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Influence of Statins and Fibrates Drugs on Bone Health and Regeneration

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Abstract

In the medical and dental field, the importance and need for the study of materials and drugs for use as bone grafts or regeneration in injured areas due to the presence of fractures, infections or tumors that cause extensive loss of bone tissue is observed. Bone is a specialized, vascularized and dynamic connective tissue that changes throughout the life of the organism. When injured, it has a unique ability to regenerate and repair without the presence of scars, but in some situations, due to the size of the defect, the bone tissue does not regenerate completely. Thus, due to its importance, there is a great development in therapeutic approaches for the treatment of bone defects through studies that include autografts, allografts and artificial materials used alone or in association with bone grafts. Pharmaceuticals composed of biomaterials and osteogenic active substances have been extensively studied because they provide potential for tissue regeneration and new strategies for the treatment of bone defects. Statins work as specific inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoAreductase). They represent efficient drugs in lowering cholesterol, as they reduce platelet aggregation and thrombus deposition; in addition, they promote angiogenesis, reduce the β-amyloid peptide related to Alzheimer's disease and suppress the activation of T lymphocytes. Furthermore, these substances have been used in the treatment of hypercholesterolemia and coronary artery disease. By inhibiting HMG-CoAreductase, statins not only inhibit cholesterol synthesis, but also exhibit several other beneficial pleiotropic effects. Therefore, there has been increasing interest in researching the effects of statins, including Simvastatin,

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on bone and osteometabolic diseases. However, statins in high doses cause inflammation in bone defects and inhibit osteoblastic differentiation, negatively contributing to bone repair. Thus, different types of studies with different concentrations of statins have been studied to positively or negatively correlate this drug with bone regeneration. In this review we will address the positive, negative or neutral effects of statins in relation to bone defects providing a comprehensive understanding of their application. Finally, we will discuss a variety of statin-based drugs and the ideal dose through a theoretical basis with preclinical, clinical and laboratory work in order to promote the repair of bone defects.

Keywords

Bone, Statins, Rosuvastatin, Sinvastatin, Fibrates, Fenofibrate, Bone Regeneration

1. Introduction

Dyslipidemia is a chronic disease that seriously affects human health. Many studies have shown that dyslipidemia could induce multiple complications and cause serious damage to human health [1] [2] [3].

It is a multifactorial clinical entity. Factors such as: Age, sex, sedentary lifestyle, smoking, alcoholism, diet rich in trans fats, saturated fats, and carbohydrates but low in proteins, minerals and vitamins, Diabetes, High Blood Pressure, family history, among others, intervene in its development [4].

Many of these risk factors can be controlled through the use of hygienic dietary measures, along with physical activity at an intensity that will depend on each patient [5]. However, in many cases, it will be necessary to add pharmacological treatment, due to the degree of the patient's condition and/or the patient's level of adherence to these measures [6] [7] [8]. **Table 1** summarizes the most common treatments currently applied for the treatment of these pathologies [9] [10] [11] [12]. There is some evidence that the treatments carried out with statins and fenofibrate could somehow interact with bone metabolism. In this review we decided to carry out a search for the most relevant works in this regard.

2. Statins

Statins revolutionized the treatment of hypercholesterolemia, and have been the most widely used hypolipidemic agents for more than 20 years, due to their efficacy in reducing blood cholesterol levels [13]. These drugs act by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA into mevalonic acid, the precursor of cholesterol [14] [15]. Inhibition of this enzyme reduces hepatic cholesterol synthesis, which increases the production of LDL receptors on the surface of hepatocytes, and adequate knowledge of its pharmacokinetics should always be

Table 1. The most common treatments currently applied for the treatment of dyslipidemia.

Statins: Rosuvastatin, Sinvastatin, Pravastatin, Lovastatin, Fluvastatin, Atorvastatin, Pitavastatin.	These drugs act by inhibiting the enzyme HMG-CoA reductase, thus reducing the synthesis of cholesterol in the liver. Through this effect, through a negative feedback mechanism, it increases the synthesis of LDL-cholesterol receptors, which increase the uptake of blood cholesterol by the liver, thereby decreasing its plasma concentration, favoring cardiovascular health.
Fibrates: Fenofibrate, Gemfibrozil, Bezafibrate	act as agonists of the PPAR-α receptor, thus decreasing the synthesis and export of TAG by the liver, in addition to increasing the production of HDL molecules.
Ezetimibe	It acts by inhibiting the NCP 1L1 enzyme, which is found at the level of intestinal vellosities (this enzyme allows the lipids to be incorporated into the micelles for later digestion and absorption). This leads to a decrease in the absorption of cholesterol from the diet. Through a negative feedback mechanism, at the liver level, it increases the synthesis of LDL-cholesterol receptors to increase the plasma uptake of the same.
PSK-9 Inhibitors: Alirocumab, Evolocumab	They are monoclonal antibodies that act by inhibiting PCSK-9, which is a mediator that acts by binding to LDL-receptors to lead them to cytoplasmic internalization and subsequent destruction. Thus, LDL-cholesterol receptors remain active in the plasma membrane.
Bile acid binders: Cholestyramine, Colestipol, Colesevelam	These drugs bind to bile acids at the level of intestinal lysis, in a non-resorbable manner, inhibiting the entero-hepatic circuit, thus increasing fecal excretion of cholesterol.
Other: Omega-3, Nicotinic acid, Phytosterols	They decrease the synthesis of LDL, LDL and TAG.

