol synthesis HMG-CoA reductase (Endo, 2010).

In 1979, lovastatin (naturally occurring) that produced from *Aspergillus terreus* proven has acceptable toxicity. After that several types of statins appeared in the market, from these types fluvastatin, atorvastatin, rosuvastatin and pitvastatin (Drake *et al*., 2008; Endo, 2010).

In 1994, simvastatin sold by Merck's Zocor and tested in 4,444 patients who have high cholesterol level, the results after 5 years, showed a reduction 35% in their cholesterol (Simons, 2003).

In 2001, cerivastatin a synthetic statin was outgoing from the market because of its side effects (Shah *et al*., 2015).

2-1-2: Mechanism of action

Statins act by competitively blocking the active site of HMG-CoA reductase which considered the first and key rate-limiting in the pathway of mevalonate. Thus inhibiting this step leads to prevent the conversion of HMG-CoA into tmevalonic acid, then reduces the hepatic synthesis of cholesterol, causing an increase in the production of microsomal HMG-CoA reductase. This elevation of this enzyme increased the expression of LDL receptors genes in cell surface, thereby increased the clearance of LDL-c from the blood circulating and consequently reduction in the level of LDL-c by 20-55% in circulating (El-Nabarawi *et al*., 2017; Kluger *et al*., 2019).

2-1-3: Structural characteristics of statins

The active unit of statins is a 3, 5-dihydroxyglutaric acid, which is structurally analogous to the HMG-CoA substrate. This active site bounds to HMG-CoA reductase and inhibit its activity in a stereosective way. Statins are different from each other in their molecular and clinical

properties which arise from the ring that attached to the active moiety (Ward *et al*., 2019).

2-1-4: Types of statins

Statins can be classified as lipophilic or hydrophilic based on their potency to dissolve in water or in fat. The mainly lipophilic statins (simvastatin, fluvastatin, pitavastatin, lovastatin and atorvastatin) have ability to enter the cells easily, whereas the hydrophilic statins (rosuvastatin and pravastatins) are more hepatoselectivity (Mueller *et al*., 2021; Climent *et al*., 2021).

Table 1: Classification of statins according to the chemical reaction

Lipophilic statin	Hydrophilic statin
Simvastatin Fluvastatin Atorvastatin Lovastatin Pitavastatin	Rosuvastatin Pravastatin

2-1-5: Atorvastatin

Atorvastatin is a synthetic drug, and has half-life 14 hours (Ahmadi *et al*., 2020). Atorvastatin has a various trade names: Arkas[®], Ator[®], Atoris[®], Lipitor[®], Torvast[®] and Totalip[®].
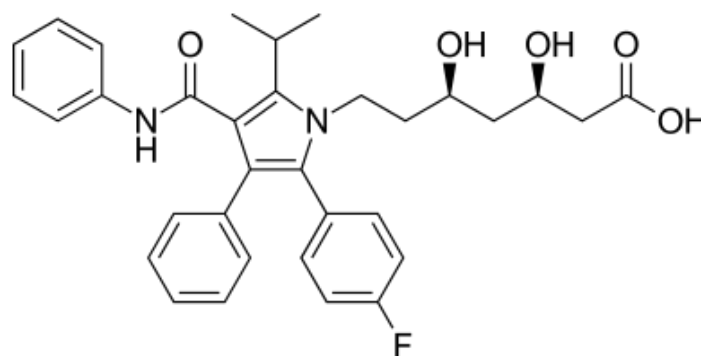
Figure 1: Structure of Atorvastatin (Ruan *et al*., 2012)

It is a lipophilic statin, most lipophilic substances are metabolized and converted to more polar metabolites (Climent *et al*., 2021).

Name: Lipitor[®] atorvastatin (as calcium) 10mg, 20mg, 40mg and 80mg tablets. Lipitor contains the active ingredient atorvastatin calcium. The molecular formula ($C_{33}H_{34}F N_2O_5$) Ca.3H2O. It is white to off white crystalline powder.

Atorvastatin is a synthetic agent which is used for lowering cholesterol levels in plasma. Atorvastatin inhibits the synthesis of cholesterol in the liver by increasing receptors of LDL. Atorvastatin is quickly absorbed after taken it orally, its optimum plasma concentration (1-2) hours. It is metabolized by CYP3A4 (Climent *et al*., 2021).

2-1-6: Rosuvastatin

Rosuvastatin is a synthetic drug (Monjo *et al*., 2010). It has different trade names including: Colcardiol[®], Colfri[®], Crativ[®], Crestor[®], Dilivas[®], Exorta[®], Koleros[®], Lipidover[®], Miastina[®], Provisacor[®], Rosastin[®], Simestat[®] and Staros[®].
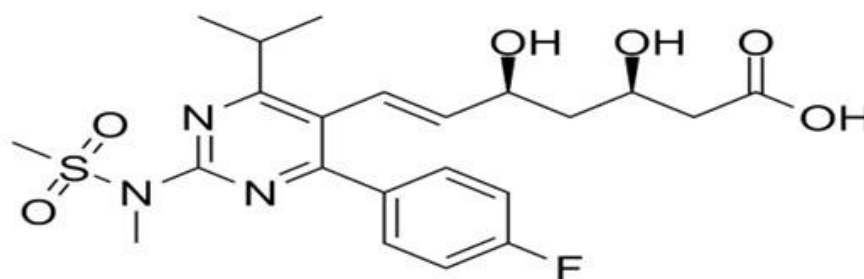
Figure 2: Structure of Rosuvastatin (Ruan *et al*., 2012)

Rosuvastatin is a hydrophilic statin. It has some pharmacologic properties as hepatic selectivity and enhanced the inhibition of HMG-CoA reductase (Monjo *et al.*, 2010).

Rosuvastatin is very similar to structure of atorvastatin. It has (about 20%) bioavailability and it has 18-19h half-life this makes it having longer plasma half-life than other types of statin. Also rosuvastatin has minimal metabolism through CYP system, this property makes it in less interaction with other medicines (Kostapanos *et al.*, 2010; Ahmadi *et al.*, 2020).

Rosuvastatin is not significantly metabolized by CYP2C9 enzymes (Bhattacharyya *et al.*, 2012). The dosages 10mg and 80mg of rosuvastatin have a good lowering activity of LDL (low density lipoprotein) compared to 10mg and 80mg of atorvastatin. Also, this drug can elevate high density lipoprotein (HDL) 8% to 12% and can lower triacylglycerol 10% to 35%. Rosuvastatin is a hydrophilic agent with less penetration in the extrahepatic cells, rosuvastatin has less potential for cytochrome P450 drug interactions. Because the differences in the polarity of statins and bone bioavailability the individual effects of these drugs may be varied (Mancini et al., 2013; Climent *et al.*, 2021).

2-1-7: Effect of statins on bone

The action of statins in reducing the intracellular cholesterol, they also reduce other products of the mevalonate pathway, including the isoprenoids farnesyl diphosphate and geranylgeranyl diphosphate. Farnesyl diphosphate and geranyl geranyl diphosphate are attached to the carboxy terminal of small GTP-binding proteins to produce prenylated proteins. The process of prenylation is necessary for the membrane localization and function of this type of proteins. The prenylation protein

including Rac and Rho are pivotal in mediating the cytoskeletal changes started by growth factors and integrins, leading to membrane ruffling, and resulting in the activation of cells as macrophages and osteoclast as shown (Ruan *et al*., 2012; El-Nabarawi *et al*., 2017; Sheridan *et al*., 2022).
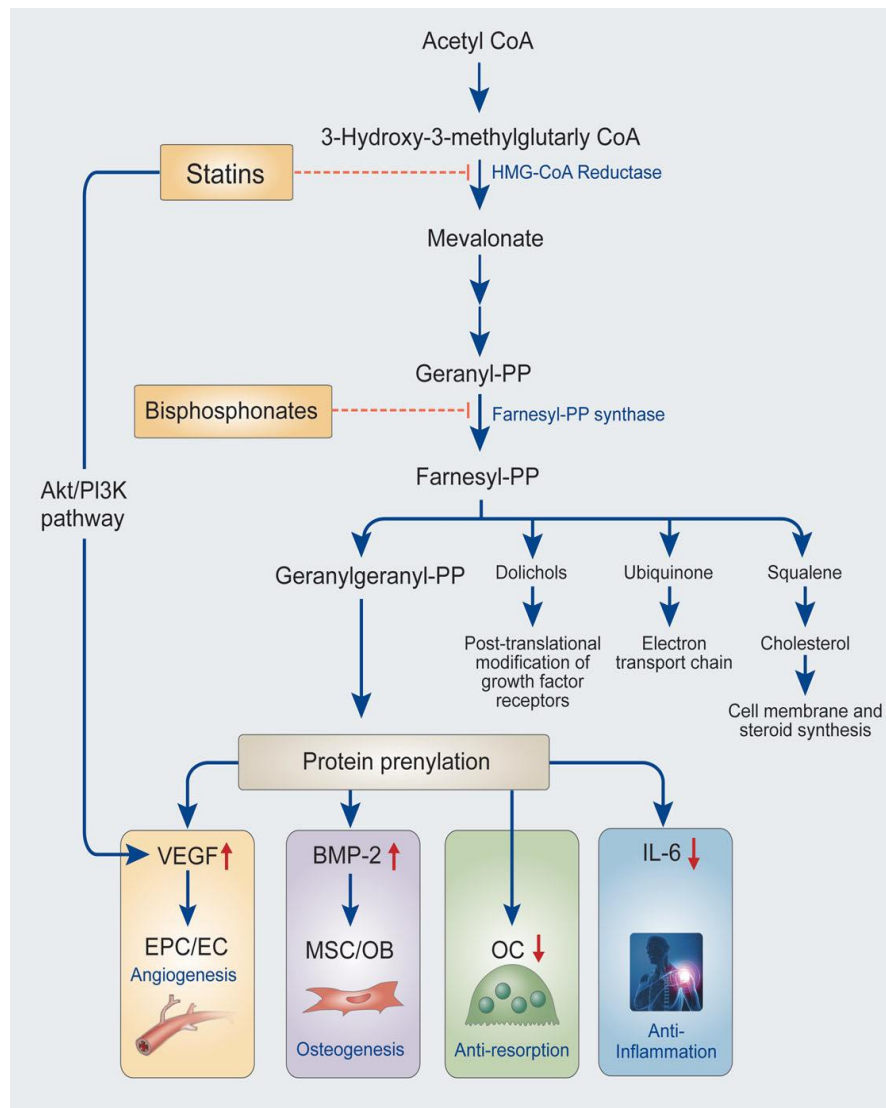
Figure 3: Biochemical Effects of Statins in Mevalonate Pathway (Shah *et al*., 2015)

Some studies observed the stimulation of protein called bone morphogenetic protein-2 (BMP-2) through the action of statins, so statins may have useful effects in osteoporosis and fractures treatment and have both the anabolic and antiresorptive effects like differentiation of osteoblasts and reduction osteoclasts numbers (Monjo *et al*., 2010; An *et al*., 2017; Morse *et al*., 2018).

On other hand, several studies suggested, that statin may increase bone mineral density (Ferreira Junior *et al*., 2018; Antonenko *et al*., 2021).

A beneficial effects noticed in some patients how used statins for treatment hypercholesterolemia from these effects preventing osteoporosis (Shah *et al*., 2015; Hong *et al*., 2020).

Some clinical studies pointed, that patients who used statin drugs for the treatment of hypercholesterolemia, perhaps a beneficial effects appeared in preventing of osteoporosis in these patients (Shah *et al*., 2015; Hong *et al*., 2020).

In a study of Shahrezaee *et al*., (2018) they showed that simvastatin and lovastatin significantly elevated serum calcium, osteogenic gene expression, and bone mineral density (BMD) in rats.

2-2: Bone

Bone is a special connective tissue made up of water 80%, protein, fat, non-collagenous protein and minerals, most of the minerals are calcium salts, hydroxyapatite crystal, Ca_{10} $(Po_4)_6$ $(OH)_2$ and contain osteoid matrix (Pikner, 2016; Kenkre and Bassett, 2018). The bone minerals considered a major storage of calcium and phosphate that necessary for homeostasis and enhance the skeleton (Clarke, 2008; Boskey and Robey, 2019).

The major protein in the organic matrix is collagen, but glycosaminoglycans, also glycoproteins considered the non-collagen molecules that comprise about 10% of the organic matrix. Type 1 collagen constitutes 80-90% of the collagen, there are another types of collagen including (type 3, 5, 11, and 13). Collagen made up the structure of matrix (Fonseca *et al*., 2014; Garnero, 2015; Daneault *et al*., 2017).

Proteoglycans such as chondroitin sulfate and proteoglycan, glycoproteins such as alkaline phosphatase and osteonectin, glycoproteins that contain arginine, glycine aspargin such as osteopontin and sialoprotein and carboxylated proteins, all considered non-collagen proteins (Morgan *et al*., 2015; Bailey *et al*., 2017).
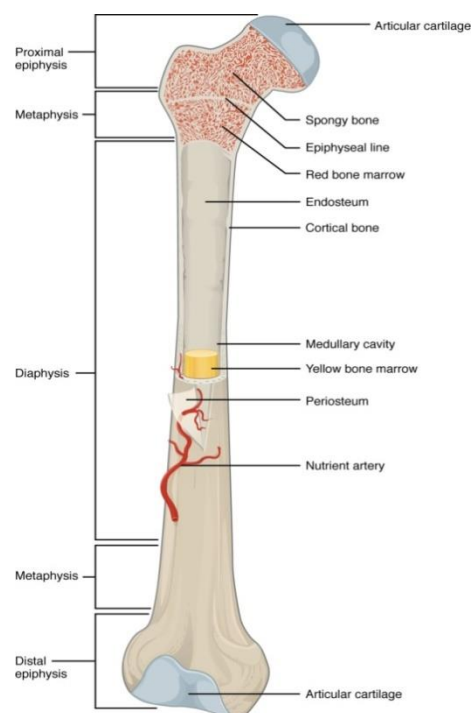
Figure 4: Anatomy of the Long Bone (Biga et al., 2019)

There are two types of bones that comprising the skeleton: (Pikner, 2016; Biga *et al*., 2019).

1. Cortical bone: it is very strong, but light weight, therefore, it undertakes mechanical functions. It makes about 80% of bone mass, cortical bone is the main constituent of the long bones. The remodeling activity of cortical bone is 10 times lower than the trabecular bone, mineralization about up 90%.

2. Trabecular bone: consists of plentiful cross-hair connections called trabeculae. It makes up 20% of bone mass, and its mineralization is about 5-20%.

2-2-1: Functions of bones

Bones have three important functions: (Ris te li *et al*., 2015; Konukoglu, 2019).

1.   Maintain the shape of the body.

2.   Protection of vital organs (heart, brain, lungs).

3.   Production of cellular part of the blood (RBCs, WBCs, and platelets).

4.   Serve a reservoir for calcium, phosphate and other minerals.

2-2-2: Types of bone cells and functions

Bone is metabolically active tissue, which is permanently remodeled.

There are three types of cells:

1. Osteoblasts: this type of cells is responsible for bone formation, they synthesize new bone matrix, proteins, growth factors and cytokines in the bone. Receptors for vitamin D, estrogen and parathyroid hormone are on the surface of these cells. Plasma

membrane of osteoblasts contains alkaline phosphatase (Pikner, 2016; Konukoglu, 2019).

2. Osteoclasts: this type of cells is responsible for bone resorption, they have multiple nucleus, and have an apical membrane which acts as a site for resorption of bone. The receptors of calcitonin are found in osteoclast membrane, therefore, calcitonin inhibited by osteoclast activity (Pikner, 2016; Konukoglu, 2019).

3. Osteocytes: these cells are type of osteoblasts, they found abundantly in the bone, they keep the bone tissue in active state. Osteocytes evolve from osteoblasts and have a role in bone matrix formation (Pikner, 2016; Konukoglu, 2019).

The function of osteoblasts is to set a new bone, but before that they initiate resorping the bone by osteoclasts, which dissolve the protein networks by using acid and enzymes, then osteoblasts start formation of bone through adding some compounds which assist construct a new bone as shown in Fig. 5 (Ris te li *et al*., 2015; Pikner, 2016; Konukoglu, 2019; Biga *et al*., 2019).
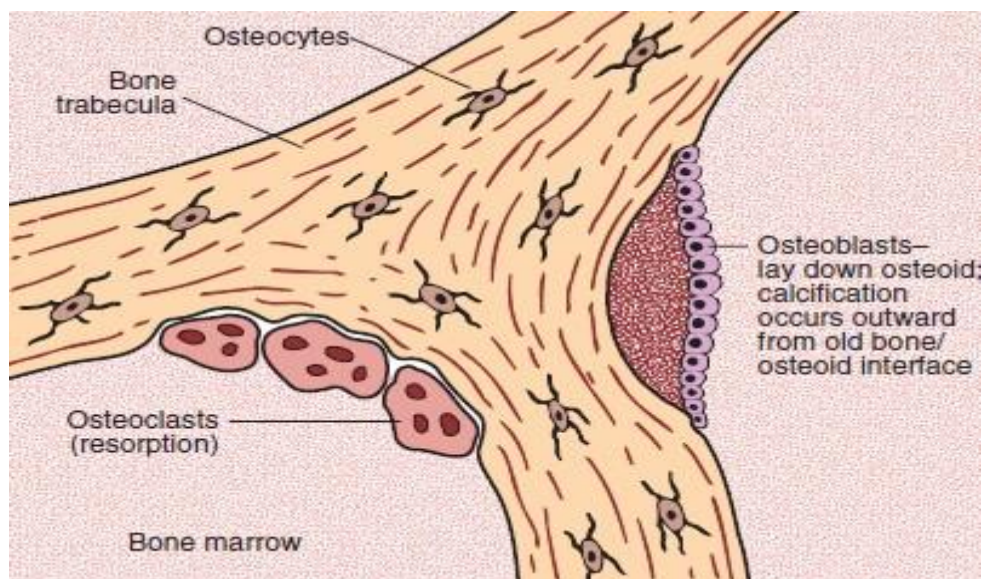
Figure 5: Bone Remodeling (Gaw *et al*., 2013a)

2-2-3: Bone remodeling

Bone is constantly undergoing, metabolic process, breakdown and reformed called bone remodeling or bone turnover.