Siglas: HMG-coA Reductase: Hidroxi-metil-glutaril coenzime A reductase; LDL: Low density lipoprotein; PPAR-α: Peroxisome proliferator-activated receptor alpha; TAG: triacylglycerides; HDL: High density lipoprotein; PCSK-9: Proprotein Convertase Subtilisin Kexin 9; NCP 1L1: Niemann C – Pick Protein 1 L 1; VLDL: Very low density protein. Bibliografía: 1) Tenenbaum, A., & Fisman, E. Z. (2012). Fibrates are an essential part of modern anti-dyslipidemic arsenal: spotlight on atherogenic dyslipidemia and residual risk reduction. Cardiovascular diabetology, 11, 125. 2) Handelsman, Y., Jellinger, P. S., Guerin, C. K., Bloomgarden, Z. T., Brinton, E. A., Budoff, M. J., Davidson, M. H., Einhorn, D., Fazio, S., Fonseca, V. A., Garber, A. J., Grunberger, G., Krauss, R. M., Mechanick, J. I., Rosenblit, P. D., Smith, D. A., & Wyne, K. L. (2020). Consensus Statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the Management of Dyslipidemia and Prevention of Cardiovascular Disease Algorithm - 2020 Executive Summary. Endocrine practice: official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists, 26 (10), 1196-1224. 3) Brunton Laurence L., Lazo John S., Parker Keith L. "Goodman & Gilman. Las bases farmacológicas de la Terapéutica". Undécima Edición. McGraw Hill. 2006.

considered to avoid cytotoxic problems associated with inadequate intake [16]. The acute vasodilator effects of statins may account, at least in part, for their beneficial effects on cardiovascular diseases associated with impaired organ blood flow [17] (**Table 2**).

3. Fenofibrates

Fibrates are effective to lower hypertriglyceridemia and hypercholesterolemia. Fibrates are used in clinical practice for about 50 years due to their ability to substantially decrease triglyceride levels and increase HDL. They affect both triglyceride-rich and cholesterol-rich particles in different ways limiting substrate

Table 2. Featured articles related to statins and their effects on bone metabolism at an experimental and clinical level.

Work title	Type of study	Authors	Year	Place	Drug	Effect
Effect of pravastatin on frequency of fracture in the LIPID study: secondary analysis of a randomised controlled trial	Clinical Trial	Reid, I. R., Hague, W., Emberson, J.	2001	New Zealand	Pravastatin	Neutral
Statin given perorally to adult rats increases cancellous bone mass and compressive strength	Pre-Clinical Trial	IN., Dalstra, M., Andreassen, T. T.	2001	Denmark	Simvastatin	Positive
The effect of atorvastatin on markers of bone turnover in patients with type 2 diabetes	Clinical Trial	Braatvedt, G. D., Bagg, W., Gamble, G.	2004	New Zealand	Atorvastatin	Neutral
Simvastatin treatment partially prevents ovariectomy-induced bone loss while increasing cortical bone formation	Pre-Clinical Trial	Oxlund, H., & Andreassen, T. T	2004	Denmark	Simvastatin	Positive
Effects of Atorvastatin on Bone in Postmenopausal Women with Dyslipidemia: A Double-Blind, Placebo-Controlled, Dose-Ranging Trial	Clinical Trial	Bone, H. G., Kiel, D. P., Lindsay, R. S.	2007	EE.UU	Atorvastatin	Neutral
A Comparison between the Effects of Hydrophobic and Hydrophilic Statins on Osteoclast Function In Vitro and Ovariectomy-Induced Bone Loss In Vivo	Pre-Clinical Trial	Hughes, A., Rogers, M. J., Idris, A. I.	2007	U.K	Rosuvastatin, Pravastatin, Cerivastatin and Sinvastatin	Neutral
Statins attenuate polymethylmethacrylate-mediated monocyte activation	Pre-Clinical Trial *In-vitro*	Laing, A. J., Dillon, J. P., Mulhall, K. J.	2008	Ireland	Cerivastatin	Positive
Effects of statins vs. non-statin lipid-lowering therapy on bone formation and bone mineral density biomarkers in patients with hiperlipidemia	Clinical Trial	Chuengsamarn, S., Rattanamongkoulgul, S., Suwanwalaikorn, S.	2010	Thailand	Simvastatin, Gemfibrozil	Positive
Simvastatin promotes osteoblast viability and differentiation via Ras/Smad/Erk/BMP-2 signaling pathway	Pre-Clinical Trial	Chen, P. Y., Sun, J. S., Tsuang, Y. H.	2010	Taiwan	Simvastatin	Positive