(Varela and Jolette, 2018; Kenkre and Bassett, 2018).
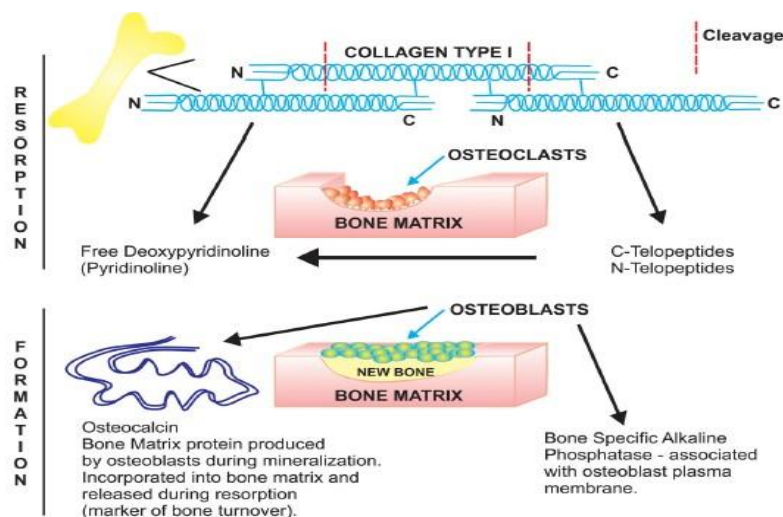
Figure 6: Illustration of Bone Turnover (Varela and Jolette, 2018)

In fact, this process is maintained by a coupled balance between bone formation that mediated by the action of osteoblasts and bone resorption of old or injured bone that mediated by the action of osteoclasts (Matsuoka *et al*., 2014; Lee *et al*., 2020).

At the physiological conditions, these two processes are in stable state. The unbalance between these process leads to abnormal architecture or function and causes diseases such as osteoporosis (Zaidi, 2007).

The old and damaged bone is replaced in bone remodeling cycle, this process is necessary for bone preservation and maintaining the homeostasis of minerals (Pikner, 2016).

Remodeling process is regulated by the following: (Pikner, 2016).

1- Hormones: like parathormone (PTH), calcitonin, thyroid hormones, cortisol and calcitriol.

2- Locally acting cytokines: sclerostin, and bone morphogenetic proteins (BMPs).

3- Minerals: calcium, magnesium and zinc.

4- Vitamins: K, C, B6 and A.

The osteoclastic resorption process is tightly coupled with osteoblastic formation during bone remodeling. Bone remodeling occurs continuously in order to repair damage in the skeleton and prevent the accumulation the fragile hyper-mineralized bone through releasing stores of calcium and phosphate, small locations of bone are resorbed by the action of osteoclasts then replaced by osteoblasts (Kenkre and Bassett, 2018).

2-3: Estrogen

Estrogens are steroidal compounds derived from cholesterol (Emmanuelle *et al*., 2021). Naturally synthesized estrogens are carbon-18 compounds (Gulati, 2018). Estrogens are hormones capable of producing certain biological effects. The naturally occurring estrogens in humans are; β-Estradiol, Estrone, and Estriol. All the three pituitary gonadotropins FSH, LH and LTH are involved in stimulation of estrogen Estrogens are also formed in the adrenal cortex, placenta and testes in small amounts. The principle estrogenic hormone in circulation and the most active form of the estrogen is β-Estradiol, which is in metabolic equilibrium with estrone. Estrogens are produced by graffian follicles in the ovary (Chatterjea and Shinde, 2021).

Estrogen is involved in both female and male reproductive, also play a role in other biological system as neuroendocrine, vascular, skeletal and immune systems, therefore it is implicated in several diseases and some conditions such as infertility, obesity, osteoporosis and some types of cancers (Hamilton *et al*., 2017).

Menopause is a natural physiological phenomenon resulting from primary ovarian failure secondary to apoptosis or programmed cell death. Ovarian function declines with age. The onset of menopause features the decreasing production of estradiol, as well as increasing levels of follicle-stimulating hormone (FSH). At menopause the normal bone turnover cycle is impaired by estrogen deficiency. This may be due to the presence of estrogen receptors in osteoclast progenitor cells and multi-nucleated osteoclasts. The osteoclastic resorption activity increases while the osteoblastic activity decreases. As a result, the amount of bone resorbed exceeds the amount deposited, which leads to a net loss of bone. The increase of overall bone resorption is due to a weakened inhibition effect due to the reduction of available estrogen on both osteoclastogenesis and osteoclast activity (Ji and Yu, 2015).

Estrogen binds with estrogen receptor to promote the expression of osteoprotegerin (OPG), and to suppress the action of nuclear factor-κβ ligand (RANKL), thus inhibiting osteoclast formation and bone resorptive activity. It can also activate Wnt/β-catenin signaling to increase osteogenesis, and upregulate BMP signaling to promote mesenchymal stem cell differentiation from pre-osteoblasts to osteoblasts, rather than adipocytes. The lack of estrogen will alter the expression of estrogen target genes, increasing the secretion of IL-1, IL-6, and tumor necrosis factor (TNF) (Cheng *et al*., 2022).

Many factors affect bone development and architecture, with endogenous estrogen being a major component in evolution of bone. Since the major cause of osteoporosis in menopause is the loss of bone due to estrogen deficiency (Levin *et al*., 2018).

Estrogen plays an essential role in skeletal growth and bone homeostasis in both men and women. Increased bone turnover during lactational amenorrhoea is one of the finest examples of modulating tissue compositions during reproduction. The associated hypo-estrogenic state tips the balance in favor of osteoclastic activity to enrich breast milk with calcium. When menstruation is resumed with rising estrogen levels, osteoclasts are suppressed, allowing osteoblasts to rebuild the skeleton in preparation for the next pregnancy (Al-Azzawi, 2013).

Estrogens have antioxidant properties which are due to their ability to bind to estrogen receptors and to upregulate the expression of antioxidant enzymes via intracellular signalling pathways (Borras *et al*., 2010). Oxidative stress is higher in postmenopausal women, suggesting that antioxidant status may be related to estrogen deficit, this is due to the action of estrogens which upregulate nuclear gene expression of antioxidant enzymes (Bellanti *et al*., 2013).

2-4: Osteoporosis

Osteoporosis is a bone disorder with remarkable changes in bone biologic material (El-Nabarawi *et al*., 2017; Abraham and Abraham, 2018).

Osteoporosis is a disease characterized by low bone mass and deterioration of the architecture of bone tissue. Hormonal changes, aging and lack nutritional status and several diseases considered the significant

risk factors (Gaw *et al*., 2013a; Mustafa *et al*., 2018; Watanabe *et al*., 2019).
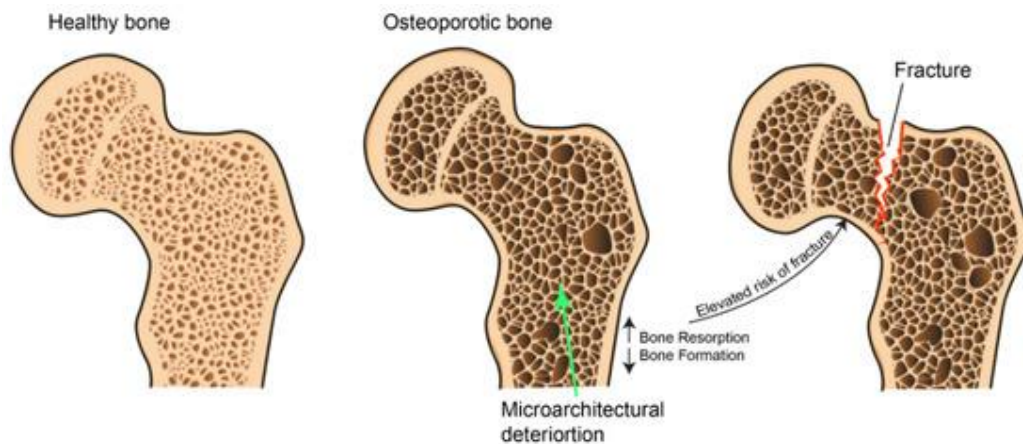
Figure 7: Healthy & Osteoporotic Bone (Salamanna et al., 2020)

The areas of cortical bones become more thin and trabecular bones become small, through life, bone shows a progressive loss in both sexes, but loss of bone in women is more quickly (Ris te li *et al*., 2015).

The most of osteoporosis cases occur in women after menopause, since estrogen deficiency that related to rapidly resorption of bone (Mirkin and Pickar, 2013). Also, osteoporosis considered as a global public health, in addition the medical cost of treating this condition and the related fractures, and remarkable reduction in life quality (Wong *et al*., 2020).

Osteoporosis is not reported thus far in large animals (farm animals). This may be due to the poor diagnosis, where animals do not receive regular check in this context. In horses, it has been recorded that they may have a loss of bone mass to a certain degree to develop osteoporosis; however, general osteoporosis in horses is rather rare compared to the localized type. Canine and feline species do not show clear signs of osteoporosis although arithritis in these species is a common syndrome.

However, some cases of osteoporotic cases have been reported in pets (Project, 2017).

In addition, lab animals have intensively been used as models to study osteoporosis. The best model, which mimics that of human is rat which exhibits ideal bone loss (Bonjour *et al*., 1999). Therefore, this study relied on rats as a biological model to investigate the effects of statins. Other models such as mice and rabbits have also been used. Furthermore, monkeys have also been subjected to the investigations in terms of characterizing and treating of osteoporosis (Renwald and Burr, 2008).

2-4-1: Ovariectomized rats as a model of Osteoporosis

The study of post-menopaused osteoporosis is difficult because it is restricted to humans, therefore animal model which mimics of postmenopausal osteoporotic women may give information about potential therapy and aid in the treatment. Animal models play an important role for understanding of osteoporosis, since clinical studies are so expensive and highly cost (Khajuria *et al*., 2012).

Ovariectomized (ovx) rats are female rats, whose ovaries have been removed, and they represent the stage of osteoporosis in humans and estrogen deficiency. Ovx rat model is widely used for studying the prevention and treatment of osteoporosis. Rats are currently the most model of osteoporosis were used because they are easy to house and growing rapidly. The bone of rats are identical to those in humans, it dynamic tissue, it formed and reconstructed through bone life (Yousefzadeh *et al*., 2020).

2-5: Biochemical markers of bone remodeling

Bone markers are blood or urine tests that detect the bone turnover products. There are several conditions and some diseases can give rise to the imbalance between bone formation and bone resorption. So bone turnover markers can be useful in the monitoring of the clinical responses for therapies of osteoporosis, also for predicting fracture risks. The biomarkers of bones are useful to detect bone loss and determine the bone loss rate. The markers of bone turnover can be used for monitor the responding for anti-resorption therapies and some bone disease as osteoporosis, rickets, osteomalacia and Paget disease. Blood or urine bone markers represent the accumulated results of the modeling and remodeling in all bone sites (Konukoglu, 2019).

The type 1 collagen is comprised 90% of bone organic matrix. Through the resorption process, the carboxy peptide and amino procollagen are released to blood stream, so the biochemical bone markers can determine these molecules which generated during both formation and resorption of bone matrix (Romero Barco, *et al*., 2012).

Biochemical markers of bone turnover can be classified as the following:

Biochemical markers of bone formation:

1. Alkaline phosphate (ALP).
2. Serum bone-specific ALP.
3. Osteocalcin reflects its rate synthesis by osteoblasts.
4. Procollagen type 1 C-terminal propeptide (P1CP).
5. Procollagen type 1 N-terminal propeptide (P1NP).

P1CP and P1NP reflect changes in synthesis of new collagen. Type 1 collagen is produced as a precursor called type 1 procollagen, which

contain two ends carboxy terminal and amino terminal, these C-terminal and N-terminal called PICP (procollagen type 1 C-terminal propeptide) and PINP (procollagen type 1 N-terminal propeptide). During formation of collagen from procollagen, these propeptides (PICP and PINP) are removed from procollagen by the action of two enzymes, laying aside on the collagen molecules in bone (Ris te li *et al*., 2015), so PICP and PINP are released in the circulation during collagen synthesis, and PINP is used as a marker for osteoporosis studies, it is increase after treatment of osteoporosis, PICP and PINP can be detected in serum and they considered markers of new bone formation (Pikner,2016; Konukoglu, 2019).

Biochemical markers of bone resorption: (Ris te li *et al*., 2015).

1. C-telopeptide (C-terminal telopeptide of type 1 collagen (CTx)) it is a peptide fragment from the carboxy terminal end of protein matrix.
2. N-telopeptide (N-terminal telopeptide of type 1 collagen (NTx)) it is a peptide fragment from the amino terminal end of the protein matrix.
3. Tartrate-resistant acid phosphatase (TRAP) is the version of acid phosphatase produced by osteoclasts.

2-5-1: Alkaline phosphatase

Alkaline phosphatase (ALP) (E.C.3.1.3.1) belongs to a group of enzymes that catalyzes the hydrolysis of a wide variety of naturally occurring substrates (Ris te li et al., 2015). It plays a critical role in the hard tissue formation, and favorite mineralization, also decrease the concentration of pyrophosphate an inhibitor of formation of mineral (Vimalraj, 2020).

There are various isoforms for ALP, including liver, bone, placenta and kidney. Bone and liver isoforms have same gene (Romero Barco *et al.*, 2012).

Clinically the determination of serum ALP has a value in the diagnosis of liver disease and bone disease that is associated with elevated osteoblasts activity (Johnson-Davis, 2018).

The bone isoenzyme is very specific for activity of osteoblasts, this isoenzyme bound to cell membrane so it provides a specific indication for bone formation and elevation of bone alkaline phosphatase levels in circulation correlated with bone formation rate (Hlaing and Compston, 2014).

Bone ALP (produced by osteoblasts), hydrolyzes inorganic pyrophosphate (ppi), during bone mineralization, and serum ALP is one of the biomarker of bone formation secondary to bone turnover elevation (Nagareddy and Lakshmana, 2006; Ris te li *et al.*, 2015; Grigoryan *et al.*, 2017).

2-5-2: Osteocalcin

Osteocalcin is the most abundant non-collagenous protein, formed by osteoblasts in the extracellular matrix and some of osteocalcin enters circulation (Romero Barco et al., 2012). Osteocalcin synthesized by osteoblasts and its carboxylation is dependent on vitamin K (Pikner, 2016).

Osteocalcin is a calcium-binding peptide and it is a small protein seen only in bone, it consists of 49 amino acid that binds to hydroxyapatite crystals of bone, it is increased in condition of bone turnover elevation (Romero Barco *et al.*, 2012). Also, osteocalcin in bone

tissue has affinity to calcium ions and has a role in mineralization of bone matrix (Pikner, 2016).

Osteocalcin is removed by the kidneys and it has a half-life about 5 minutes, osteocalcin concentration changes in cases of bone turnover. Osteocalcin contains 3 glutamyl residues, it is incorporated in matrix of bone also it released from matrix during resorption of bone (Ris te li *et al.*, 2015).

The serum level of the osteocalcin reflects the bone formation rate, therefore it is considered as an important indicator for osteoblasts function (Karsenty, 2017).

Two forms of osteocalcin are found, carboxylate osteocalcin and the non-carboxylate osteocalcin, the later form breakdown more rapidly (Vlot *et al.*, 2018).

2-5-3: N-telopeptide of type 1 collagen

N-telopeptide (N-terminal telopeptide of type 1 collagen (NTx)) is one of the telopeptides of type 1 collagen. It is released during collagen degradation of collagen, it can be used as a marker for bone resorption (Vasikaran *et al.*, 2011). Proteolytic enzymes of osteoclasts are degraded the type 1 collagen, and the most proteolytic enzyme is cathepsin K, this enzyme cleaves fiber collagen into different fragments that detected in the circulation as N-terminal cross-linking telopeptide of type 1 collagen (NTx) or C-terminal cross-linking telopeptide (CTx) (Pikner, 2016).

NTx is a peptide fragment from the amino terminal end of the protein matrix (Romero Barco *et al.*, 2012; Stock, 2015). Serum NTx has been elevated in postmenopausal women (Romero Barco *et al.*, 2012). It has been suggested that NTx is a bone resorption marker and elevated in bone metastasis and postmenopausal women (Vasikaran *et al.*, 2011).

2-5-4: Vitamin D

Vitamin D is a prohormone which is essential for calcium homeostasis, it is formed by ultraviolet-B (UVB) irradiation on the skin. 7-dehydrocholesterol is the substrate that produced vitamin D. vitamin D is produced by 7-dehydrocholesterol an intermediate in the cholesterol synthesis pathway. It is converted to 25-hydroxy vitamin D [25(OH) D] in the liver, then it is converted to 1,25-dihydroxy vitamin D [1,25 $(OH)_2$ D] in the kidney, that is the active form (Duchow *et al*., 2021).
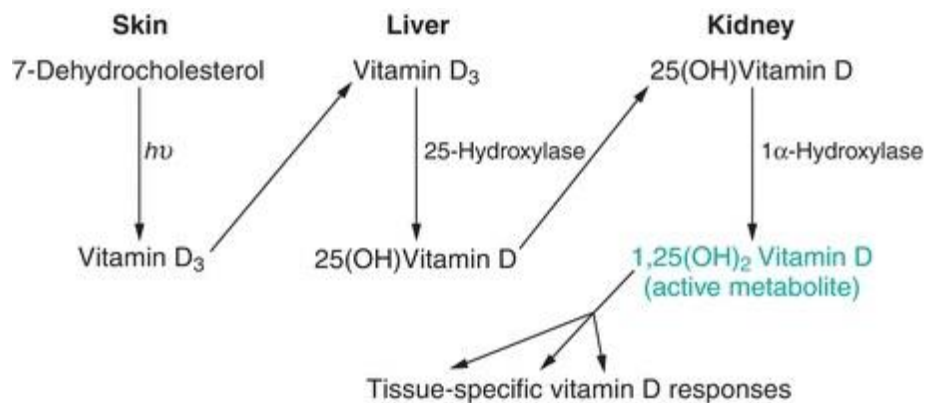
Figure (8: Vitamin D3 Metabolism (Abraham and Abraham, 2018)

2-5-4-1: Biochemical effects of vitamin D

The biochemical effects of vitamin D are (Abraham and Abraham, 2018):

1. It promotes calcium and phosphorus absorption from the intestine.
2. It is effect on bones through the stimulation of osteoblasts which secrete the enzyme ALP this caused an increase in the phosphate concentration, with calcium ion all this lead to mineralization.

Serum 25(OH) vitamin D is the most circulating metabolites of vitamin D and it is reflects the inputs from synthesis and dietary intake (Pennisi *et al*., 2019).

Vitamin D deficiency considered one of the factors that affect remodeling of bone mass and maintenance the mineralization of bone (Marini *et al*., 2022).

Vitamin D plays a role in an increasing bone mass and preventing loss of bone (Park, 2019).

There is a suggestion that statins may impair vitamin D state that in turn can increase the risk of several types of cancers and chronic diseases (Mazidi *et al*., 2017). Some studies showed that rosuvastatin and atorvastatin only but not fluvastatin can increase the level of $25(OH)_2D3$ in the circulation (Yavuz *et al*., 2009; Ertugrul *et al*., 2011). Although some studies showed that statins do not impair levels of vitamin D (Rejnmark *et al*., 2010; Mazidi *et al*., 2016).

2-5-5: Calcium

Calcium is the most important mineral in the bones. Calcium considered as biomarker to control bone metabolism (Mohamed *et al*., 2021).About 1-1.5kg of calcium found in the human body, 99% of it exists in the bone, and 1% in the extracellular fluid. Bone considered the reservoir for calcium (Vasudevan *et al*., 2011).