Continued

Rosuvastatin Promotes Osteoblast Differentiation and Regulates SLCO1A1 Transporter Gene Expression in MC3T3-E1 Cells	Pre-Clinical Trial	Monjo, M., Rubert, M., Ellingsen, J. E.	2010	Spain	Rosuvastatin	Positive
Bisphosphonate- and statin-induced enhancement of OPG expression and inhibition of CD9, M-CSF, and RANKL expressions via inhibition of the Ras/MEK/ERK pathway and activation of p38MAPK in mouse bone marrow stromal cell line ST2	Pre-Clinical Trial	Tsubaki, M., Satou, T., Itoh, T.	2012	Japan		Positive
Rosuvastatin inhibits spontaneous and IL-1-induced interleukin-6 production from human cultured osteoblastic cells	Pre-Clinical Trial and Clinical Trial	Lazzerini, P. E., Capperucci, C., Spreafico, A.	2013	Italy	Rosuvastatin	Positive
Discontinuation of simvastatin leads to a rebound phenomenon and results in immediate peri-implant bone loss	Pre-Clinical Trial	Li, X., Wu, F., Zhang, Y.	2016	Japan, China	Simvastatin	Negative
The effect of atorvastatin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (HMG-CoA), on the prevention of osteoporosis in ovariectomized rabbits	Pre-Clinical Trial	Zhou, H., Xie, Y., Baloch, Z.	2016	China	Atorvastatin	Positive
Anti-inflammatory effect of rosuvastatin decreases alveolar bone loss in experimental periodontitis	Pre-Clinical Trial	Kırzıoğlu, F. Y., Tözüm Bulut, M., Doğan, B.	2016	Turkey	Rosuvastatin	Positive
1.2% Rosuvastatin versus 1.2% Atorvastatin Gel local drug delivery and re-delivery in treatment of intrabony defects in chronic periodontitis: A randomized placebo controlled clinical trial	Clinical Trial	Pradeep, A. R., Garg, V., Kanoriya, D.	2016	India	Rosuvastatin and Atorvastatin	Positive

Continued

Rosuvastatin 1.2 mg in Situ Gel Combined it 1:1 Mixture of autologous platelet-rich fibrin and porus-hydroxyappatite bone graft in surgical treatment of mandibular degree ii furcation defects: a randomized clinical control trial	Clinical Trial	Pradeep, A. R., Karvekar, S., Nagpal, K.	2016	India	Rosuvastatin	Positive
Rosuvastatin Regulates Odontoblast Differentiation by Suppressing NF-jB Activation in an Inflammatory Environment	Pre-Clinical Trial	Feng, X., Wang, C., Gu, Z.	2019	China	Rosuvastatin	Positive
Diagnosis of osteoporosis in statin-treated patients is dose-dependent	Clinical Trial	1) Leutner, M., 2) Matzhold, C., 3) Bellach, L.	2019	Austria	Lovastatin, Sinvastatin, Pravastatin and Rosuvastatin	Dosis-dependent could be positive or not
Atorvastatin promotes bone formation in aged apoE–/– mice through the Sirt1–Runx2 axis	Pre-Clinical Trial	Hong, W., Wei, Z., Qiu, Z.	2020	China	Atorvastatin	Positive
Comparison the effects of chitosan and hyaluronic acid-based thermally sensitive hydrogels containing rosuvastatin on human osteoblast-like MG-63 cells	Pre-Clinical Trial *In-vitro*	Akbari, V., Reza-zadeh, M., & Ebrahimi, Z.	2020	Iran	Rosuvastatin	Positive
Evaluation of the effects of locally applied rosuvastatin on bone formation in a three dimensional reconstruction rabbit xenograft model	Pre-Clinical Trial	Özer, T., Aktaş, A., Avağ, C.	2021	Turkey	Rosuvastatin	Positive
Peri-implant marginal bone loss and systemic statin use: A retrospective cohort pilot study	Clinical Trial	Bahrami-Hessari, B., & Jansson, L.	2022	Sweden		Negative
Comparative evaluation of autogenous bone graft and autologous platelet—Rich fibrin with and without 1.2 mg in situ rosuvastatin gel in the surgical treatment of intrabony defect in chronic periodontitis patients	Clinical Trial	Gautam, K., Ka-poor, A., Mathur, S.	2022	India	Rosuvastatin	Positive

availability for triglyceride synthesis in the liver; promoting the action of lipoprotein lipase; modulating low density lipoprotein (LDL) receptor/ligand interaction; and stimulating reverse cholesterol transport [18] [19] [20]. Fibrates activate PPARα, which binds to a peroxisome proliferator activated receptor (PPAR)α response element in conjunction with the retinoid X receptor (20 - 21). PPARs α, -γ and -β/δ are ligand-activated transcription factors and members of the superfamily of nuclear hormone receptors [21] (**Table 3**).

4. Interrelationship of Bone and Lipid Metabolism

There has long been evidence that fat and bone cells share a common progenitor in MSCs of the bone marrow [22] [23] [24]. Scientific literature offers a lot of work showing a relationship between osteoblasts and cholesterol metabolism [25] [26] [27] [28], and disorders of lipid metabolism could be related to bone metabolic disorders [29] [30] [31]. There is also in vivo evidence suggesting that lipids are involved in the development of osteoporosis [32], and that bone and lipid tissue could respond to mechanosensitive [33] [34] [35]. The skeleton is able to continually adapt to mechanical loading by adding new bone to withstand increased amounts of loading, and by removing bone in response to unloading or disuse [32]-[36].

Recently, high levels of total cholesterol and triacylglycerol were associated with a greater risk of osteoporosis in a cross-sectional study [36]. Bone fractures represent a deleterious consequence of osteoporosis, whose increasing incidence constitutes a major health and socio-economic problem that threatens to explode in the next decade worldwide [37]. In fact, approximately 40% of women and 13% of men over the age of 50 will suffer an osteoporotic fracture of the hip, wrist or spine [38].

There is not a single proposal that can be established as a universal strategy to improve the fracture healing, further than the standard care [39] but considering the above, this review updates the main current studies on the bone cells ant tissue impact of statins and fibrates, given that there is preliminary evidence that there would be some type of relationship, and that they are the most used lipid-lowering drugs.

5. Statins and Bone

About 20 years ago, it was demonstrated that statins increased bone density in mice after both subcutaneous administration over the calvaria and systemic oral dosing [40]. Thereafter, a great deal of work has been done trying to obtain information on the effects of these drugs on bone health and the potential prevention of fractures. One of the first papers published in a high impact journal, back in 2001, was about a clinical trial made in New Zealand: 9014 patients (17% women, median age 62 years) with ischemic heart disease were randomly assigned to either pravastatin 40 mg daily or placebo, and followed up for a mean of 6 years. In this study, they failed to show any evidence of a reduced frequency

Table 3. Featured articles related to fibrates and their effects on bone metabolism.

Work title	Type of study	Authors	Year	Place	Drug	Effect
PPAR agonists modulate human osteoclast formation and activity in vitro	*In vitro*	Chan, B.Y., Gartland, A., Wilson, P.J., Buckley, K.A., Dillon, J.P., Fraser, W.D., Gallagher, J.A.	2006	U.K	Fenofibrate	Neutral
The peroxisome proliferator activator receptor alpha/delta agonists linoleic acid and bezafibrate upregulate osteoblast differentiation and induce periosteal bone formation in vivo	*In-vitro* and *In-vivo*	Still, K., Grabowski, P., Mackie, I., Perry, M., Bishop, N.	2008	U.K	Fenofibrate	Positive
Bezafibrate prevents palmitate-induced apoptosis in osteoblastic MC3T3-E1 cells through the NF-κB signaling pathway	*In-vitro*	Zhong, X., Xiu, L., Wei, G., Pan, T., Liu, Y., Su, L., Li, Y., Xiao, H.	2011	China	Bezafibrate	Positive
Bezafibrate enhances proliferation and differentiation of osteoblastic MC3T3-E1 cells via AMPK and eNOS activation	*In-vitro*	Zhong, X., Xiu, L.L., Wei, G.H., Liu, Y.Y., Su, L., Cao, X.P., Li, Y.B., Xiao, H.P.	2011	China	Bezafibrate	Positive
The peroxisome proliferator-activated receptor (PPAR) alpha agonist fenofibrate maintains bone mass, while the PPAR gamma agonist pioglitazone exaggerates bone loss, in ovariectomized rats	*In-vitro*	Stunes, A.K., Westbroek, I., Gustafsson, B.I., Fossmark, R., Waarsing, J.H., Eriksen, E.F., Petzold, C., Reseland, J.E., Syversen, U.	2011	Norway	Fenofibrate	Negative
Combination treatment with pioglitazone and fenofibrate attenuates pioglitazone-mediated acceleration of bone loss in ovariectomized rats	*In-vitro*	Samadfam, R., Awori, M., Bénardeau, A., Bauss, F., Sebokova, E., Wright, M., Smith, S.Y.	2012	Canada	Fenofibrate	Positive
PPAR agonists stimulate adipogenesis at the expense of osteoblast differentiation while inhibiting osteoclast formation and activity	*In-vitro*	Jessal, J. P., Oliver, R. B., Timothy, R. A.	2014	U.K.	Fenofibrate	Neutral
Effects of pioglitazone and fenofibrate co-administration on bone biomechanics and histomorphometry in ovariectomized rats	Pre-Clinical Trial	Susan, Y. S., Rana, S., Luc, C.	2015	Canada	Fenofibrate	Negative