Calcium has a significant role in protection from osteoporosis (Mustafa *et al*., 2018). Calcium and phosphorus concentrations are regulated by $1,25(OH)_2D3$ (Ris te li *et al*., 2015; Song, 2017).

When calcium level decreases in the blood, parathyroid glands secrete PTH hormone, this hormone cause bone tissue decompose by osteoclasts, so lead to relase of calcium into blood stream (Mistler, 2018).

The optimal excitability of neuronal and muscular tissue, as well as the coordinated operation of many organ systems in the human body depends on the maintenance of a steady serum calcium level. Calcium levels are important for nerve impulse transmission, contraction, blood clotting, hormone secretion and intercellular adhesion (Abraham and Abraham, 2018).

Calcium level is regulated by three major organs, bone, kidney and intestine through the action of the following hormones, parathyroid hormone (PTH), vitamin D (calcitriol or cholecalciferol) and calcitonin. Since PTH hormones stimulates calcium mobilization from the bone by osteoclast stimulation, the osteoclast activity leads to bone demineralization, so calcium uptake by bone reduce, this causes increased calcium level in the blood. The effect of PTH in kidney occurs by increasing calcium reabsorption and decreasing excretion. PTH also indirectly effect on the intestine through calcitriol formation, since PTH enhances calcitriol production, which increases calcium absorption from the intestine. Thus, the overall action of PTH also calcitriol elevated calcium level in blood. In contrast, calcitonin is secreted when serum calcium level increases, calcitonin in this case inhibits the mobilization of calcium from the bones and increase calcification of bone by the stimulation of osteoblast activities, and also calcitonin enhances the calcium excretion by kidney, so decreases calcium level, as shown in Fig 9 (Naik, 2012) .
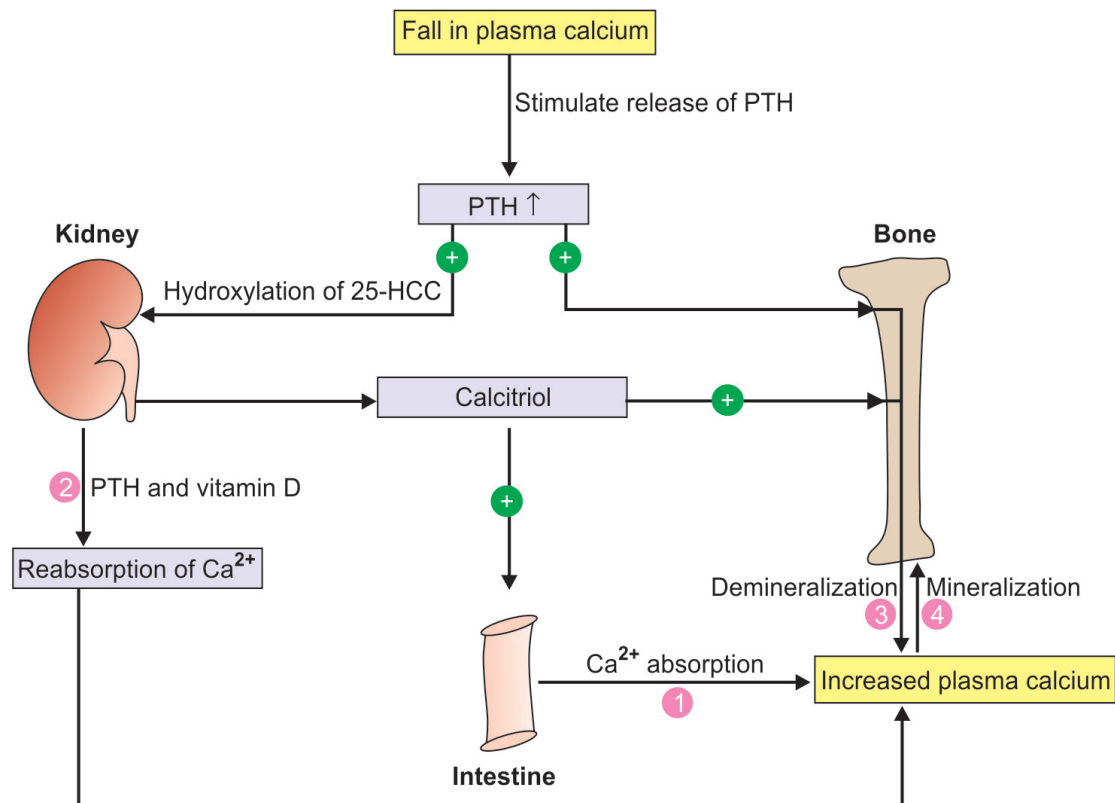
Figure 9: Regulation of Plasma Calcium where, 25-HCC: 25-Hydroxycholecalciferol (Naik, 2012)

2-5-6: Phosphorus

Phosphorus is an essential mineral for bones and teeth development. Adult body contains nearly 1kg of phosphate and it is found in each cell of body. About 80% of phosphorus occurs in bones with combination with calcium. Phosphate in serum exist in three forms, (40%) as free ions, (50%) combined with Ca^{2+}, Mg^{2+}, Na^+ and K^+, and (10%) of phosphate bound with proteins (Satyanarayana and Chakrapani, 2013).

Factors that causes hypophosphatemia are hyperparathyroidism and deficiency of vitamin D (Song, 2017; Pickering *et al*., 2022). Phosphate homeostasis is controlled by parathyroid hormone (PTH), $1,25(OH)_2D3$ and the fibroblast growth factor 23 (FGF-23).

2-5-7: Magnesium

Magnesium ions are the most abundant cations in the cell. There are about 300 enzymes activated by magnesium. Magnesium influences PTH secretion by parathyroid gland and hypomagnesaemia may leads to hypoparathyroidism (Gaw *et al*., 2013b).

Similar to calcium magnesium found in bone 50%-60%. Magnesium plays a role in hormone signaling, and regulating the intracellular calcium concentration (Dent and Selvaratnam, 2022).

Chapter Three

Materials and Methods

3-1: Instruments

Table 2: list of instruments used in the experiments

instrument	origin
Centrifuge	GIMA/ Italy
Electrical Balance	aeADAM/ UK
Muffle Furnace	Stuart/ UK
Microplate Reader	Biotek/ USA
Microplate Washer	BioTek/ USA
Incubator	Jrad/ Germany
Electronic Scale	SF-400C/ China
Spectrophotometer	721-VIS/ China
Spectrophotometer UV-9200	Biotech Engineering Management/ UK
Microtome	Certified Coasco/ India
Abbott/Architect system chemistry analyzer	Architect / Germany

3-2: Chemicals

Table 3: list of chemicals used in the experiments

chemical	origin
Atorvastatin (Lipodar®)	Dar aldawa/Jordan
Rosuvastatin (Crestor®)	AstraZeneca/UK
Ketamine hydrochloride 5%	Germany
Xylazine 2%	Interchemie (Netherlands)
Wounds Spray OTC	Jordan
Povidine iodine	France
Silk 2/0	China
Vicryl 2/0	China

3-3: Statins dose preparation

The tablet (20 mg of statins) was crushed and ground by a morter, weighed and dissolved in 5 ml of distilled water to obtain the required concentration.

3-4: Laboratory animals

Forty adult female albino rats aged (2.5 – 3 months), weighing (200 – 220 g) were obtained from the laboratory animals house of the College of Veterinary Medicine, University of Mosul, they were housed and kept in controlled conditions of ($22 \pm 2°C$), (12h-12h light/dark) cycles.

All animals received a commercially available feed and water through the study, water in polyethylene bottle was available as drinking water.

3-5: Experimental design

After one week of adaptation, 10 rats were sham operated, 30 rats were ovariectomized (ovx) and divided as the following:

Group 1: Sham: It consisted of 10 rats which underwent a dummy surgical intervention, where only a piece of fat around the ovaries were removed, without removing ovaries. They left 30 days after surgery (Yousefzadeh, *et al*., 2020) and they were given distilled water through the period of study.

Group 2: OVX: It consisted of 10 rats which underwent ovariectomy, and left for 30 days after surgery (Yousefzadeh, *et al*., 2020), they served as a model of osteoporosis, they given distilled water through period of study.

Group 3: It consisted of 10 rats which underwent ovariectomy, and left for 30 days after surgery (Yousefzadeh, *et al*., 2020), then orally gavaged at a dose of 20 mg/kg/day (Shahrezaee, *et al*., 2018) atorvastatin (Lipodar®/dar aldawa/Jorden) for 60 days (Shahrezaee, *et al*., 2018).

Group 4: It consisted of 10 rats which underwent ovariectomy, and left for 30 days after surgery, then orally gavaged at a dose of 20 mg/kg/day (Wang, *et al*., 2019) rosuvastatin (Crestor®/AstraZeneca/UK) for 60 days (Shahrezaee, *et al*., 2018).

3-6: Ovariectomy

A mixture of 7.5 mg/kg of xylazine 2% and 75 mg/kg of ketamine hydrochloride 10% was given intraperitoneally to rats for anesthesia (Shahzamani *et al*., 2021), and the failure of the wink reflex and the lack reaction to pinching of the foot was used to evaluate the anesthesia success, aseptic conditions were used to make a skin incision, hair was removed from the abdominal area using a sharp blade and soap, the area was disinfected with povidine iodine, the abdominal area was opened by making a longitudinal incision down to the abdominal cavity, then the ovaries were excised, followed by suturing of abdominal layers and skin using (vicryl 2/0, silk 2/0) respectively by surgical threads then disinfected with OTC (AL-Mamari, 2010).

In the sham group, the same procedure was performed, but the ovaries were not removed. All rats were returned to their cages after a complete recovery from anesthesia.

3-7: Blood collection

Blood samples were collected after 30 and 60 days from the retro-orbital plexus (Fitzner Toft *et al*., 2006; Arnold and Langhans, 2010) of all rats by using the capillary glass tubes, then put it in gel containing tubes, left for clotting, then centrifuged at 3000 r/min for 15 minutes. After that, serum samples were separated, placed in clean plastic tubes (Eppendorf tubes) and kept at -18°C for biochemical analysis.

3-8: Biochemical markers assessment

3-8-1: Determination of rat alkaline phosphatase activity (ALP)

Rat alkaline phosphatase (ALP) activity in serum was determined using the Sandwich-ELISA kit (Catalog No: E-EL-R1109, Elabscience company).

The Sandwich-ELISA method is employed by this ELISA kit. Standards or samples are added to the micro ELISA plate wells and mixed with the relevant antibody, which has been pre-coated on the micro ELISA plate included in this kit. After that, each microplate well receives an addition of an Avidin-Horseradish Peroxidase (HRP) combination and a biotinylated detection antibody specific for Rat ALPL. Free parts are removed during washing. To each well, the substrate solution is applied. There will only be blue coloration in the wells that have Rat ALPL, biotinylated detection antibody, and Avidin-HRP conjugate. The addition of stop solution stops the enzyme-substrate reaction, and the color changes to yellow. At a wavelength of 450 nanometers, the optical density (OD) is measured spectrophotometrically (Aydin, 2015).

3-8-2: Determination of rat N-telopeptide type I collagen (NTx)

Rat N-telopeptide type I collagen (NTx) in serum was determined by using the Sandwich-ELISA kit (Catalog No: E-EL-R0276, Elabscience company). Method as explained in ALP activity.

3-8-3: Determination of rat osteocalcin (OC)

Rat osteocalcin in serum was determined using the Sandwich-ELISA kit (Catalog No: E-EL-R0243, Elabscience company). Method as explained in ALP activity.

3-8-4: Determination of rat vitamin D3

Rat DHVD3 (1,25-Dihydroxyvitamin D3) in serum was determined using the Sandwich-ELISA kit (Catalog No: E-EL-0016, Elabscience company). Method as explained in ALP activity.

3-8-5: Determination of calcium in serum

Calcium in serum was determined spectrophotometrically by using the diagnostic reagent kit (Biolabo, France) which includes reacting of o-cresol phtalein complex (CPC) one with calcium to form a dark-read colored complex which absorbance measured at 570nm (Ris te lie *et al*., 2015).

$$Ca^{+2} + CPC \xrightarrow[\text{Medium}]{\text{Alkaline}} Ca\text{-}CPC \text{ complex}$$
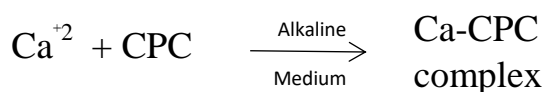
Calculation

Calcium level was calculated in (mg/dl) in the sample according to the following low:

$$\text{Conc. (mg/dl) x } \frac{\text{Test} - \text{Blank}}{\text{Standard}}$$

3-8-6: Determination of phosphorus in serum

Total serum phosphorus level (mg/dl), was estimated using Abbott Architect System clinical chemistry analyzer (Abbott GmbH & Co. KG. Max-Plank-Ring2, 65205-Wiesbaden, Germany) at 340nm wave length.

Quality control measures were taken into consideration. That is, a fresh pipette tip was used for each sample and both reagents 1 and 2 of the kit, as well as estimation steps per the manufacture's instruction in 7D71-G96000R03-B7DS10 phosphorus reagent kit (Ris te lie *et al.*, 2015).

3-9: Bone ashing

At the end of the study, after 60 days of treatment, 3 rats from each group were sacrificed, then the right femur was taken, cleaned and soft tissues around each femur were removed in order to convert it to ash. Ash content of the bone represents the inorganic materials (minerals) that remaining after the complete oxidation and ignition of the organic materials. To obtain ash, the right femur bones were weighted before ashing, then heated in ceramic lid in a muffle apparatus (oven) at 600°C for 18h (Lee *et al.*, 2021).

After observing the ash, they weighed, solubilized with in 0.1M/L HCl and then diluted with deionized water up to 100 ml (Nagareddy and Lakshmana, 2006; Mustafa *et al.*, 2018).

The final solutions were used to estimate calcium, phosphorus and magnesium in bone ash.

3-9-1: Determination of calcium, phosphorus and magnesium in bone ash

Calcium was determined in bone ash (preparing in previous paragraph) by Ca-EDTA method.

This method called complexometric titration and it is used to estimate calcium content in various solid materials. The principle of this method based on the reaction of EDTA (Ethelene Diamine-Tetra Acetate) with calcium ions to produce colorless complex (Bird *et al.*, 1961).

Inorganic phosphate was determined by the method that depends on the reaction of inorganic phosphorous with molydic acid to form yellow phosphomolybdic acid which reduced to give a blue color detected spectrophotometrically (Association of official analytical chemists, 2000).

The percentage of magnesium in bone ash was estimated by eriochromic black T method, which includes preparation of eriochrome black dye, preparation the magnesium standard solution, buffer solution 9.6 and then read the absorbance at wavelength 520 nm against the blank. The result was read by using standard curve (Al-Hashemi, 2007; Association of official analytical chemists, 2000).

3-10: Histological examination

The rats were dissected and left femurs bone were cleaned from the surrounding musculature and fixed with 10% formalin for 24h, followed by decalcification by (10% nitric acid, 5% EDTA, and distilled water), and then dehydrated in alcohol, then cleared in xylene and embedded in paraffin and cut into longitudinal sections of 5μm (thickness) and stained with hematoxylin and eosin for observation (Luna, 1968). The thickness of trabecular bone was measured using the software of a microscope camera in a micrometer as a mean of 5 measurements /field (100X) for 5 fields for each animal in the group.

3-11: Statistical analysis

The results were statistically analyzed by using the statistical sigma plot version 12.5, all results were expressed as mean ± standard error (mean ± SE). One-way analysis of variance (ANOVA) was used to find any significant difference among the groups followed by Duncan's

multiple comparisons within probability at (P≤ 0.05) (El-Nabarawi, *et al*., 2017).

Chapter Four

Results

4-1: Effect of ovariectomy on the body Weight of the female rats

The results in table (4) show, there was no differences in body weight (g) before ovariectomy in all groups. After 30 days of ovariectomy the results showed a significant elevation in body weight of all ovariectomized rats (30 rats) compared with G1 (sham group).

The results showed a significant elevation at (p≤ 0.05) in body weight of G2 (ovx) and G3 (ovx + atorvastatin) and G4 (ovx + rosuvastatin) after 60 days of treatment with statins compared with the sham group. Body weight of all groups significantly increased at (p≤ 0.05) through the period of the experiment.

Table 4: Effect of ovariectomy on the body weight of the female rats

Groups	Body weight (g)		
	Before ovariectomy	30 days after ovariectomy	60 days after treatment
Sham	206.50 ± 2.23 aA	222.50 ± 2.28 bA	260.60 ± 4.20 cA
OVX	206.40 ± 2.23 aA	244.80 ± 3.80 bB	287.60 ± 3.80 cB
OVX + Atorvastatin	206.40 ± 2.20 aA	244.30 ± 3.60 bB	284.20 ± 3.48 cB
OVX + Rosuvastatin	206.22 ± 2.59 aA	244.22 ± 3.39 bB	293.78 ± 7.34 cB

Each value represents the mean ± SE, values superscripts with different capital letters represent comparison in the column and small letters represent comparison in the row were significantly at P≤ 0.05.

4-2: Effect of statins on serum rat ALP Activity after 30 and 60 days of treatment

The results in table (5) show, there was a significant elevation in serum ALP activity in the ovx rats, in the ovx rats treated with atorvastatin and ovx rats treated with rosuvastatin compared with the sham group, and no significant differences in serum ALP activity between ovx rats, ovx rats treated with atorvastatin and ovx rats that treated with rosuvastatin after 30 days of treatment.

After 60 days of treatment with atorvastatin, a significant reduction at ($p \leq 0.05$) in serum ALP activity was observed in comparison to the ovx group. Also, the ovx rats that treated with rosuvastatin exhibited a significant reduction at ($p \leq 0.05$) in serum ALP activity compared with the ovx group.

Serum ALP activity in rats treated with rosuvastatin decreased, but not significantly from ovx rats that treated with atorvastatin also there was no differences in serum ALP activity between the ovx rats treated with rosuvastatin and the sham group.

Table 5: Serum rat ALP activity after 30 and 60 days of treatment with statins

Groups	ALP (ng/ml)	
	After 30 days	After 60 days
Sham	9.18 ± 0.53 [b]	11.64 ± 0.56 [c]
OVX	12.77 ± 1.10 [a]	16.78 ± 0.39 [a]
OVX+ Atorvastatin	12.15 ± 0.65 [a]	14.18 ± 0.65 [b]
OVX + Rosuvastatin	11.55 ± 0.54 [a]	12.76 ± 1.02 [bc]

Each value represents the mean $\pm$ SE, values superscripts with different letters in the column were significantly at $P \leq 0.05$.

4-3: Effect of statins on serum rat NTx after 30 and 60 days of treatment

Table (6) shows a significant elevation at (p≤ 0.05) in serum NTx in the ovx rats, ovx rats treated with atorvastatin, and ovx rats with rosuvastatin compared with the sham group after 30 days of treatment, but there was no significant differences between the ovx rats, ovx rats treated with atorvastatin and ovx rats treated with rosuvastatin in NTx level after 30 days of treatment. Treatment with statins after 30 days of treatment could not reduce NTx level.

The ovx group showed a significant rise in NTx level after 60 days of treatment in comparison to the sham group. But treating the ovx rats with atorvastatin also treatment with rosuvastatin showed a significant reduction at (p≤ 0.05) in NTx levels after 60 days compared with the ovx group. No differences in serum NTx levels between the ovx rats treated by atorvastatin and ovx rats treated with rosuvastatin compared with the sham group.

Table 6: Serum NTx after 30 and 60 days of treatment with statins

Groups	NTx (ng/ml)	
	After 30 days	After 60 days
Sham	4.76 ± 0.38 [b]	5.11 ± 0.39 [b]
OVX	7.05 ± 0.41 [a]	6.71 ± 0.23 [a]
OVX+ Atorvastatin	6.20 ± 0.30 [a]	5.19 ± 0.51 [b]
OVX + Rosuvastatin	6.35 ± 0.19 [a]	5.12 ± 0.35 [b]

Each value represents the mean ± SE, values superscripts with different letters in the column were significantly at P≤ 0.05.

4-4: Effect of statins on serum rat OC after 30 and 60 days of treatment

As shown in table (7), after 30 days of treatment the ovx rats showed a significant elevation at ($p \leq 0.05$) in serum osteocalcin compared with the sham group. Treatment with atorvastatin caused a non-significant reduction in serum osteocalcin compared with the ovx group, but no differences in osteocalcin levels between the ovx rats treated by rosuvastatin, ovx rats and ovx rats treated with atorvastatin.

After 60 days of treatment, the results showed a significant increase at ($p \leq 0.05$) in serum osteocalcin of the ovx rats compared with the sham group. The treatment with atorvastatin has non-significant decrease in serum osteocalcin when compared with the ovx rats, but the treatment of the ovx rats with rosuvastatin for 60 days leads to a significant decrease in serum osteocalcin in comparison to the ovx rats and ovx rats treated with atorvastatin. Treatment with rosuvastatin could reduce the level of the osteocalcin and reach its level of the sham group, because there were no significant differences between the ovx rats treated with rosuvastatin and the sham group in osteocalcin level.

Table 7: Serum OC after 30 and 60 days of treatment with statins

Groups	OC (ng/ml)	
	After 30 days	After 60 days
Sham	10.92 ± 0.52 [b]	13.20 ± 0.72 [b]
OVX	14.43 ± 0.94 [a]	17.64 ± 0.87 [a]
OVX+ Atorvastatin	12.93 ± 0.35 [ab]	16.9 ± 0.40 [a]
OVX + Rosuvastatin	13.44 ± 1.20 [a]	13.57 ± 1.09 [b]

Each value represents the mean ± SE, values superscripts with different letters in the column were significantly at $P \leq 0.05$.

4-5: Effect of statins on serum vitamin D3 after 30 and 60 days of treatment

The results in table (8) show, that ovariectomy significantly reduce at ($p \leq 0.05$) the level of $1,25(OH)_2$ D3 when compared with the sham group, also the treatment with atorvastatin and treatment with rosuvastatin did not increase the level of $1,25(OH)_2$ D3 when compared with the ovx group after 30 days of treatment.

After 60 days of treatment serum $1,25(OH)_2$ D3 in the ovx rats still low when compared with the sham group. Treatment of the ovx rats with atorvastatin increased but not significantly serum $1,25(OH)_2$ D3 when compared with ovx rats, but treating the ovx rats with rosuvastatin caused a significant elevation at ($p \leq 0.05$) in serum $1,25(OH)_2$ D3 when compared with the ovx rats. Also the results showed no significant differences between the groups treated with atorvastatin with the group that treated with rosuvastatin.

Table 8: Serum $1,25(OH)_2$ D3 after 30 and 60 days of treatment with statins

Groups	Vit D3 (pg/ml)	
	After 30 days	After 60 days
Sham	196.10 ± 5.36 [a]	223.70 ± 4.52 [a]
OVX	144.01 ± 7.50 [b]	164.19 ± 4.62 [c]
OVX+ Atorvastatin	154.00 ± 4.76 [b]	177.24 ± 5.39 [bc]
OVX + Rosuvastatin	151.63 ± 5.64 [b]	192.65 ± 6.59 [b]

Each value represents the mean ± SE, values superscripts with different letters in the column were significantly at $P \leq 0.05$.

4-6: Effect of Statins on Serum Calcium Level after 30 and 60 days of treatment

The results in table (9) show, that ovariectomy, treatment with atorvastatin and treatment with rosuvastatin did not affects the level of serum calcium after 30 days of treatment, when compared with the sham group. But after 60 days of treatment serum calcium significantly elevated at (p≤ 0.05) in the ovx rats compared with the sham group. Treatment of the ovx rats with atorvastatin caused a non-significant reduction in serum calcium in comparison with the ovx rats, while treatment of the ovx rats with rosuvastatin resulted in a significantly reduction at (p≤ 0.05) in serum calcium compared with theovx rats. There was a non-significant difference in serum calcium of the ovx rats that treated with rosuvastatin with ovx rats that treated with atorvastatin.

Table 9: Serum calcium level after 30 and 60 days of treatment with statins

Groups	Serum calcium (mg/dL)	
	After 30 days	After 60 days
Sham	10.60 ± 0.10 [a]	10.90 ± 0.08 [c]
OVX	10.70 ± 0.10 [a]	11.51 ± 0.11 [a]
OVX + Atorvastatin	10.90 ± 0.20 [a]	11.28 ± 0.10 [ba]
OVX + Rosuvastatin	10.60 ± 0.20 [a]	11.19 ± 0.09 [b]

Each value represents the mean ± SE, values superscripts with different letters in the column were significantly at P≤ 0.05.

4-7: Effect of statins on serum phosphorus level after 30 and 60 days of treatment

The results in the table (10) show, there was no significant differences in serum phosphorus among all studied groups after 30 days of treatment. A significant increase at (p≤ 0.05) in serum phosphorus of the ovx rats was observed after 60 days of treatment, and treatment the ovx rats with atorvastatin did not reduce this elevation in phosphorus level. But the treatment of ovx rats with rosuvastatin reduce the level of serum phosphorus but not significantly, although there were a non-significant differences in serum phosphorus between the ovx rats treated with rosuvastatin and the sham group.

Table 10: Serum phosphorus after 30 and 60 days of treatment with statins

Groups	Serum phosphorus (mg/dL)	
	After 30 days	After 60 days
Sham	5.70 ± 0.20 [a]	4.15 ± 0.42 [b]
OVX	6.10 ± 0.17 [a]	5.74 ± 0.13 [a]
OVX + Atorvastatin	6.10 ± 0.30 [a]	5.30 ± 0.27 [a]
OVX + Rosuvastatin	6.00 ± 0.20 [a]	4.75 ± 0.24 [ba]

Each value represents the mean ± SE, values superscripts with different letters in the column were significantly at P≤ 0.05.

4-8: Effect of statins on the bone ash weight after 60 days of treatment

The weight of the right femur bone ash of the ovx rats at the end of experiment was significantly reduced at (p≤ 0.05) compared with the weight of the bone ash of the sham group, this refers to reduce bone mass. Treatment of the ovx rats with atorvastatin leads to non-significant

elevation in the weight of the bone ash compared with the bone ash weight of the ovx rats. But treatment of the ovx rats with rosuvastatin caused a significant elevation in the bone ash weight in comparison to the bone ash weight of the ovx rats.

There was non-significant reduction in the weight of the bone ash of the ovx rats treated with rosuvastatin compared with the weight of the bone ash in the sham group, as shown in table (11). As well as, there were no significant differences between the ovx rats treated with atorvastatin and ovx rats treated with rosuvastatin in the weight bone ash.

Table 11: Weight of the bone ash after 60 days of treatment with statins

Groups	Bone ash weight (g)
Sham	0.24 ± 0.01 [a]
OVX	0.19 ± 0.00 [c]
Atorvastatin	0.21 ± 0.00 [bc]
Rosuvastatin	0.22 ± 0.00 [ab]

Each value represents the mean ± SE, values superscripts with different letters were significantly at P≤ 0.05.

4-9: Effect of statins on Ca, P, and Mg percentage in bone ash after 60 days of treatment

The results in table (12) refer to the percentage of calcium, phosphorus and magnesium in the bone ash content of all studied groups. Ovariectomy caused a significant reduction at ($p \leq 0.05$) in calcium, phosphorus and magnesium in the bone ash when compared with the bone ash of the sham group. Calcium and phosphorus content in the bone ash of the ovx rats treated with atorvastatin and in the ovx rats group treated with rosuvastatin significantly elevated at ($p \leq 0.05$) in comparison

to calcium and phosphorus content in the bone ash of the ovx group, even reached their levels of the sham group as shown there was no significant difference between these two treated groups and the sham group.

Treatment of the ovx rats with atorvastatin could not increase level of magnesium in the bone ash. A non-significant elevation in magnesium content was observed in the ovx rats treated with rosuvastatin compared with the ovx rats and ovx rats treated with atorvastatin. The level of magnesium content of the bone ash of the ovx rats treated with rosuvastatin increased, but not significantly when compared with the sham group, it not reaches the level content of this group.

Table 12: Percentage of Ca, P and Mg in the bone ash after 60 days of treatment with statins

Groups	Meam ± SE		
	Ca %	P %	Mg %
Sham	42.07 ± 1.48 [a]	22.10 ± 0.97 [a]	1.40 ± 0.11 [a]
OVX	34.20 ± 2.10 [b]	16.87 ± 0.98 [b]	0.93 ± 0.08 [b]
OVX + Atorvastatin	39.90 ± 0.62 [a]	20.13 ± 0.56 [a]	1.10 ± 0.58 [b]
OVX + Rosuvastatin	40.67 ± 0.57 [a]	21.87 ± 0.22 [a]	1.20 ± 0.05 [ab]

Each value represents the mean ± SE, values superscripts with different letters in the column were significantly at P≤ 0.05.

4-10: Effect of statins on the thickness of trabecular bone after 60 days of treatment

The results in table (13) indicate a significant reduction at (p≤ 0.05) in the trabecular bone thickness of ovx rats in comparison to the sham group. While, treatment of the ovx rats with atorvastatin. Also, the

treatment of ovx rats with rosuvastatin caused a significant elevation at (p≤ 0.05) in trabecular bone thickness compared with the ovx rats, in which reach the level of the sham group. There were no significant differences between the ovx rats treated with atorvastatin, ovx rats treated with rosuvastatin with the sham group.

Table 13: Thickness of trabecular bone after 60 days of treatment with statins

Groups	Thickness of trabecular bone/ µm
Sham	86.6 ± 12.4 [a]
OVX	50 ± 4.2 [b]
OVX + Atorvastatin	69.6 ± 8.2 [a]
OVX + Rosuvastatin	73.8 ± 3.7 [a]

Each value represents the mean ± SE, values superscripts with different letters were significantly at P≤ 0.05. Mean ± SE with different superscript letters in the column are significant at P≤0.05 using one way ANOVA test.

4-11: Histological examination

The results in fig. 10and fig. 11 showed the rat femur bone in the sham group. There was a high density and trabecular thickness bone, also the blood vessels were high, few osteoclasts, and normal osteoblast.

The ovariectomy caused low density, thin trabecular bone, few blood vessels and poor developed osteogenic tissue, with low osteoblasts numbers, and high osteoclasts numbers as shown in fig. 12 and fig 13 .

The treatment of the ovx rats with atorvastatin, showed high density trabecular bone, medium developed osteogenic tissue, medium numbers of osteoclasts and medium osteoblasts numbers as shown in fig. 14 and fig. 15 .