Continued

Ovariectomized rats' femur treated with fibrates and statins. Assessment of pore-size distribution by ^{1}H-NMR relaxometry	*In-vitro*	Şipoş, R.S., Fechete, R., Chelcea, R.I., Moldovan, D., Pap, Z., Pávai, Z., Demco, D.E.	2015	Romania	Fenofibrate	Neutral
The PPAR agonist fenofibrate improves the musculoskeletal effects of exercise in ovariectomized rats	*In vitro*	Mosti, M.P., Ericsson, M., Erben, R.G., Schüler, C., Syversen, U., Stunes, A.K.	2016	Norway	Fenofibrate	Positive
Fenofibrate decreases the bone quality by down regulating Runx2 in high-fat-diet induced Type 2 diabetes mellitus mouse model	*In vitro*	Shi, T., Lu, K., Shen, S., Tang, Q., Zhang, K., Zhu, X., Shi, Y., Liu, X., Teng, H., Li, C., Xue, B., Jiang, Q.	2017	China	Fenofibrate	Negative
Fenofibrate induces PPARα and BMP2 expression to stimulate osteoblast differentiation	*In vitro*	Kim, Y.H., Jang, W.G., Oh, S.H., Kim, J.W., Lee, M.N., Song, J.H., Yang, J.W., Zang, Y., Koh, J.T.	2019	Republic of Korea	Fenofibrate	Positive

of bone fracture in those patients treated with pravastatin [41].

Statins were shown to have a positive role in bone formation as they modulate inflammation and increase osteogenesis and angiogenesis [42]. Oxlund, H *et al*. investigating biomechanical aspects, concluded that statin given perorally to adult rats increased cancellous bone mass and increased cancellous bone compressive strength. The cancellous bone was found to possess normal biomechanical competence after the statin treatment [43]. This same author published an interesting work in a high-impact journal in 2004, on the effects of systemic treatment with simvastatin in ovariectomized rats (OVX). The study was very thorough, since it was carried out through dynamic histomorphometry studies, with tetracycline and calcein treatment. The cancellous bone volumes in the proximal tibia and vertebral body were reduced in OVX rats, but the reduction was less if they had received a chronic treatment of 3 months with simvastatin. Simvastatin did not affect the endocortical bone formation but concerning to cortical bone, the tibial diaphysis bending strength was increased by 8% and the periosteal bone formation rate at the mid-diaphysis increased by twofold in the OVX rats who received simvastatin, compared with the ovariectomized group that no received these drugs [44].

In 2007 it was conducted a prospective, randomized, double-blind, placebo-controlled, dose-ranging comparative clinical trial at 62 sites in the United States, with Clinically doses of atorvastatin that lower lipid levels in post-menopausal women with dyslipidemia. The authors concluded that these doses had no effect on bone mineral density or biochemical indices of bone metabolism in this study, suggesting that such oral agents are not useful in the prevention of osteo-

porosis [45]. They proposed that one of the causes of this apparent lack of impact of atorvastatin at the bone level is due to the small amount that reaches the bone, because they are mainly absorbed by the liver, very little amount reaches the general circulation and perhaps with the use of other routes of administration or higher oral doses, the bone impact may be greater [46].

Another interesting work was conducted [47] based on the idea that statins decrease the biosynthesis of cholesterol by inhibiting the synthesis of phenyl groups that would be important for membrane targeting of small GTPase proteins involved in osteoclast function [48]. Indeed, these investigators demonstrated in vitro that hydrophobic and hydrophilic statins can inhibit osteoclast function by preventing the prenylation of small GTPases. However, despite these findings, given the predominantly liver-specific targeting of orally administered statins [48] it appears unlikely that sufficient circulating levels of statin (particularly hydrophilic statins) would reach the bone microenvironment following oral administration of normal doses of statins to substantially affect bone remodeling in humans. Although these were results obtained from in vitro work, we should highlight the contributions in 2008 made by the group of Laing AJ *et al.* who concluded that the anti-inflammatory properties of statins may suppressed the osteoclast activation and osteolysis, because the pretreatment with cerivastatin significantly abrogates the production of inflammatory cytokines TNF-alpha and MCP-1 by human monocytes in response to polymethylmethacrylate particle activation, the authors propose that intervening at the upstream activation stage, subsequent osteoclast activation and osteolysis can be suppressed, playing a prophylactic role [49]. Starting in 2010, a succession of pre-clinical works, offered new favorable perspectives for the potential effect of statins on bone status. In a very interesting clinical work published in Bone, Chuengsamarn *et al.* [50] made a prospective randomized control trial study enrolling 212 hyperlipidemic patients with osteopenia during 2006-2008. All subjects were randomized to 2 groups treated or untreated with simvastatin, and they demonstrated that high dose of these lipophilic statin had a beneficial effect by increasing bone mineral density and could be additive use for prevention of bone loss in in hyperlipidemia patients. In the same year Cem P-Y *et al.* demonstrated that simvastatin can promote osteoblast viability and differentiation via membrane-bound Ras/Smad/Erk/BMP-2 pathway [51]. Another interesting work in 2010, demonstrate that rosuvastatin induced osteoblast differentiation, as measured by increased BMP-2 gene expression and secretion, and ALP activity in MC3T3-E1 osteoblast cells, without significantly affecting cell proliferation within the concentration range of 0.001 - 10 μM [52], and also regulates the expression of Slco1a1 which may constitute the transport system for these drug across the cell membrane in mature osteoblasts. two years later, Tsubakwt *et al.* had shown that statins enhanced osteoprogeterin expression, inhibited the expression of CD9, M-CSF, and RANKL through blocking the Ras/ERK pathway and activating p38MAPK in mouse bone marrow stromal cell line ST2 [53], providing molecular basis for