The rat femur bone in the group that treated with rosuvastatin, showed high density trabecular bone, high blood vessels and well osteogenic tissue, low numbers osteoclasts and high numbers osteoblasts as shown in fig. 16 and fig. 17 .the results in table (13) show a significant reduction in thickness trabecular bone/µm in the ovx rats compared with the sham group. But the treatment with atorvastatin also treatment with rosuvastatin leads to a significant elevation in trabecular bone thickness compared with the ovx group and no differences between atorvastatin group compared with rosuvastatin group.
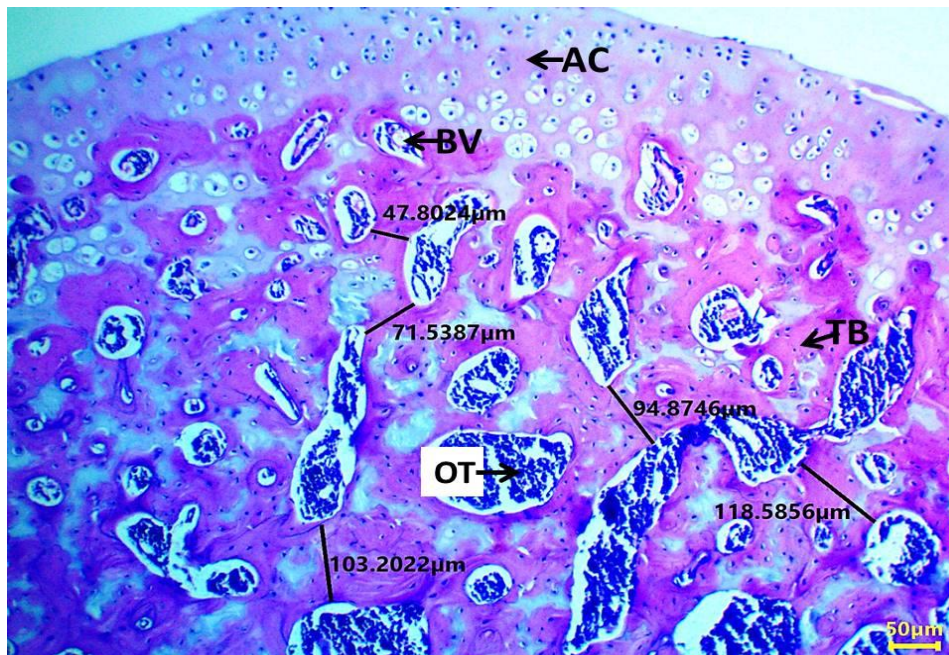
Figure 10: photomicrograph of rat femur bone of control negative (Sham) group shows intact articular cartilage (AC), high density and thickness trabecular bone (TB), blood vessels (BV) and well developed osteogenic tissue (OT). H&E stain, scale bar=50µm, 100X.
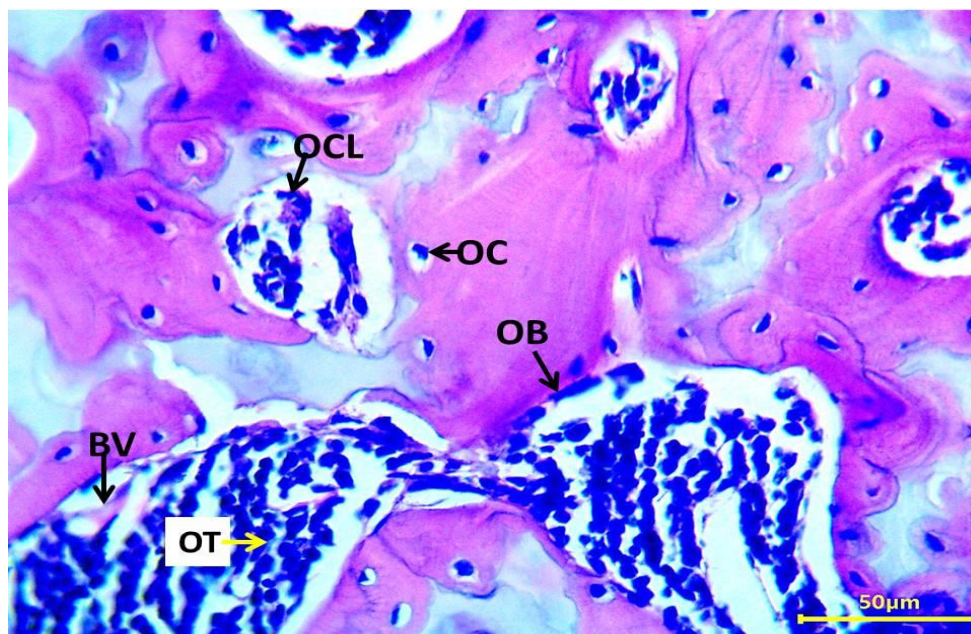
Figure 11: photomicrograph of rat femur bone of control negative (Sham) group shows normal architecture representing by osteoblasts (OB), osteocytes (OC), few osteoclasts (OCL) and blood vessels (BV). H&E stain, scale bar=50µm, 400X.
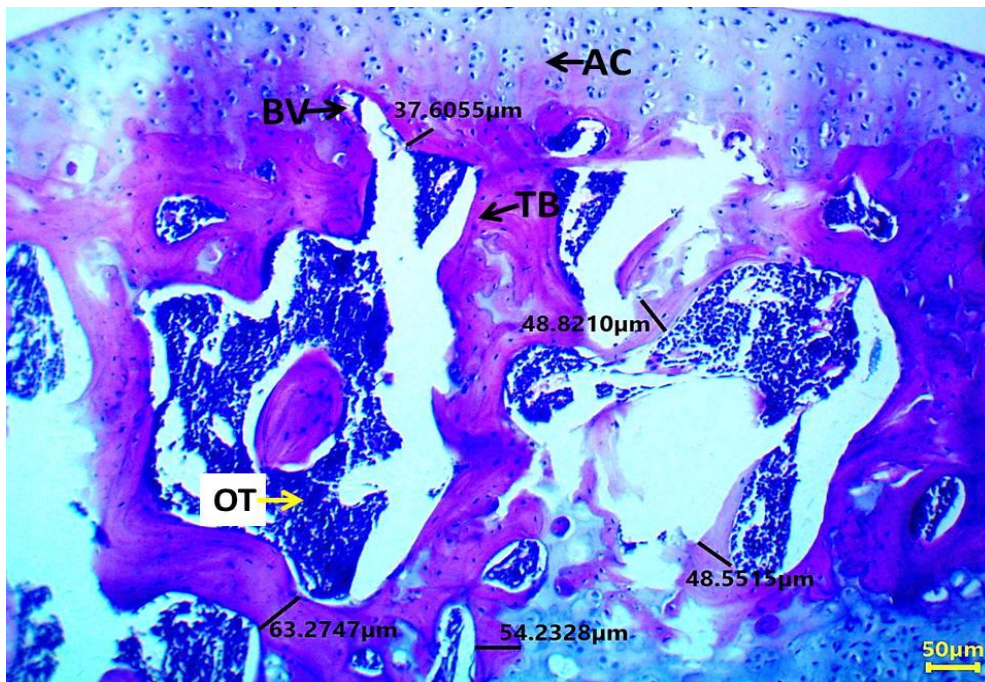
Figure 12: photomicrograph of rat femur bone of control positive (OVX) group shows articular cartilage (AC), low density and thin trabecular bone (TB), few blood vessels (BV) and poor developed osteogenic tissue (OT). H&E stain, scale bar=50µm, 100X.
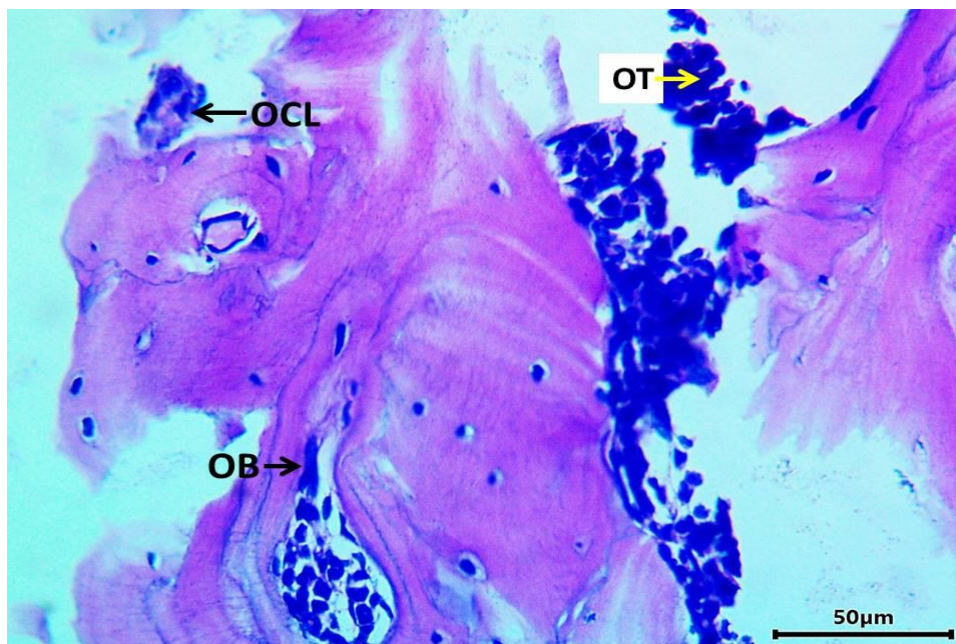
Figure 13: photomicrograph of rat femur bone of control positive (OVX) group shows presence of low numbers osteoblasts (OB), high numbers osteoclasts (OCL) and poor developed osteogenic tissue (OT). H&E stain, scale bar=50µm, 400X.
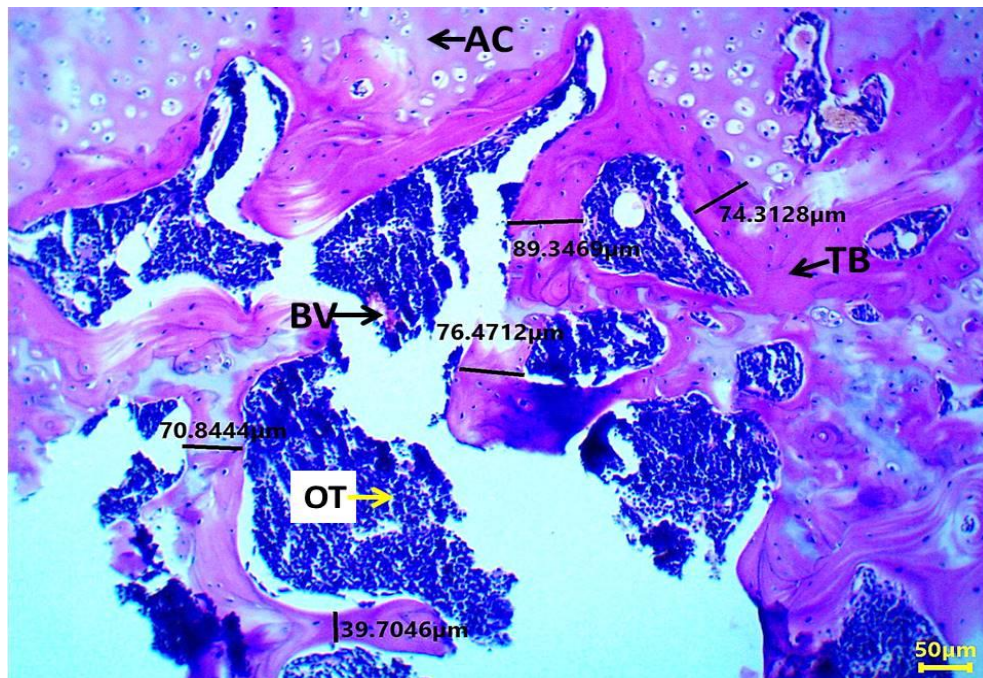
Figure 14: photomicrograph of rat femur bone of Atrovastatin treated group shows articular cartilage (AC), high density trabecular bone (TB), few blood vessels (BV) and medium developed osteogenic tissue (OT). H&E stain, scale bar=50μm, 100X
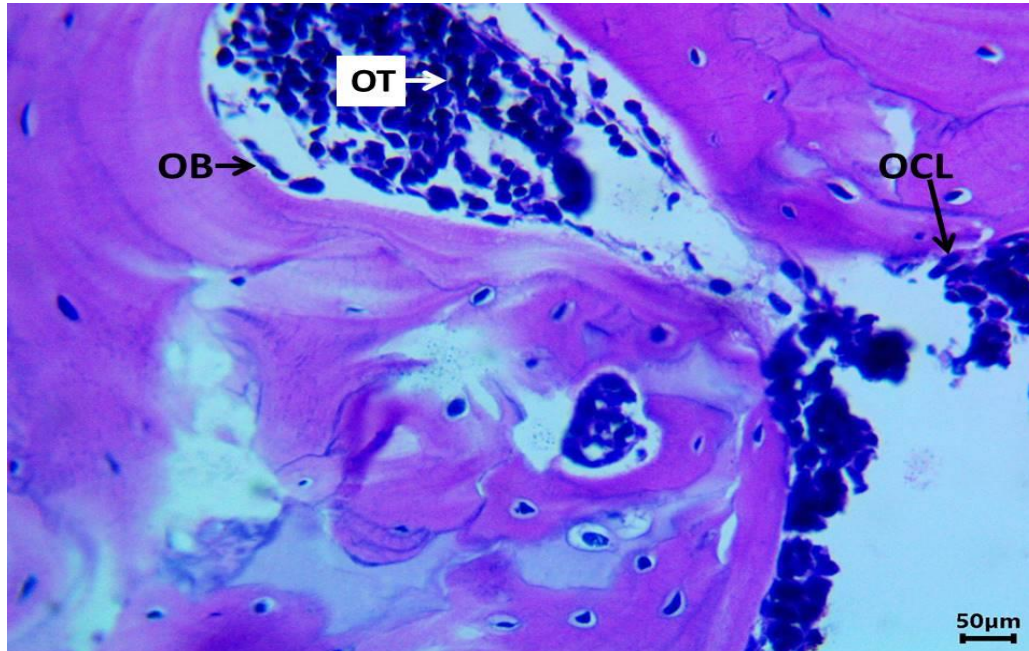
Figure 15: photomicrograph of rat femur bone of Atrovastatin treated group shows presence of medium numbers osteoblasts (OB), medium numbers osteoclasts (OCL) and osteogenic tissue (OT). H&E stain, scale bar=50μm, 400X.
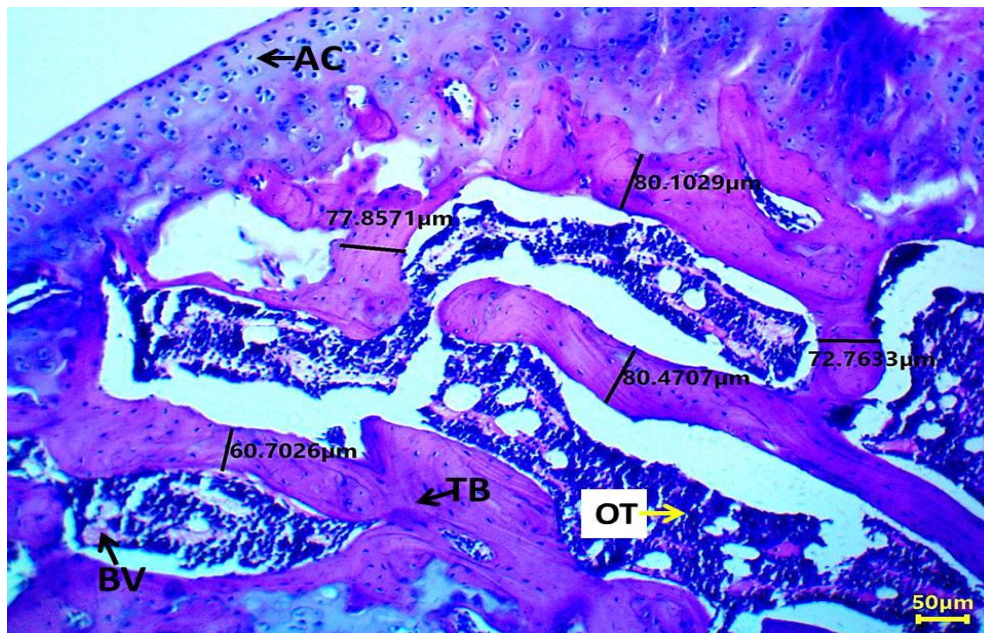
Figure 16: photomicrograph of rat femur bone of Rosuvastatin treated group shows articular cartilage (AC), high density trabecular bone (TB), blood vessels (BV) and well developed osteogenic tissue (OT). H&E stain, scale bar=50µm 100X.
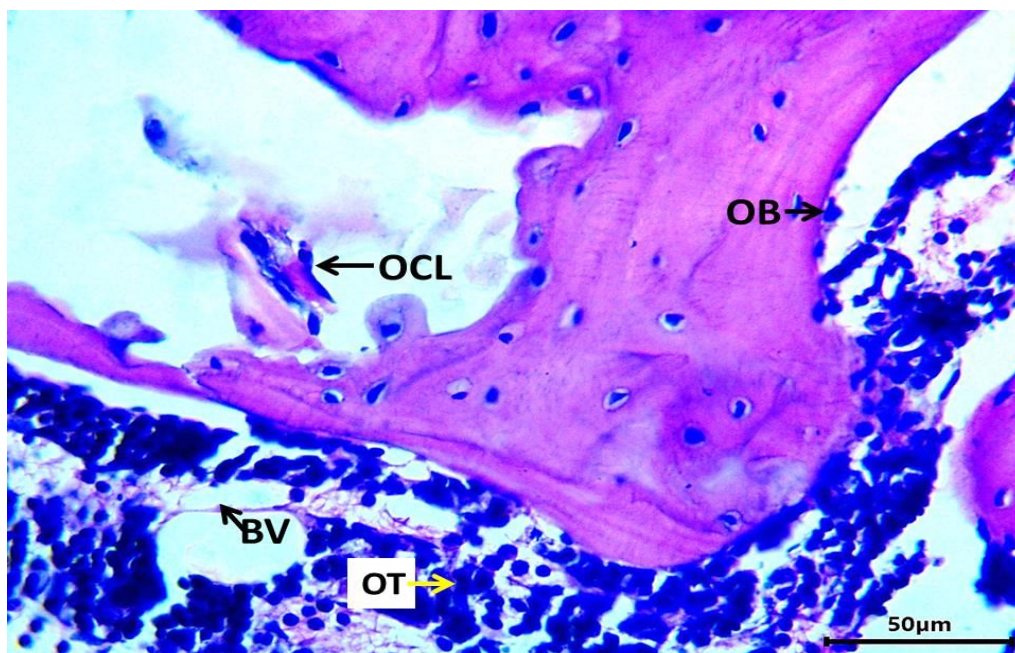
Figure 17: photomicrograph of rat femur bone of Rosuvastatin treated group shows presence of high numbers osteoblasts (OB), low naumbers osteoclasts (OCL), blood vessels (BV) and well developed osteogenic tissue (OT). H&E stain, scale bar=50µm, 400X.

Chapter five

Discussion

The aim of this study was to I nvestigate the effect of two types of statins, one lipophilic statin (atorvastatin) and the other is hydrophilic statin (rosuvastatin) on bone formation and bone resorption of rats suffered from osteoporosis. Female rats in this study developed bone changes after ovariectomy: because the ovariectomy results in estrogen deficiency, this case is similar and mimics bone deterioration that occur in osteoporosis in women after menopause.

The ovariectomized rats are used as a model of osteoporotic women and they used for studing postmenopausal bone loss (El-Nabarawi *et al*., 2017; Torrubia *et al*., 2020).

Estrogen is a good inhibitor for bone resorption, and prevents osteoporosis. Deficiency of estrogen leads to bone remodeling elevation, and both bone formation and resorption are increased, but uncoupling between these process causes bone resorption exceeds bone formation (Manolagas *et al*., 2013). In addition, apoptosis in osteoclast cells is produced by estrogen (Mackie *et al*., 2008).

Sham rats in this study were subjected to the same surgery of ovarectomy, but without removing their ovaries, in order to exposure to the same stress of surgery (Razzoli *et al*., 2015).

The weights of the rats were equal at the beginning of the experiment. But ovariectomy operation results in a significant body weight gain compared with sham group, these results agree with results of Chou *et al*., (2022).

Who noticed an increase in the body weight in ovx rats, due to the relation between ovariectomy and energy expenditure reduction. Our results also agree with results of Anbinder *et al*., (2007) and Sharma *et al*., (2017). Ovariectomy may affect several types of proteins that have related with some brown adipose tissue (Chou *et al*., 2022). Also Saul *et al*., (2021) showed that all ovariectomized rats had higher body weight compared with control group. The elevation of body weight in ovx group rats due to estrogen deficiency and leads to obesity (Girgoryan *et al*., 2017). The body weight gain in this study is similar to study reported by Mustafa *et al*., (2018) since they found that ovx rats gained more body weight than the sham group and the body weight gain was 19.23% in ovx compared with sham 11.32 %, also similar to study of Torrubia *et al*., (2020), they revealed that ovariectomy caused increased in body weight in rats.

Alkaline phosphatase activity in serum ovx rats was significantly elevated compared with sham rats, this result agrees with Morgan *et al*., (2021) who noticed the elevation in serum ALP activity in ovx rats. Also agrees with the study of Hamdoon *et al*.,(2020) that showed an increase in ALP activity in serum of ovx rats. Estrogen deficiency leads to a high bone turnover, due to the low production of estrogen by ovaries.

Ovariectomy caused ALP elevation, this elevation may be due to imbalance between bone formation and bone resorption, in which bone resorption exceeding bone formation. Osteoclastogenesis is inhibited by estrogen, and this process involving many signaling pathways, as osteoprotegerin (OPG), receptor activator of nuclear factor-KB (RANK) and RANA ligand (RANKL). RANKL stimulates osteoclasts activation and differentiation (Alrowaili *et al*., 2021).

The elevation in ALP activity refers to a high bone turnover in ovx rats. After 60 days of treatment ovx rats with atorvastatin show a significant reduction in serum ALP activity, but treatment with rosuvastatin caused more reduction than atorvastatin. This refers to a significant improvement in bone formation and decreased bone resorption after treatment with statins. ALP is one of the bone biomarkers related to bone formation, and its activity increased in some metabolic diseases and osteoporosis (Romero Barco, *et al*., 2012). Results of this study indicate that rosuvastatin has a better effect on bone formation than atorvastatin. Although the two types of statins that used in this study efficiently ameliorated osteoporosis that done by ovariectomy.

The reduction in ALP activity after treatment with atorvastatin agrees with El-Nabarawi *et al*., (2017) they noticed a reduction in ALP activity after atorvastatin treatment.

ALP plays an important role in hard tissue formation, and it increases the mineralization, also it reduces pyrophosphate level in the extracellular fluid. It has a role in the calcification mechanism (Vimalraj, 2020). The results of this study indicate the utility effect of using atorvastatin and using of rosuvastatin in ameliorating the ovx-induced osteoporosis. The effectiveness of rosuvastatin in reducing ALP activity more than atorvastatin may be due to its polarity and half-life. Rosuvastatin induced the proliferation of osteoblast, it has been shown that statin enhanced formation of bone through bone morphogenetic protein-2 (BMP-2) induction. Rosuvastatin is not only enhancing the BMP-2 expression, but it also enhances ALP activity in the later stages of differentiation of osteoblast (Monjo *et al*., 2010).

Ovariectomy of rats in this study caused the elevation in NTx levels also after 30 and 60 days. This elevation of NTx is in agreement with the

study of Alrowaili *et al*., (2021), who noticed an increase in NTx level in osteoporotic rats when compared with the sham group, and they suggested that the increased activity of osteoclasts due to the estrogen deficiency after ovariectomy. During remodeling of bone, some molecules are released into the blood during bone resorption, so the NTx is released during bone resorption. Serum NTx level also CTx have been suggested to be increased in postmenopausal women (Kanterewicz *et al*., 2013). The elevation in NTx in this study refers to a significant increase in bone resorption. NTx is one of the biomarkers of bone turnover and considered the best marker with CTx for bone resorption (Romero Barco, *et al*., 2012). NTx mobilized from bone by osteoclasts and elevated its levels in serum indicate increase in bone resorption (Alrowaili *et al*., 2021). In addition, the elevation in NTx in this study agrees with the results of Alrowaili *et al*., (2021). The treatment with atorvastatin, also the treatment with rosuvastatin after 30 days did not affect NTx level, because no differences between these groups with ovx group. This means that the duration was not enough to reduce the level of NTx that elevated as a result of ovariectomy. But after 60 day of treatment with 20mg/kg of atorvastatin and rosuvastatin, NTx levels reduced compared with the ovx group, this means these two types of statins have ability to reduce bone resorption done by ovariectomy, and both types of statins atorvastatin (lipophilic) and rosuvastatin (hydrophilic) were similar in their effectiveness in reducing bone resorption. NTx is released through type 1 collagen degradation by osteoclasts, so NTx and CTx released to the circulation and these proteins that related to type 1 collagen are specific to collagen turnover, since osteoclasts are not effective in degradation of other type 1 collagen containing tissues (Varela and Jolette, 2018). These results indicated that two types of statin used in this study have ability to reduce the resorption of bone through their action that similar to action of

some drugs that used to treatment of osteoporosis, because they reduce products that produced from mevalonate pathway as farnesyl pyrophosphate (FPP) and geranyl geranyl pyrophosphate (GGPP) which considered precursors for isoprenoids, so isoprenoids can modulate some cellular proteins as oncoproteins (Ras), nuclear proteins (Laminins) and small guanosine triphosphate (GTP)-binding proteins as (rho-rac-rab) to form prenylated proteins. This is essential for activation of motile cells as osteoclasts. For this reason alendronate (a bisphosphonate contain nitrogen) used for treatment osteoporosis, because it inhibits the prenylation and finally they inhibit osteoclasts that responsible for bone resorption (Maritz *et al*., 2001; Ahmadi *et al*., 2020).

Osteocalcin levels in ovariectomized rats in this study were elevated and continue to elevate after 30 and 60 days. This means ovariectomy caused elevation in osteocalcin, this agrees with the previous study of Saul *et al*., (2021) also agree with El-Nabarawi *et al*., (2017) and Morgan *et al*., (2021) who observed an increase in osteocalcin levels after ovariectomy due to estrogen deficiency.

After 30 days of atorvastatin treatment anon-significant reduction in osteocalcin was observed also no difference was observed the the treatment with rosuvastatin after 30 days. But treatment with rosuvastatin decreased the level of osteocalcin significantly compared with ovx group and with group treated atorvastatin after 60 days, although atorvastatin after 60 days of treatment could not reduce the elevation of osteocalcin that seen in ovx rats. This result refers to the effectiveness of rosuvastatin to improve bone formation more than atorvastatin. Rosuvastatin could lower bone turnover, because its ability to reduce the elevation of serum NTx and osteocalcin that seen in the ovx rats. Osteocalcin is a non-collagenous present in bone matrix, it is synthesized by osteoblast cells

and it is a marker of bone turnover, because it is produced from newly synthesized osteocalcin released from bone formation and osteocalcin released from matrix during resorption, therefore osteocalcin considered a marker of bone turnover (Gaw *et al*., 2013a). In study reported by Monjo *et al*., (2010) rosuvastatin could stimulate the expression and secretion of BMP-2, since this protein has important role in mediating action of statins in bone.

El-Nabarawi *et al*., (2017) showed, that atorvastatin not only has a positive effect on markers of bone formation, but also has ability to reduce markers of bone resorption, this means atorvastatin has double effects, anabolic and anti-resorptive. Some studies have shown that lipophilic statins had better effect than hydrophilic statin, the results in this study did not agree with the studies of Hatzigeorgiou and Jackson, (2005) and Jadhav and Jain, (2006) they showed that lipophilic statins as simvastatin had better activity than hydrophilic statins. The results of El-Nabarawi *et al*., (2017) showed that atorvastatin administration to ovx rats produce improvement in metabolic markers of bone.

Ovariectomized rats in this study showed a significant reduction in serum $1,25(OH)_2D3$. Ovariectomy results in estrogen deficiency that known to decrease $1,25(OH)_2D3$ (Nagareddy and Lakshmana, 2006). The reduction in serum $1,25(OH)_2D3$ of ovx rats is in agreement with result of Muhammad *et al*., (2020) who noticed a decrease in serum $1,25(OH)_2D3$ in ovx group. Vitamin D is considered a pro-hormon, which is necessary for calcium homeostasis. Vitamin D stimulates calcium and phosphorus absorption from intestine, therefore mineralized of the skeleton. In addition, $1,25(OH)_2D3$ with the parathyroid hormone stimulate mobilization of calcium from bone in order to prevent hypocalcemia (Duchow *et al*., 2021). Treatment of the ovx after 30 days with

atorvastatin also treatment of the ovx rats with rosuvastatin could not change the level of $1,25(OH)_2D3$ in the ovx rats. This means the time period was not enough to affect the level of vitamin D, but after 60 days of treatment with rosuvastatin leads to a significant elevation in serum $1,25(OH)_2D3$ compared with the ovx group, this result agrees with results of Yavuz *et al*., (2009) who reported that rosuvastatin increased the levels of 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D in study preformed on 91 patients with hyperlipidemia. However, treatment with 20mg/kg of atorvastatin leads to non-significant elevation, so rosuvastatin treatment showed a greater efficiency than atorvastatin. These results indicate the efficiency and excellence of rosuvastatin in bone formation compared with the atorvastatin. This role may be due to its structure, also atorvastatin is lipophilic that has low bioavailability.

The results of Torrubia *et al*., (2020) showed that treatment with vitamin D and calcium for long time didn't affect the bone mineral density, so the use of rosuvastatin may be useful for the density of bone as shown in this study.

Some researchers have shown a valuable role of statins on levels of $1,25(OH)_2D3$, but other studies have not. Anagnostis *et al*., (2014) were showed that rosuvastatin and atorvastatin could not reveal a significant influence in serum $1,25(OH)_2D3$ level. But in a study on patients with hyperlipidemia, they were given 10 mg/kg rosuvastatin a significant increase in $1,25(OH)_2D3$ was observed (Sathyapalan *et al*., 2013).

In fact, the accurate mechanism of statins effect on $1,25(OH)_2D3$ is not understood, but some studies have suggested that 7-hydrocholesterol is the precursor of vitamin D and cholesterol, the inhibition of HMG-CoA reductase leads to an elevation in 7-hydrocholesterol levels, this may offer an substrate for 25-hydroxy vitamin synthesis (Orces *et al*., 2020).

The results after 30 days of treatment referred, that there is no differences between all groups in serum calcium and phosphorus, however after 60 day of treatment, a significant elevation in serum calcium and phosphorus was observed in ovx rat. These results agree with Ohlsson *et al*., (2014) and Yousefzadeh *et al*., (2020) they showed that calcium and phosphorus were elevated in serum after ovariectomy. This refers to the bone turnover due to estrogen deficiency, which causes a reduce in $1,25(OH)_2D3$ that leads to decrease in calcium absorption by intestine, the decline in serum calcium stimulate parathyroid hormone PTH secretion, that causes releasing of calcium and phosphorus from the skeleton to normalize serum calcium and phosphorus levels, this leads to excretion calcium and phosphorus in the urine and then loss of bone. So, calcium levels in serum depend on the level of estrogen deficiency (Nagareddy and Lakshmana, 2006). In this study, the treatment with 20mg/kg rosuvastatin significantly decreased the elevation in serum calcium that happened by ovariectomy but not returned to the sham level, this agree with Gokdemir *et al*., (2021), while the same statin could not affect phosphorus level. Calcium and phosphorus have important role in many functions of the body, therefore their regulations in plasma done by the action of resorption/excretion in the kidney with absorption by the intestine and exchange from bone that considered the reservoir for calcium and phosphorus (Nagareddy and Lakshmana, 2006; El-Nabarawi *et al*., 2017). The result indicates to a high level of calcium in the atorvastatin treatment group after 60 day that similar to its level in ovx group. Ipekci *et al*., (2014) were noticed a condition of hypercalcemia in patients that using atorvastatin, and they suggested that atorvastatin caused a high level of calcium in serum. In addition, serum calcium elevate significantly in rats that treated with atorvastatin (Kawane *et al*., 2004). Treatment with atorvastatin could not affect phosphorus level, but

treatment with rosuvastatin non-significantly reduced the level of phosphorus level compared with ovx group.

The ovariectomy operation in this study caused a significant reduction in bone ash weights after approximately 90 days of operation, this results indicate decreased bone density due to lack of estrogen, This study was confirmed of results found by Nagareddy and Lakshmana, (2006) who showed a significant decrease in weight of bone ash in ovx rats and they mentioned that the reason for this decrease was increased bone turnover also loss of bone due to elevation in bone resorption. The finding in this study about bone ash weight can be reinforced by the reduction in the percentage of calcium, phosphorus and magnesium. Therefore, the decreasing in calcium, phosphorus and magnesium in ash of ovx rats lead to reduction of bone ash weight. Ovariectomy leads to accumulation of ROS that lead to this reduction in bone ash, increased production of cytokines like IL-1 beta and IL-6, these interleukins causes the generation of osteoclast and then bone loss (Mustafa *et al*., 2018).

However, after 60 days of treatment with atorvastatin a non-significant elevation in weight of bone ash was noticed when compared with the ovx rats, but treatment with rosuvastatin caused a significant elevation in bone ash weight compared with ovx rats. These results indicated to effectiveness role of rosuvastatin to improve bone formation and decrease bone resorption compared with atorvastatin due to the ability of rosuvastatin to increase weight of bone ash until reach its level in sham group. This result may be attributed to stimulate calcium and phosphorus to be accumulated in bone and then increasing bone density.

Ovariectomy leads to a reduction in the calcium, phosphorus and magnesium percentage in bone ash. Calcium and phosphorus are utilized as markers for bone formation, they have a critical role in bone

calcification (Choi and Seo, 2013) and calcium is associated with decrease mass of bone and causing osteoporosis. Calcium is important element for bone formation, and more than 99% of calcium found in bone and teeth (Mao *et al*., 2021).

The reduction in calcium, phosphorus and magnesium percentage in bone ash of ovx rats was in accordance with findings of Nagareddy and Lakshmana, (2006), who reported a reduction in bone ash weight, percentage ash, calcium, phosphorus and magnesium in the ovx rats, also the results of this study were in agreement with Mustafa *et al*., (2018), who pointed to a significant reduction in femur ash weight and decrease calcium and phosphorus in bone ash of ovx rats. The decrease of calcium and phosphorus in bone ash may be due to the low level of vitamin D in ovx rats, because vitamin D enhance the absorption of calcium and phosphorus that essential for building of bones.

The results of this study refer to improve in percentage of calcium and phosphorus in bone ash after treatment with atorvastatin and rosuvastatin, and the two types of statin that used in this study were similar in their action compared with the ovx group. These results showed the effectiveness of statins in stimulating bone formation. Findings of this study showed a significant elevation in percentage of calcium, phosphorus and magnesium content of bone ash in ovx rats that treated with rosuvastatin, this indicates the positive effect of rosuvastatin on bone formation and decrease bone resorption and these results enhanced by the elevation in the thickness of trabecular bone after treatment with rosuvastatin. The results in this study also refer to high level of serum calcium in the group that treated with atorvastatin and in group that treated with rosuvastatin. These results indicate that statins may be elevated serum calcium level.

The histological findings of this study showed a reduction in trabecular bone thickness and low number of osteoblasts, in addition to high number of osteoclasts in ovx group, these results were in agreement with results of Zhang *et al*., (2003) and El-Nabarawi *et al*., (2017) who noticed a significantly decrease 60% in trabecular bone volume after ovariectomy, also there was increment of osteoclastogenesis. The result of Lane *et al*., (2003) showed a 50% reduction in trabecular bone also in the connectivity of trabecular bone that result from osteoporosis. This means ovariectomy causes bone loss. Also, the reduction of trabecular bone thickness in this study agrees with Torrubia *et al*., (2020).

Estrogen deficiency increases the number of osteoclasts and decreases the number of osteoblasts resulting in overall bone resorption. Estrogen binds with estrogen receptor to promote osteoproteogerin (OPG) and to suppress the action of nuclear factor-KB ligand RANKL, thus inhibiting osteoclast formation (Cheng *et al*., 2022).

Treatment with atorvastatin leads to a medium developed in osteogenic tissue and increasing in the thickness of trabecular bone, however the treatment with rosuvastatin showed a better improvement in trabecular thickness of bone than atorvastatin although this improvement not significantly elevated from group that treated atorvastatin.

Osteoblasts, osteocytes and osteoclasts all express estrogen receptors. In addition, estrogen effects bone directly through cytokines and local growth factors. Estrogen inhibits IL-6 secretion and IL-6 contributes to the recruitment of osteoclasts, thus contributing of osteoporosis (Alrowaili *et al*., 2021).

The receptor activator of nuclear factor-kappa B ligand RANKL / receptor avtivator of nuclear factor-kappa B (RANK) osteoprotegerin

(OPG) system is the final common pathway for bone resorption. RANKL binds to RANK expressed by osteoclasts and osteoclasts precursors to promote osteoclast differentiation. OPG is a soluble decoy receptor that inhibits RANK-RANKL by binding and sequestering RANKL (Tanaka, 2019; Cheng *et al*., 2022).

Chapter Six

Conclusions and Recommendations

Conclusions:

1. The two types of statins lipophilic (atorvastatin) and hydrophilic (rosuvastatin) ameliorated the osteoporosis changing that developed by ovariectomy.

2. Rosuvastatin has a greater effect than atorvastatin in bone metabolism.

3. Rosuvastatin has a double mode of action anabolic and antiresorpative influence on bone.

4. Rosuvastatin has a good effect on the level of vitamin D and this property enhances the role of rosuvastatin in bone formation.

5. The possibility of using Rosuvastatin to reduce the symptoms of osteoporosis in addition to lowering cholesterol in the blood.

Recommendations:

1. Exploration the effect of another type of statins on bone metabolism.

2. Estimation of estrogen, parathyroid hormones, and hormones of thyroid gland and its relation with treatment with statins.

3. Screening the effect of statins in level of insulin, glucose and HbA1c.

References:

Abraham, D., and Abraham, J. (2018). Calcium Homeostasis and Hormonal Regulation. In: Bishop, M. L., Fody, E. P., Schoeff, L. E. (eds.) Clinical Chemistry. 8th edition. Philadelphia: Wolters Kluwer, pp. 1228-1265.

Ahmadi, M., Amiri, S., Pecic, S., Machaj, F., Rosik, J., Łos, M. J., Alizadeh, J., Mahdian, R., da Silva Rosa, S. C., Schaafsma, D., Shojaei, S., Madrakian, T., Zeki, A. A., and Ghavami, S. (2020). Pleiotropic effects of statins: A focus on cancer. Biochim Biophys Acta Mol Basis Dis, 1866(12): 165968. DOI: 10.1016/j.bbadis.2020.165968

Al-Azzawi F. (2013). The clinical choice between estrogen and bisphosphonates to prevent bone loss: are women being misled?. Womens Health (Lond), 9(3): 221–224. DOI: 10.2217/whe.13.16

Alenghat, F. J., and Davis, A. M. (2019). Management of Blood Cholesterol. JAMA, 321(8): 800–801. DOI: 10.1001/jama.2019.0015

Al-Hashemi, H. M. (2007). Effect of Magnesium Deficiency on Bone Metabolism in Female rats. MSc Dissertation: College of Veterinary Medicine, University of Mosul, in partial fulfillment of the requirement for the degree of Master of Science in Veterinary Biochemistry 6th, November, 2007. Mosul- Iraq.

AL-Mamari, A. H. (2010). Effect of Strontium Chloride in some Biochemical Parameters in Normal and Ovariectomized Rats. MSc Dissertation: College of Veterinary Medicine, University

of Mosul, in partial fulfillment of the requirement for the degree of Master of Science in Veterinary Biochemistry 9th, June, 2010. Mosul – Iraq.

Alrowaili, M. G., Hussein, A. M., Eid, E. A., Serria, M. S., Abdellatif, H., and Sakr, H. F. (2021). Effect of Intermittent Fasting on Glucose Homeostasis and Bone Remodeling in Glucocorticoid-Induced Osteoporosis Rat Model. J Bone Metab, 28(4): 307–316. DOI: 10.11005/jbm.2021.28.4.307

An, T., Hao, J., Sun, S., Li, R., Yang, M., Cheng, G., and Zou, M. (2017). Efficacy of statins for osteoporosis: a systematic review and meta-analysis. Osteoporos   In, 28(1):   47–57.   DOI: 10.1007/s00198-016-3844-8

Anagnostis, P., Adamidou, F., Slavakis, A., Polyzos, S. A., Selalmatzidou, D., Panagiotou, A., Athyros, V. G., Karagiannis, A., and Kita, M. (2014). Comparative Effect of Atorvastatin and Rosuvastatin on 25-hydroxy-Vitamin D Levels in Non-diabetic Patients with Dyslipidaemia: A Prospective Randomized Open-label Pilot Study. Open cardiovasc   Med   J, 8,   55–60.   DOI: 10.2174/1874192401408010055

Anbinder, A. L., Prado, F., Prado, M., Balducci, I., and Rocha, R. F. (2007). The influence of ovariectomy, simvastatin and sodium alendronate on alveolar bone in rats. Braz Oral Res, 21(3): 247–252. DOI: 10.1590/s1806-83242007000300010

Antonenko, A., Leahy, A., Babenko, M., and Lyons, D. (2021). Low dose hydrophilic statins are the preferred agents for females at risk

of osteoporosis. Bone Rep, 16, 101152. DOI: 10.1016/j.bonr.2021.101152

Arnold, M., and Langhans, W. (2010). Effects of anesthesia and blood sampling techniques on plasma metabolites and corticosterone in the rat. Physiol Behav, 99(5): 592–598. Doi: 10.1016/j.physbeh.2010.01.021

Association of official analytical chemists (A.O.A.C). (2000). 7[th]edition. VII, USA.

Aydin S. (2015). A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides, 72, 4–15. Doi: 10.1016/j.peptides.2015.04.012

Bailey, S., Karsenty, G., Gundberg, C., and Vashishth, D. (2017). Osteocalcin and osteopontin influence bone morphology and mechanical properties. Ann N Y Acad Sci, 1409(1): 79–84. DOI: 10.1111/nyas.13470

Bellanti, F., Matteo, M., Rollo, T., De Rosario, F., Greco, P., Vendemiale, G., and Serviddio, G. (2013). Sex hormones modulate circulating antioxidant enzymes: Impact of estrogen therapy. Redox Biology, 1(2013): 340-346. Doi: 10.1016/j.redox.2013.05.003

Bhattacharyya, S., Bhattacharyya, K., and Maitra, A. (2012). Possible mechanisms of interaction between statins and vitamin D. QJM, 105(5): 487–491. DOI: 10.1093/qjmed/hcs001

Biga, M., Dawson, S., Harwell, A., Hopkins, R., Kaufmann, J., LeMaster, M., Matern, P., Graham, K. M., Quick, D., and Runyeon, J.

(2019). Bone Tissue and the skeletal System. In: Biga, M., Dawson, S., Harwell, A., Hopkins, r., Kaufmann, J. et al. (eds.). Anatomy and Physiology. 1th edition. USA. Oregon state university. Available from: https://openstax.org/details/books/anatomy-and-physiology. [Accessed 26 Sep 2019].

Bird, E. W., Weber, J., Cox, C. P., and Chen, T. C. (1961). Determination of calcium and magnesium in milk by E.D.T.A. titration. J Dai Sci, 44(6): 1036-1046. DOI: 10.3168/jds.S0022-0302(61)89854-8

Bonjour, J. P., Ammann, P., and Rizzoli, R. (1999). Importance of preclinical studies in the development of drugs for treatment of osteoporosis: a review related to the 1998 WHO guidelines. Osteoporosis Int, 9(5): 379–393. DOI: 10.1007/s001980050161

Borras, C., Gambini, J., Lopez-Grueso, R., Pallardo, F. V., and Vina, J. (2010). Direct antioxidant and protective effect of estradiol on isolated mitochondria. Biochim Biophys Acta, 1802(1): 205–211. DOI: 10.1016/j.bbadis.2009.09.007

Boskey, A. L., and Robey, P. G. (2019). The composition of bone. In: John, P., Bilezikian, M. D., Bouillon, R., Juliet, C. O., Douglas C., et al., (eds.). Primer on the metabolic bone diseases and disorders of mineral metabolism. 9th edition. USA, American Society for Bone and Mineral Research, pp. 84-92.