future clinical studies. The differentiation and activation of osteoclasts, specialized cells that degrade bone matrix, are decisively regulated by the paracrine system osteoprotegerin (OPG)-receptor activator of nuclear factor kB (RANKL). Osteoprotegerin is a soluble protein, similar to other members of the tumor necrosis factor superfamily, that acts as a decoy receptor for RANKL. Its biological activity counteracts the effects of RANKL by competing for the activation of the nuclear factor kB activating receptor and thus inhibits the differentiation and activation of osteoclasts and decreases bone resorption. Statins act on receptors that are not completely known (as can be seen in the graph), through which they generate synthesis of some nuclear mediators such as Sip1, Sp7/Osx, Runx2/Cbfa1/Sirt1 and these increase the synthesis of Osteoprotegerin and other proteins that promote bone formation [54].

Another interesting molecular study was driven by Lazzerini *et al*., with Osteoblasts from osteoarthritic patients, showing that rosuvastatin decreases IL-6 production by osteoblasts, thereby suggesting a possible inhibiting activity on osteoclast function in an indirect way [55], providing original data on the effect of this drug on osteoblasts with regard to the production of this cytokine. Although in 2016 a work in a mouse model warned about the brief and discontinued use of simvastatin [56], in that same years, many results were presented in reference to the beneficial effects of statins: is in the intensive study in ovariectomized rabbits, where it was observed that atorvastatin was observed to significantly increase the mechanical parameters such as maximum load, stiffness, and energy-absorbing capacity, and it improved the microarchitecture [57], the interesting results of Kırzıoğlu F *et al.* showing rosuvastatin treatment decreases alveolar bone loss in experimental periodontitis [58] or the interesting clinical works of the group of Pradeep *et al.* [57]-[61], studying the positive effect of rosuvastatin in patients with chronic periodontitis , or the in situ effect in a gel local drug delivery system as surgical treatment of mandibular class II furcation defects, in a randomized clinical control trial. These preliminary results to rosuvastatin effects were reinforced at the molecular level by the impactful work carried out in 2019 [61]. The authors examined the effects of rosuvastatin on human odontoblast differentiation and mineralized nodule formation by real-time polymerase chain reaction, western blot, alizarin red S staining, and alkaline phosphatase staining. The extent of anti-inflammation was determined by RT-PCR and analysis of the expression of tumor necrosis factor α, interleukin 1β (IL-1β), and IL-6. They conclude that rosuvastatin may enhance odontoblast differentiation capacity by inhibiting NF-kappaB signaling pathway.

In that same year, an Australian group showed the epidemiological results carried out in patients treated with different types of statins during the years 2006-2007 in Australia, concluding that statin treatments could be beneficial if performed in low doses but they would not be recommended for bone status in high doses [62]. In 2020, a very interesting work was carried out with apoE−/− mice, characterized by severe hyperlipidemia and spontaneous development of

atherosclerosis, treated with atorvastatin. The results indicate that atorvastatin could act as a bone anabolic agent to increase bone mass in aged apoE−/−, by regulating the Sirt1-Runx2 axis [63]. Given the advances generated in bone tissue engineering, promoting scaffolds as an in situ delivery system for certain drugs [64], we must highlight two recent works, in which the authors present evidence of the beneficial effect of rosuvastatin linked to a quitosan hydrogel [65] or when applied locally under a titanium barrier on an area to be repaired with bone grafts, increases new bone and total bone volume [66]. A work carried out in 2016 should be highlighted here, were the authors made a meta-analysis of randomized controlled trials to evaluate the effects of statins on bone mineral density (BMD) and fracture risk among adults with dyslipidemia [67]. They provided evidence that statins could be used to increase BMD other than decreasing fracture risk in participant. In 2021 it was made a retrospective cohort pilot study made in Sweden were the authors compared clinical parameters of peri-implantitis in human subjects exposed and non-exposed to systemic statins treatment. They concluded that peri-implant bone loss was significantly correlated to use of statin after compensation for age and se; A non-significant correlation between statin use and severity of peri-implantitis was found may partly be due to time on medication, doses or types of statins, because these were not variables in this study, as they were not consistently specified in patients' records and however, very few patients were considered in the study [67] [68].

A recently published work should also be considered pointing to a dose-dependent biphasic effect of simvastatin: combining mouse experiments with big data analysis of the Austrian population, the authors studied the association between high-dose simvastatin treatment and bone quality. They concluded that high-dose simvastatin reduces bone quality in obese male and ovariectomized female mice, suggesting that direct drug action accounts for the association between high dosage and increased risk of osteoporosis as observed in comparable human cohorts [69]. In parallel, other works have highlighted the beneficial effect of sub-gingival delivery of simvastatin and rosuvastatin for treatment of chronic periodontitis with diabetes mellitus [70]. On the other hand, recently prospective randomized split-mouth study was done to assess the efficacy of simvastatin in bone regeneration in extraction sockets of mandibular third molars using cone beam computed tomography (CBCT) at 6th post-operative month, and it was demonstrated a statistically significant beneficial difference in bone regeneration with simvastatin treatment [71].

Many effects of statins were seen in situ. It is important to consider its use for matrix-linked treatments in regenerative medicine. Summarizing the mechanisms that statins would use to stimulate bone formation: Stimulation of the activity of the TGF-β/Smad signaling pathway, which would lead to the inhibition of osteoblast apoptosis. By acting on the cholesterol synthesis pathway, specifically inhibiting the enzyme HMG-CoA reductase, in this way metabolites of the pathway such as Farnesyl-pp (with its derived proteins and Small-G proteins and

Ras family proteins) are reduced. And Geranylgeranyl-pp (with its derived proteins and Rho family proteins), the latter leads, on the one hand, to an anti-inflammatory effect, and to an increase in activity (through stimulation of protein RNA synthesis, detected by detection techniques). PCR) of Runx2, BMP-2 and PI3 kinase/PKB (both latter act through VEGF), which would generate stimulation of the synthesis of a series of proteins such as Alkaline Phosphatase, Osteoprotegerin, Osteocalcin, Osteopontin, Collagen 1, but would decrease the synthesis of Matrix-mineraloproteinases such as 1 and 13.

Acting on the ERK pathway (from which other pathways within the nucleus are stimulated such as E2F, favoring osteoblastic proliferation, M-tor (which inhibits Bim and stimulates Mift, from which Bcl-2 is produced and This way, osteoblastic survival is also stimulated), Sp1 (which stimulates Sp7/Osx) and Runx-2/Cbfα1/Sirt1, the latter would stimulate Osteocalcin, Osteopontin, Alkaline Phosphatase and Osteoprotegerin to produce bone formation and osteoblastic differentiation.

The Wnt pathway activates B-catenin, which is capable of stimulating a transcription factor at the nuclear level (TCF1) and thus favoring osteoblastic differentiation. Regarding the mechanisms that decrease osteoclastic activity, we found that statins generate an increase in the synthesis of Osteoprotegerin by Osteoblasts, Osteocytes and stromal cells, this protein binds to RANKL (produced by the same cells), acting as a decoy, and in this way prevents its union with its respective receiver. On the other hand, these drugs also stop the cascade triggered by RANK + RANKL (TRAF6-ROS-JNK-NFK-β-NFATC1), specifically with the inhibition of ROS and NFK-β.

Statins are also capable of increasing the number of estrogen receptors (the effects of estrogen are an increase in Osteoprotegerin and a decrease in the synthesis of RANKL).

6. Fibrates and Bone

In 2008 an interesting work brings results results about the interrelation between fibrates and bone pathways [72]: The authors had shown first that prostaglandin A2 (PGA2) increases osteoprogenitor number in a similar way to the known bone anabolic compound PGE2 and that PGA2 mediates its effects in tissues by binding and activating members of the peroxisome proliferator activator receptors family (PPARs). The PPARs are promiscuous receptors, and few specific agonists are known. PPAR binding assays show that PGA2 preferentially activates the PPAR ά/δ isoforms. These isoforms can also be activated with varying specificity by natural ligand such as bezafibrate (Bez). They therefore set out to test the hypothesis that bone anabolic effects similar to those engendered by PGA2 could be shown for Bez in an in vitro model of osteogenesis (with Bone marrow cells obtained from tibia rats; BMC) and in an intact male rat model. Interestingly, the authors note that BMSCs from Bez-treated rats produced greater numbers of osteoblastic colonies that expressed ALP, produced a colla-

genous matrix, and became calcified compared to BMSCs from vehicle-treated rats. The in vivo studies revelated that Bez induced a striking increase in periosteal bone formation, elevated serum osteocalcin and greater metaphyseal BMD as assessed by DXA, suggesting an increase in bone formation and suggesting these agents may have therapeutic potential in human skeletal disorders characterized by low bone mass such as osteoporosis. One year later Syversen U *et al*., reinforced the idea, studing fenofibrates effects on bone in intact female rats, namely resulting in significantly higher femoral BMD [73].

New evidence on this was provided by the work carried out by Zhong's group [74] who proposed that since it is known that saturated fatty acid (SFA) intake is negatively associated with bone mineral density, they made and interesting experiment in vitro, were they find that that bezafibrate inhibits palmitate-induced apoptosis in osteoblast via the NF-κB signaling pathway. Their results point to bezafibrate as a new strategy to attenuate bone loss associated with high fat diet beyond its lipid-lowering actions. The same group in the same year published another interesting result, demonstrating that bezafibrate stimulates proliferation and differentiation of MC3T3-E1 cells, mainly via a PPARβ-dependent mechanism [75]. They concluded that bezafibrate can induce the proliferation and differentiation of osteoblastic MC3T3-E1 cells and augments the expression of BMP-2 and Runx-2 in the cells. Adding to these results, those obtained by the group from Stunes AK *et al*., based on the idea that activation seems to have positive skeletal effects, should be note [76]: using ovariectomized rats with and without treatment with fenofibrates, PPARalpha agonist, they concluded that fenofibrate maintained BMD and bone architecture at sham levels in ovariectomized rats. It was the first evidence proving that the administration of fenofibrate reverses the loss of bone effects in an ovariectomized rat model. It means fenofibrate produced a preventive effect on bone in this rat model of osteoporosis, as femoral and whole-body BMC and BMD were maintained at the same levels as for sham-operated rats, and this group also exhibited significantly higher femoral BMC and BMD than ovariectomized controls. The authors proposed that if fenofibrate is currently used to treat hyperlipidemia, could be beneficial for the skeleton of menopause hyperlipidemic patients on treatment. These data were reinforced through work published by a group from Canada [77].