Chamani, S., Liberale, L., Mobasheri, L., Montecucco, F., Al-Rasadi, K., Jamialahmadi, T., and Sahebkar, A. (2021). The role of statins

in the differentiation and function of bone cells. Eur J Clin Invest, 51(7): e13534. DOI: 10.1111/eci.13534

Chatterjea, M. N., and Shinde, R. (2012). Hormones-chemistry, mechanism of action and metabolic role. In: Chatterjea, MN., Shinde, R. (eds.). Textbook of Medical Biochemistry. 8th edition. New Delhi: JAPEE, pp. 562-607

Cheng, C. H., Chen, L. R., and Chen, K. H. (2022). Osteoporosis Due to Hormone Imbalance: An Overview of the Effects of Estrogen Deficiency and Glucocorticoid Overuse on Bone Turnover. Int J Mol Sci, 23(3): 1376. DOI: 10.3390/ijms23031376

Choi, M. J., and Seo, J. N. (2013). Effect of taurine feeding on bone mineral density and bone markers in rats. Adv Exp Med Biol, 776, 51–58. DOI: 10.1007/978-1-4614-6093-0_6

Chou, T. J., Lu, C. W., Liao, C. C., Chiang, C. H., Huang, C. C., and Huang, K. C. (2022). Ovariectomy Interferes with Proteomes of Brown Adipose Tissue in Rats. Int J Med Sci, 19(3): 499–510. DOI: 10.7150/ijms.66996

Clarke B. (2008). Normal bone anatomy and physiology. Clin J Am Soc Nephrol, S131–S139. DOI: 10.2215/CJN.04151206

Climent, E., Benaiges, D., and Pedro-Botet, J. (2021). Hydrophilic or Lipophilic Statins?. Front Cardiovasc Med, 8, 687585. DOI: 10.3389/fcvm.2021.687585

Cruz, R., Pesce, G., Calasans-Maia, J., Moraschini, V., Calasans-Maia, M. D., and Granjeiro, J. M. (2020). Calcium Phosphate Carrying Simvastatin Enhances Bone Regeneration: A

Systematic Review. Braz Dent J, 31(2): 93–102. DOI: 10.1590/0103-6440202002971

Daneault, A., Prawitt, J., Fabien Soule, V., Coxam, V., and Wittrant, Y. (2017). Biological effect of hydrolyzed collagen on bone metabolism. Crit Rev Food Sci Nutr, 57(9): 1922–1937. DOI: 10.1080/10408398.2015.1038377

Dent, A., and Selvaratnam, R. (2022). Measuring magnesium - Physiological, clinical and analytical perspectives. Clin Biochem, S0009-9120(22)00099-6. Advance online publication. DOI: 10.1016/j.clinbiochem.2022.04.001

Drake, M. T., Clarke, B. L., and Khosla, S. (2008). Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clin Proc, 83(9): 1032–1045. DOI: 10.4065/83.9.1032

Duchow, E. G., Duchow, M. W., Plum, L. A., and DeLuca, H. F. (2021). Vitamin D binding protein greatly improves bioactivity but is not essential for orally administered vitamin D. Physiol Rep, 9(23): e15138. DOI: 10.14814/phy2.15138

El-Nabarawi, N., El-Wakd, M., and Salem, M. (2017). Atorvastatin, a double weapon in osteoporosis treatment: an experimental and clinical study. Drug Des Devel Ther, 11, 1383–1391. DOI: 10.2147/DDDT.S133020

Emmanuelle, N. E., Marie-Cecile, V., Florence, T., Jean-Francois, A., Francoise, L., Coralie, F., and Alexia, V. (2021). Critical Role of Estrogens on Bone Homeostasis in Both Male and Female: From Physiology to Medical Implications. Int J Mol Sci, 22(4): 1568. DOI: 10.3390/ijms22041568

Endo A. (2010). A historical perspective on the discovery of statins. Proc Jpn Acad Ser B Phys Bio Sci, 86(5): 484–493. DOI: 10.2183/pjab.86.484

Ertugrul, D. T., Yavuz, B., Cil, H., Ata, N., Akin, K. O., Kucukazman, M., Yalcin, A. A., Dal, K., Yavuz, B. B., and Tutal, E. (2011). Statin-D study: comparison of the influences of rosuvastatin and fluvastatin treatment on the levels of 25 hydroxyvitamin D. Cardiovasc Ther, 29(2): 146–152. DOI: 10.1111/j.1755-5922.2010.00141.x

Ferreira Junior, D. B., Pizziolo, V. R., Oliveira, T. T., Matta, S., Piccolo, M. S., and Queiroz, J. H. (2018). Biometric, histomorphometric, and biochemical profile in atorvastatin calcium treatment of female rats with dexamethasone-induced osteoporosis. Rev Bras Ortop, 53(5): 607–613. DOI: 10.1016/j.rboe.2018.07.007

Fitzner Toft, M., Petersen, M. H., Dragsted, N., and Hansen, A. K. (2006). The impact of different blood sampling methods on laboratory rats under different types of anaesthesia. Lab Anim, 40(3): 261–274. DOI: 10.1258/002367706777611433

Fong, C. W. (2014). Statins in therapy: understanding their hydrophilicity, lipophilicity, binding to 3-hydroxy-3-methylglutaryl-CoA reductase, ability to cross the blood brain barrier and metabolic stability based on electrostatic molecular orbital studies. Eur J Med Chem, 85, 661–674. DOI: 10.1016/j.ejmech.2014.08.037

Fonseca, H., Moreira-Gonçalves, D., Coriolano, H. J., and Duarte, J. A. (2014). Bone quality: the determinants of bone strength and fragility. Sports Med, 44(1): 37–53. DOI: 10.1007/s40279-013-0100-7

Garnero, P. (2015). The Role of Collagen Organization on the Properties of Bone. Calcif Tissue Int, 97(3): 229–240. DOI: 10.1007/s00223-015-9996-2

Gaw, A., Murphy, M. J., Srivastava, R., Cowan, R. A., and O´Reilly, D. S. J. (2013a). Bone disease, In: Gaw, A., Murphy, M. J., Srivastava, R., Cowan, R. A., and O´Reilly, D. S. J. (eds.). Clinical Biochemistry. 5th edition. British, Churchill livingstone, pp. 76-78.

Gaw, A., Murphy, M. J., Srivastava, R., Cowan, R. A., and O´Reilly, D. S. J. (2013b). Phosphate and magnesium, In: Gaw, A., Murphy, M. J., Srivastava, R., Cowan, R. A., and O´Reilly, D. S. J. (eds.). Clinical Biochemistry. 5th edition. British, Churchill livingstone, pp. 74-75.

Gokdemir, G. S., Gokdemir, M. T., Kayhan, H., Yokus, B., Tasdemir, E., Gul, C., and Baylan, M. (2021). The effects of rosuvastatin and pravastatin on bone metabolism in diabetic rats. Ann Med Res. 28(10): 1933–1938. DOI: 10.5455/annalsmedres.2020.11.1108

Grigoryan, A. V., Dimitrova, A. A., Kostov, K. G., Ruseva, A.L., Atanasova, M. A., Blazhev, A. B., and Betova, T. M. (2017). Serum concentrations of matrix metalloproteinase-p, -13 and timp-1 in an ovariectomized wistar rat model of osteoporosis. Journal of the Balkan Medical Union. 52(4): 391-396

Gulati, M. (2018). Gonadal Function. In: Bishop, M. L., Fody, E. P., and Schoeff, L. E. (eds.). Clinical Chemistry. 8th edition. Philadelphia: Wolters Kluwer, pp. 1157-1198

Hamdoon, A.A., Al-khashab, E.M., and Al-hashemi, H.M. (2020). Effect of saponin extract of Glycyrrihiza glabra in activity of hepatic enzymes and some biochemical parameters in serum of adults ovariectomized female rats. Iraqi J Vet Sci, 34(2): pp. 411-415. DOI: 10.33899/ijvs.2019.126127.1239

Hamilton, K. J., Hewitt, S. C., Arao, Y., and Korach, K. S. (2017). Estrogen Hormone Biology. Curr Top Dev Biol, 125, 109–146. DOI: 10.1016/bs.ctdb.2016.12.005

Hatzigeorgiou, C., and Jackson, J. L. (2005). Hydroxymethylglutaryl-coenzyme A reductase inhibitors and osteoporosis: a meta-analysis. Osteoporos Int, 16(8): 990–998. DOI: 10.1007/s00198-004-1793-0

Hlaing, T. T., and Compston, J. E. (2014). Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem, 51(Pt 2): 189–202. DOI: 10.1177/0004563213515190

Hong, W., Wei, Z., Qiu, Z., Li, Z., Fu, C., Ye, Z., and Xu, X. (2020). Atorvastatin promotes bone formation in aged apoE^{-/-} mice through the Sirt1-Runx2 axis. J Orthop Surg Res, 15(1): 303. DOI: 10.1186/s13018-020-01841-0

Hughes, A., Rogers, M. J., Idris, A. I., and Crockett, J. C. (2007). A comparison between the effects of hydrophobic and hydrophilic statins on osteoclast function in vitro and ovariectomy-induced bone loss in vivo. Calcif Tissue Int, 81(5): 403–413. DOI: 10.1007/s00223-007-9078-1

Ipekci, S. H., Baldane, S., Sozen, M., and Kebapcilar, L. (2014). Can atorvastatin calcium cause asymptomatic hypercalcemia?. Turk Kardiyol Dern Ars, 42(7): 662–666. DOI: 10.5543/tkda.2014.05995

Jadhav, S. B., and Jain, G. K. (2006). Statins and osteoporosis: new role for old drugs. J Pharm Pharmacol, 58(1): 3–18. DOI: 10.1211/jpp.58.1.0002

Ji, M. X., and Yu, Q. (2015). Primary osteoporosis in postmenopausal women. Chronic Dis Transl Med, 1(1): 9–13. DOI: 10.1016/j.cdtm.2015.02.006

Johnson-Davis, K. L. (2018). Enzymes. In: Bishop, M. L., Fody, E. P., and Schoeff, L. E. (eds.). Clinical Chemistry. 8th edition. Philadelphia: Wolters Kluwer, pp. 674-739.

Johnston, B. D., and Ward, W. E. (2015). The ovariectomized rat as a model for studying alveolar bone loss in postmenopausal women. Biomed Res Int, 2015, 635023. DOI: 10.1155/2015/635023

Kanterewicz, E., Peris, P., Puigoriol, E., Yanez, A., Rosique, P., Del Rio, L., and FRODOS Research Group. (2013). Distribution of serum βCTX in a population-based study of postmenopausal women taking into account different anti-osteoporotic therapies (the FRODOS Cohort). J Bone Miner Metab, 31(2): 231–239. DOI: 10.1007/s00774-012-0410-0

Karsenty, G. (2017). Update on the biology of osteocalcin. Endocr Pract, 23(10): 1270–1274. DOI: 10.4158/EP171966.RA

Kawane, T., Terashima, S., Kurahashi, I., Yanagawa, T., Yoshida, H., and Horiuchi, N. (2004). Atorvastatin enhances bone density in ovariectomized rats given 17beta-estradiol or human parathyroid hormone (1-34). Endocrine, 24(2): 121–129. DOI: 10.1385/ENDO:24:2:121

Kenkre, J. S., and Bassett, J. (2018). The bone remodelling cycle. Ann Clin Biochem, 55(3): 308–327. DOI: 10.1177/0004563218759371

Khajuria, D. K., Razdan, R., and Mahapatra, D. R. (2012). Description of a new method of ovariectomy in female rats. Rev Bras Reumatol, 52(3): 462–470.

Kluger, A. Y., Tecson, K. M., Sudhakaran, S., Zhang, J., and McCullough, P. A. (2019). Statin Update: Intolerance, Benefit, and Beyond. Hypert J, 5(1). DOI: 10.15713/ins.johtn.014

Konukoglu, D. (2019). Bone Markers. Int J Med Biochem, 2(2): 65-78. DOI: 10.14744/ijmb.2019.60362

Kostapanos, M. S., Milionis, H. J., and Elisaf, M. S. (2010). Rosuvastatin-associated adverse effects and drug-drug interactions in the clinical setting of dyslipidemia. Am J Cardiovasc Drugs, 10(1): 11–28. DOI: 10.2165/13168600-000000000-00000

Lane, N. E., Yao, W., Kinney, J. H., Modin, G., Balooch, M., and Wronski, T. J. (2003). Both hPTH(1-34) and bFGF increase trabecular bone mass in osteopenic rats but they have different effects on trabecular bone architecture. J Bone Miner Res, 18(12): 2105–2115. DOI: 10.1359/jbmr.2003.18.12.2105

Larsson, B., Sundh, D., Mellström, D., Axelsson, K. F., Nilsson, A. G., and Lorentzon, M. (2019). Association between Cortical Bone Microstructure and Statin Use in Older Women. J Clin Endocrinol Metab, 104(2): 250–257. DOI: 10.1210/jc.2018-02054

Lee, S. A., Lagos, L. V., Bedford, M. R., and Stein, H. H. (2021). Quantities of ash, Ca, and P in metacarpals, metatarsals, and tibia are better correlated with total body bone ash in growing pigs than ash, Ca, and P in other bones. J Anim Sci, 99(6): skab149. DOI: 10.1093/jas/skab149

Lee, Y. S., Kwak, M. K., Moon, S. A., Choi, Y. J., Baek, J. E., Park, S. Y., Kim, B. J., Lee, S. H., & Koh, J. M. (2020). Regulation of bone metabolism by megakaryocytes in a paracrine manner. Scientific reports, 10(1): 2277. DOI: 10.1038/s41598-020-59250-6

Levin, V. A., Jiang, X., and Kagan, R. (2018). Estrogen therapy for osteoporosis in the modern era. Osteoporos Int, 29(5): 1049–1055. DOI: 10.1007/s00198-018-4414-z

Luna, L. G. 1968. Manual of histologic staining methods of the armed forces institute of pathology 34th ed. McGraw-Hill book company, New York, pp. 67-70.

Mackie, E. J., Ahmed, Y. A., Tatarczuch, L., Chen, K. S., and Mirams, M. (2008). Endochondral ossification: how cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol, 40(1): 46–62. DOI: 10.1016/j.biocel.2007.06.009

Mancini, G. B., Tashakkor, A. Y., Baker, S., Bergeron, J., Fitchett, D., Frohlich, J., Genest, J., Gupta, M., Hegele, R. A., Ng, D. S.,

Pearson, G. J., and Pope, J. (2013). Diagnosis, prevention, and management of statin adverse effects and intolerance: Canadian Working Group Consensus update. Can J Cardiol, 29(12): 1553–1568. DOI: 10.1016/j.cjca.2013.09.023

Manolagas, S. C., O'Brien, C. A., and Almeida, M. (2013). The role of estrogen and androgen receptors in bone health and disease. Nat Rev Endocrinol, 9(12): 699–712. DOI: 10.1038/nrendo.2013.179

Mao, H., Wang, W., Shi, L., Chen, C., Han, C., Zhao, J., Zhuo, Q., Shen, S., Li, Y., and Huo, J. (2021). Metabolomics and physiological analysis of the effect of calcium supplements on reducing bone loss in ovariectomized rats by increasing estradiol levels. Nutr Metab (Lond), 18(1): 76. DOI: 10.1186/s12986-021-00602-y

Marini, F., Giusti, F., Iantomasi, T., Cioppi, F., and Brandi, M. L. (2022). Bone phenotypes in multiple endocrine neoplasia type 1: survey on the MEN1 Florentine database. Endocr connect, 11(5): e210456. DOI: 10.1530/EC-21-0456

Maritz, F. J., Conradie, M. M., Hulley, P. A., Gopal, R., and Hough, S. (2001). Effect of statins on bone mineral density and bone histomorphometry in rodents. Arterioscler Thromb Vasc Biol, 21(10): 1636–1641. DOI: 10.1161/hq1001.097781

Matsuoka, K., Park, K. A., Ito, M., Ikeda, K., and Takeshita, S. (2014). Osteoclast-derived complement component 3a stimulates osteoblast differentiation. J Bone Miner Res, 29(7): 1522–1530. DOI: 10.1002/jbmr.2187

Mazidi, M., Rezaie, P., Vatanparast, H., and Kengne, A. P. (2017). Effect of statins on serum vitamin D concentrations: a systematic

review and meta-analysis. Eur J Clin Invest, 47(1): 93–101. DOI: 10.1111/eci.12698

Mazidi, M., Rokni, H., Sahebkar, A. H., Mohammadi, A., Ghayour-Mobarhan, M., and Ferns, G. A. (2016). Simvastatin Treatment Does Not Affect Serum Vitamin D Concentrations in Patients with Dyslipidemia: A Randomized Double-blind Placebo-controlled Cross-over Trial. Int J Prev Med, 7, 80. DOI: 10.4103/2008-7802.183652

Mirkin, S., and Pickar, J. H. (2013). Management of osteoporosis and menopausal symptoms: focus on bazedoxifene/ conjugated estrogen combination. Int J Womens Health, 5, 465-475. DOI: 10.2147/IJWH.S39455

Mistler, J. M. (2018). Electrolets. In: Bishop, M. L., Fody, E. P., and Schoeff, L. E. (eds.). Clinical Chemistry. 8th edition. Philadelphia: Wolters Kluwer, pp. 864-940.

Mohamed, M. E., Keshak, W. A., Hosny, A., and Elsisi, H. F. (2021). Parathormone and Serum Calcium Response to Moderate Intensity Treadmill Exercise in Elderly: A Randomized Controlled Trial. Egy J Phys Ther, 8(1): 8-14.

Monjo, M., Rubert, M., Ellingsen, J. E., and Lyngstadaas, S. P. (2010). Rosuvastatin promotes osteoblast differentiation and regulates SLCO1A1 transporter gene expression in MC3T3-E1 cells. Cell Physiol Biochem, 26(4-5): 647–656. DOI: 10.1159/000322332

Morgan, E. N., Alsharidah, A. S., Mousa, A. M., and Edrees, H. M. (2021). Irisin Has a Protective Role against Osteoporosis in

Ovariectomized Rats. Biomed Res Int, 2021, 5570229. DOI: 10.1155/2021/5570229

Morgan, S., Poundarik, A. A., and Vashishth, D. (2015). Do Non-collagenous Proteins Affect Skeletal Mechanical Properties?. Calcif Tissue Int, 97(3): 281–291. DOI: 10.1007/s00223-015-0016-3

Morse, L. R., Coker, J., and Battaglino, R. A. (2018). Satins and bone health: a mini review. Actual osteol, 14(1): 31–35.

Mueller, A. M., Liakoni, E., Schneider, C., Burkard, T., Jick, S. S., Krähenbühl, S., Meier, C. R., and Spoendlin, J. (2021). The Risk of Muscular Events Among New Users of Hydrophilic and Lipophilic Statins: an Observational Cohort Study. J Gen intern Med, 36(9): 2639–2647. DOI: 10.1007/s11606-021-06651-6

Muhammad, M. H., Hussien, N. I., and Elwia, S. K. (2020). Vitamin D Replacement Mitigates Menopause-Associated Dyslipidaemia and Atherogenic Indices in Ovariectomized Rats; A Biochemical Study. Exp Clin Endocrinol Diabetes, 128(3): 144–151. DOI: 10.1055/a-0934-5666

Mustafa, R. A., Alfky, N. A., Hijazi, H. H., Header, E. A., and Azzeh, F. S. (2018). Biological effect of calcium and vitamin D dietary supplements against osteoporosis in ovariectomized rats. J Nutr Int Med, 20(1): 86-93. DOI: 10.23751/pn.v20i1.5223

Nagareddy, P. R., and Lakshmana, M. (2006). Withania somnifera improves bone calcification in calcium-deficient ovariectomized rats. J Pharm Pharmacol, 58, 513-519. DOI: 10.1211/jpp.58.4.0011

Naik, P. (2012). Mineral metabolism. In: Naik Pankaja. (ed.). Essential of Biochemistry. 1st edition. New Delhi: Jaypee Brothers, pp. 276-292.

Ohlsson, C., Engdahl, C., Fak, F., Andersson, A., Windahl, S. H., Farman, H. H., Moverare-Skrtic, S., Islander, U., and Sjogren, K. (2014). Probiotics protect mice from ovariectomy-induced cortical bone loss. PloS One, 9(3): e92368. DOI: 10.1371/journal.pone.0092368

Orces, C. H., Montalvan, M., and Tettamanti, D. (2020). The Effect of Statins on Serum Vitamin D Concentrations Among Older Adults. Cureus, 12(7): e8950. DOI: 10.7759/cureus.8950

Park C. Y. (2019). Vitamin D in the Prevention and Treatment of Osteoarthritis: From Clinical Interventions to Cellular Evidence. Nutrients, 11(2): 243. DOI: 10.3390/nu11020243

Pennisi, M., Di Bartolo, G., Malaguarnera, G., Bella, R., Lanza, G., and Malaguarnera, M. (2019). Vitamin D Serum Levels in Patients with Statin-Induced Musculoskeletal Pain. Dis Markers, 2019, 3549402. DOI: 10.1155/2019/3549402

Pickering, M. E., Bouvier, D., Puravet, A., Soubrier, M., Sapin, V., & Oris, C. (2022). Hypophosphatemia related to a neuro-endocrine tumor of the pancreas: A case report. Clin Biochem, 104, 62–65. DOI: 10.1016/j.clinbiochem.2022.03.006

Pikner, R. (2016). Bone Metabolism, In: Racek, J. and Rajdl, D. (eds.) Clinical Biochemistry. 4th edition. Prague, University of Charles Karolinum, pp. 237-261.

Pinal-Fernandez, I., Casal-Dominguez, M., and Mammen, A. L. (2018). Statins: pros and cons. Med Clin (Barc), 150(10): 398–402. DOI: 10.1016/j.medcli.2017.11.030

Project, L. H. (2017). Strong bone, weak bones and osteoporosis, In: Project, L. H. (ed.) The Boneporosis Book. 2th edition. Latina Health Project, pp. 140-160.

Razzoli, M., Frontini, A., Gurney, A., Mondini, E., Cubuk, C., Katz, L. S., Cero, C., Bolan, P. J., Dopazo, J., Vidal-Puig, A., Cinti, S., and Bartolomucci, A. (2015). Stress-induced activation of brown adipose tissue prevents obesity in conditions of low adaptive thermogenesis. Mol Metab, 5(1): 19–33. DOI: 10.1016/j.molmet.2015.10.005

Reinwald, S., and Burr, D. (2008). Review of nonprimate, large animal models for osteoporosis research. J Bone Miner Res, 23(9): 1353–1368. DOI: 10.1359/jbmr.080516

Rejnmark, L., Vestergaard, P., Heickendorff, L., and Mosekilde, L. (2010). Simvastatin does not affect vitamin d status, but low vitamin d levels are associated with dyslipidemia: results from a randomised, controlled trial. Int J Endocrinol, 2010, 957174. DOI: 10.1155/2010/957174

Ris te li, J., Winter, W. E., Kleerekoper, M., and Ris te li, L. (2015). Disorders of Bone and Mineral Metabolism. In: Burtis, C. A., and Bruns, D. E. (eds.). Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 7th edition. USA, Elsevier, pp. 741-768.

Romero Barco, C. M., Manrique Arija, S., and Rodriguez Perez, M. (2012). Biochemical markers in osteoporosis: usefulness in

clinical practice. Reumatol Clin, 8(3): 149–152. DOI: 10.1016/j.reuma.2011.05.010

Ruan, F., Zheng, Q., and Wang, J. (2012). Mechanisms of bone anabolism regulated by statins. Biosci Rep, 32(6): 511–519. DOI: 10.1042/BSR20110118

Salamanna, F., Maglio, M., Sartori, M., Tschon, M., and Fini, M. (2020). Platelet Features and Derivatives in Osteoporosis: A Rational and Systematic Review on the Best Evidence. Int J Mol Sci, 21(5): 1762. DOI: 10.3390/ijms21051762

Sathyapalan, T., Shepherd, J., Atkin, S. L., and Kilpatrick, E. S. (2013). The effect of atorvastatin and simvastatin on vitamin D, oxidative stress and inflammatory marker concentrations in patients with type 2 diabetes: a crossover study. Diabetes Obes Metab, 15(8): 767–769. DOI: 10.1111/dom.12074

Satyanarayana, U., and Chakrapani, U. (2013). Mineral metabolism, In: Satyanarayana, U and Chakrapani, U. (eds.) Biochemistry. 4th edition. India, Elsevier, Books and Allied (P) Ltd., pp. 403-424.

Saul, D., Hohl, F. E., Franz, M. K., Meyer, I., Taudien, S., Roch, P. J., Sehmisch, S., and Komrakova, M. (2021). Inhibition of Lipoxygenases Showed No Benefit for the Musculoskeletal System in Estrogen Deficient Rats. Front Endocrinol (Lausanne), 12, 706504. DOI: 10.3389/fendo.2021.706504

Schachter M. (2005). Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundam Clin Pharmacol, 19(1): 117–125. DOI: 10.1111/j.1472-8206.2004.00299.x

Shah, S. R., Werlang, C. A., Kasper, F. K., and Mikos, A. G. (2015). Novel applications of statins for bone regeneration. Natl Sci Rev, 2(1): 85–99. DOI: 10.1093/nsr/nwu028

Shahrezaee, M., Oryan, A., Bastami, F., Hosseinpour, S., Shahrezaee, M. H., and Kamali, A. (2018). Comparative impact of systemic delivery of atorvastatin, simvastatin, and lovastatin on bone mineral density of the ovariectomized rats. Endocrine, 60(1): 138–150. DOI: 10.1007/s12020-018-1531-6

Shahzamani, S., Jahandideh, A., Abedi, G., Akbarzadeh, A., Hesaraki, S., and Parsaei, P. (2021). Histopathological assessment of nano n-acetyl cysteine effect on postoperative adhesion in rats, Iraqi J Vet Sci, 35(3): 589-597. DOI: 10.33899/ijvs.2020.126857.1400

Sharma, R., Sharma, N. K., and Thungapathra, M. (2017). Resveratrol regulates body weight in healthy and ovariectomized rats. Nutr Metab (Lond), 14, 30. DOI: 10.1186/s12986-017-0183-5

Sheridan, A., Wheeler-Jones, C. P. D., and Gage, M. C. (2022). The Immunomodulatory Effects of Statins on Macrophages. Immuno, 2(2): 317-343. DOI: 10.3390/immuno2020021

Simons J. (2003). The $10 Billion Pill Hold the fries, please. Lipitor, the cholesterol-lowering drug, has become the bestselling pharmaceutical in history. Here's how Pfizer did it. Fortune Magazine, 147(1): 58–68.

Song L. (2017). Calcium and Bone Metabolism Indices. Adv Clin Chem, 82, 1–46. DOI: 10.1016/bs.acc.2017.06.005

Stock S. R. (2015). The Mineral-Collagen Interface in Bone. Calcif Tissue Int, 97(3): 262–280. DOI: 10.1007/s00223-015-9984-6

Tanaka, S. (2019). Molecular understanding of pharmacological treatment of osteoporosis. EFORT Open Rev, 4(4): 158-164. DOI: 10.1302/2058-5241-4-180018

Tonelli, M., Lloyd, A., Clement, F., Conly, J., Husereau, D., Hemmelgarn, B., Klarenbach, S., McAlister, F. A., Wiebe, N., Manns, B., and Alberta Kidney Disease Network. (2011). Efficacy of statins for primary prevention in people at low cardiovascular risk: a meta-analysis. CMAJ, 183(16): E1189–E1202. DOI: 10.1503/cmaj.101280

Torrubia B., Martin-Fernandez M., Rubert M., Gomez-Chinchón M., Sosa M, Diez-Perez, A., Visedo, P.R., and Piedrs, C. (2020). Effects of Calcium and Vitamin-D Supplementation on Bone Quality in an Ovariectomized Rat Model. J Surg 5: 1276. DOI: 10.29011/2575-9760.001276

Toth, P. P., and Banach, M. (2019). Statins: Then and Now. Methodist Debakey Cardiovasc J, 15(1): 23–31. DOI: 10.14797/mdcj-15-1-23

Varela, A., and Jolette, J. (2018). Bone Toolbox: Biomarkers, Imaging Tools, Biomechanics, and Histomorphometry. Toxicol Pathol, 46(5): 511–529. DOI: 10.1177/0192623318779565

Vasikaran, S., Eastell, R., Bruyere, O., Foldes, A. J., Garnero, P., et al. (2011). Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int, 22(2): 391–420. DOI: 10.1007/s00198-010-1501-1

Vasudevan, D. M., Sreekumari, S., and Vaidyanathan, k. (2011). Mineral Metabolism and Abnormalities. In: Vasudevan, D. M.,

Sreekumari, S., and Vaidyanathan, k. (eds.) Textbook of Biochemistry for Medical Students. 6th edition. New Delhi, Jaypee Brothers Medical Publishers, pp. 411-431.

Vimalraj S. (2020). Alkaline phosphatase: Structure, expression and its function in bone mineralization. Gene, 754, 144855. DOI: 10.1016/j.gene.2020.144855

Vlot, M. C., Den Heijer, M., De Jongh, R. T., Vervloet, M. G., Lems, W. F., De Jonge, R., Obermayer-Pietsch, B., and Heijboer, A. C. (2018). Clinical utility of bone markers in various diseases. Bone, 114, 215–225. DOI: 10.1016/j.bone.2018.06.011

Wang, B. X., Li, K. P., Yu, T., and Feng, H. Y. (2019). Rosuvastatin promotes osteogenic differentiation of mesenchymal stem cells in the rat model of osteoporosis by the Wnt/β-catenin signal. Eur Rev Med Pharmacol Sci, 23(22): 10161–10168. DOI: 10.26355/eurrev_201911_19586

Ward, N. C., Watts, G. F., and Eckel, R. H. (2019). Statin Toxicity. Circ Res, 124(2): 328–350. DOI: 10.1161/CIRCRESAHA.118.312782

Watanabe, A., Katsuhiro, M., Noriko, K., Uchiyama, H., Takayuki, A., Iwata, H., Anzai, R., and Nakae, D. (2019). Impact of dietary calcium and phosphorus levels on an ovariectomized Sprague-Dawley rat model of osteoporosis. Funda Toxicol Sci, 6(7): 253-258. DOI: 10.2131/fts.6.253

Wong, S. K., Ima-Nirwana, S., and Chin, K. Y. (2020). Can telomere length predict bone health? A review of current evidence. Bosn

J   Basic   Med   Sci, 20(4):   423–429.   DOI:
10.17305/bjbms.2020.4664

Yavuz, B., Ertugrul, D. T., Cil, H., Ata, N., Akin, K. O., Yalcin, A. A.,
Kucukazman, M., Dal, K., Hokkaomeroglu, M. S., Yavuz, B.
B., and Tutal, E. (2009). Increased levels of 25 hydroxyvitamin
D and 1,25-dihydroxyvitamin D after rosuvastatin treatment: a
novel   pleiotropic   effect   of   statins?. Cardiovasc   Drugs
Ther, 23(4): 295–299. DOI: 10.1007/s10557-009-6181-8

Yousefzadeh, N., Kashfi, K., Jeddi, S., and Ghasemi, A. (2020).
Ovariectomized   rat   model   of   osteoporosis: a   practical
guide. EXCLI J, 19, 89–107. DOI: 10.17179/excli2019-1990

Zaidi M. (2007). Skeletal   remodeling   in   health   and   disease. Nat
Med, 13(7): 791–801. DOI: 10.1038/nm1593

Zhang, L., Feng, X., and McDonald, J. M. (2003). The role of calmodulin
in   the   regulation   of
osteoclastogenesis. Endocrinology, 144(10): 4536–4543. DOI:
10.1210/en.2003-0147

الخلاصة

تم اجراء هذه الدراسة للتقصي عن تأثير نوعين من الستاتينات الاتورفاستاتين (محب للدهون) والروزفاستاتين (محب للماء) في بعض المؤشرات الحيوية لبناء العظم (انزيم الفوسفاتيز القاعدي ALP، 25,1 ثنائي هيدروكسي فيتامين D3، اوستيوكالسينOC، الكالسيوم CaوالفسفورP)، واحد مؤشرات ارتشاف العظم (NTx) في مصل دم اناث الجرذان البالغة المستأصلة المبايض. بالاضافة الى مستوى الكالسيوم، الفسفور والمغنسيوم في رماد عظم الفخذ الايمن كذلك تم إجراء الفحوصات النسجية في عظم الفخذ الايسر في هذه الجرذان. اربعون من اناث الجرذان البالغة تراوحت اعمارها (2.5-3 اشهر) و اوزانها (200-220 غم) تمت تربيتها تحت ظروف مسيطر عليها من درجة حرارة (22-25 °م) و (12 ساعة ظلام-12 ساعة ضوء) في بيت الحيوانات التابع لكلية الطب البيطري لجامعة الموصل. تم تقسيم الحيوانات الى أربعة مجاميع متساوية والتي تضمنت المجموعة الشاهدة sham، المجموعة المستأصلة المبايض والتي اخذت كنموذج لهشاشة العظام، المجموعة المستأصلة المبايض المعاملة ب 20 ملغم/كغم فموياً من الاتورفاستاتين والمجموعة المستأصلة المبايض المعاملة ب 20 ملغم/كغم فموياً من الروزفاستاتين ولمدة 60 يوم. تم سحب الدم بعد 30 و 60 يوم من المعاملة، تم جمع نماذج الدم من كل المجاميع وفصل مصل الدم لغرض الفحوصات الكيموحيوية، ونزع عظم الفخذ الايمن وتم تحويله الى رماد لغرض تقدير النسبة المئوية للكالسيوم، الفسفور والمغنسيوم ونزع عظم الفخذ الايسر لاجراء الفحوصات النسجية لكل مجموعة.

اشارت النتائج الى وجود ارتفاع معنوي في فعالية انزيم الفوسفاتيز القاعدي، الاوستيوكالسين و NTx، الكالسيوم، الفسفور و وزن الجسم مع وجود انخفاض معنوي في مستوى 1,25 ثنائي هيدروكسي فيتامين D3 في مصل الجرذان المستأصلة المبايض مقارنة مع المجموعة الشاهدة، لكن المعاملة ب 20 ملغم/كغم من الاتورفاستاتين ادت الى انخفاض معنوي في فعالية انزيم الفوسفاتيز القاعدي، الاوستيوكالسين و NTx، وارنفاع غير معنوي في مستوى 1,25 ثنائي هيدروكسي فيتامين D3 مقارنة مع المجموعة المستأصلة المبايض. في حين ادت المعاملة ب 20 ملغم/كغم من الروزفاستاتين الى انخفاض معنوي في كل من ALP, OC, NTx والكالسيوم مع وجود ارتفاع معنوي في مستوى 1,25 ثنائي هيدروكسي فيتامين D3 مقارنة مع المجموعة المستأصلة المبايض بعد 60 يوم من المعاملة.

كما أظهرت النتائج انخفاضا ملحوظا في وزن رماد العظم، النسبة المئوية لمحتوى الكالسيوم، الفسفور والمغنسيوم في رماد العظم للمجموعة المستأصلة المبايض مقارنة مع المجموعة الشاهدة، لكن المعاملة ب 20 ملغم/كغم من الروزفاستاتين ادت الى ارتفاع معنوي في وزن رماد العظم، النسبة المئوية للكالسيوم والفسفور في رماد العظم مقارنة مع المجموعة المستأصلة المبايض. كما ان المعاملة ب 20 ملغم/كغم من الاتورفاستاتين ادت الى زيادة محتوى الكالسيوم والفسفور في رماد العظم مقارنة مع المجموعة المستأصلة المبايض.

اظهرت النتائج النسجية كثافة منخفضة وعظم تربيقي رقيق، وقلة الاوعية الدموية، واعداد كبيرة من الخلايا الهادمة للعظم، مع انخفاض اعداد الخلايا البانية للعظم في مجموعة الجرذان المستأصلة المبايض مقارنة مع الجرذان الشاهدة، لكن معاملة الجرذان المستأصلة المبايض بالاتورفاستاتين ادى الى زيادة سمك العظم التربيقي وتطور متوسط لانسجة العظم، وكذلك عدد متوسط من الخلايا البانية للعظم والخلايا الناقضة للعظم مقارنة مع المجموعة المستأصلة المبايض. وتسببت معاملة الجرذان المستأصلة المبايض بالروزفاستاتين ارتفاع معنوي في سماكة العظام التربيقية، الانسجة العظمية متطورة بشكل جيد، وارتفاع اعداد الخلايا البانية للعظم وانخفاض اعداد الخلايا الناقضة للعظم.

من خلال نتائج الدراسة الحالية، نستنتج ان المعاملة بالستاتينات لها تأثير في منع ارتشاف العظام وتعزيز تكوين العظام في اناث الجرذان المستأصلة المبايض. كما ان الروزفاستاتين تأثيره في ايض العظام افضل من الاتورفاستاتين.

تأثير الأتورفاستاتين والروزفاستاتين في بعض المؤشرات الحيوية لهشاشة العظام المحدثة عن طريق استئصال المبايض في الجرذان

سمية سلطان صالح

رسالة ماجستير
الطب البيطري / الكيمياء الحياتية البيطرية

بإشراف
الأستاذ المساعد
الدكتورة الهام محمد الخشاب

تأثير الأتورفاستاتين والروزفاستاتين في بعض المؤشرات الحيوية لهشاشة العظام المحدثة عن طريق استئصال المبايض في الجرذان

رسالة تقدمت بها

سمية سلطان صالح

إلى

مجلس كلية الطب البيطري في جامعة الموصل
وهي جزء من متطلبات نيل شهادة الماجستير
في اختصاص الطب البيطري / الكيمياء الحياتية البيطرية

بإشراف
الأستاذ المساعد
الدكتورة الهام محمد الخشاب

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