In 2015 a group investigated the effects of pioglitazone, a peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist that is an effective therapy for type 2 diabetes, but has been associated with increased risk for bone fracture, and fenofibrate on bone strength and bone histomorphometry parameters in osteogenic ovariectomized (OVX) rats [78]. Pioglitazone significantly reduced biomechanical strength at the lumbar spine and femoral neck compared with rats administered fenofibrate. Co-treatment with pioglitazone + fenofibrate had no significant effect on bone strength in comparison with OVX vehicle controls.

In 2015, an experimental study made in rats concluded that the positive or negative effects of treatments with simvastatin and fenofibrates are strongly de-

pendent on the duration of treatment [79]. The following year, a group of researchers carried out interesting work related to bone and biomechanics, based on the fact that the musculoskeletal effects of exercise are attenuated by estrogen deficiency [80]. They work in an interesting experimental model with ovariectomized rats that underwent eight weeks of high-intensity jump training, 3 days a week, for a total of 24 sessions. This was the first study who investigated the combined effect of a PPAR agonist and exercise on musculoskeletal properties. The study demonstrated that eight weeks of fenofibrate administration and jumping exercise, alone or in combination, improved femoral BMD in ovariectomized rats.

Despite what has been described so far, we must name the negative results that were obtained by Shi T *et al.* in 2017 [81], with a mouse diabetes type 2 model. T2DM mouse model was induced by high-fat-diet, and the mice were treated with fenofibrate (100 mg/kg) (DIO-FENO) or PBS (DIO-PBS) for 4 weeks. The authors concluded that the biomechanical properties of bones from DIO-FENO group were significantly lower than those in the control and DIO-PBS groups, the trabecular number was lower than those of the other groups, though the cortical porosity was decreased compared with that of DIO-PBS group because of the increase of apoptotic cells. The expression of osteocalcin and collagen I were decreased after treatment with fenofibrate in T2DM mice. The expression of Runx2 decreased after treated with high dose of fenofibrate. These results were totally contrary to what was obtained by other authors before and after that article but would require consideration.

In 2019 Kim *et al.* published that fenofibrate increased BMP2 expression by inducing direct binding of PPARα to the BMP2 promoter region. Taken together, they suggest that fenofibrate has a stimulatory effect on osteoblast differentiation via the elevation of PPARα levels and the PPARα-mediated BMP2 expression. Given that fenofibrate induced osteogenic differentiation was abolished by the knockdown of PPARα to the basal level, the authors surmise that the fenofibrate induced PPARa expression is pre-requite for osteogenic induction. It was suggested then that fenofibrate can promote bone health directly by stimulating the osteogenic differentiation of osteogenic precursor cells, supporting the idea of fenofibrate as a useful agent for controlling hypercholesterolemic patients with osteoporosis [82].

There are not yet enough studies at an epidemiological level that allow us to conclude about treatment with fenofibrates on the bone health of patients.

In summary: Through the PPAR family of receptors, they increase the synthesis of proteins such as BMP-2, ONC, ALP, type 2 collagen fibers and Runx-2. With the increase in the synthesis of the latter, the formation of other proteins such as Osteoprotegerin, Osteocalcin, and Osteopontin increases and the synthesis of metalloproteinases and RANK-L decreases [83]. There are other drugs that can promote bone formation, such as progestogens. According to only some studies, they could prevent osteoporosis in premenopausal or ovariectomized

women and in postmenopausal women, while in premenopausal women they cause bone loss that appears reversible when treatment is stopped. Diuretics such as Indapamide have been described as generating a decrease in bone resorption caused by the direct effect of this drug on the differentiation of osteoclasts. Thiazides: can attenuate bone loss due to their effect of increasing calcium reabsorption in the renal tubule. However, routine administration of thiazides is not recommended to prevent or treat osteoporosis, but a thiazide diuretic is a reasonable treatment of choice in patients with osteoporosis who have hypertension, edema, or nephrolithiasis. Loop diuretics have an opposite effect to thiazides on calcium excretion, since they inhibit calcium reabsorption in the loop of Henle, increasing urinary calcium losses. The list of drugs that have a negative impact on bone formation is quite extensive, among them we can mention: Heparins. Loop diuretics, chemotherapy drugs, anticonvulsants, oral anticoagulants, high-dose thyroid hormones, etc [84] [85] [86].

Camal Ruggieri *et al.* schematized different possible crucial signaling pathways

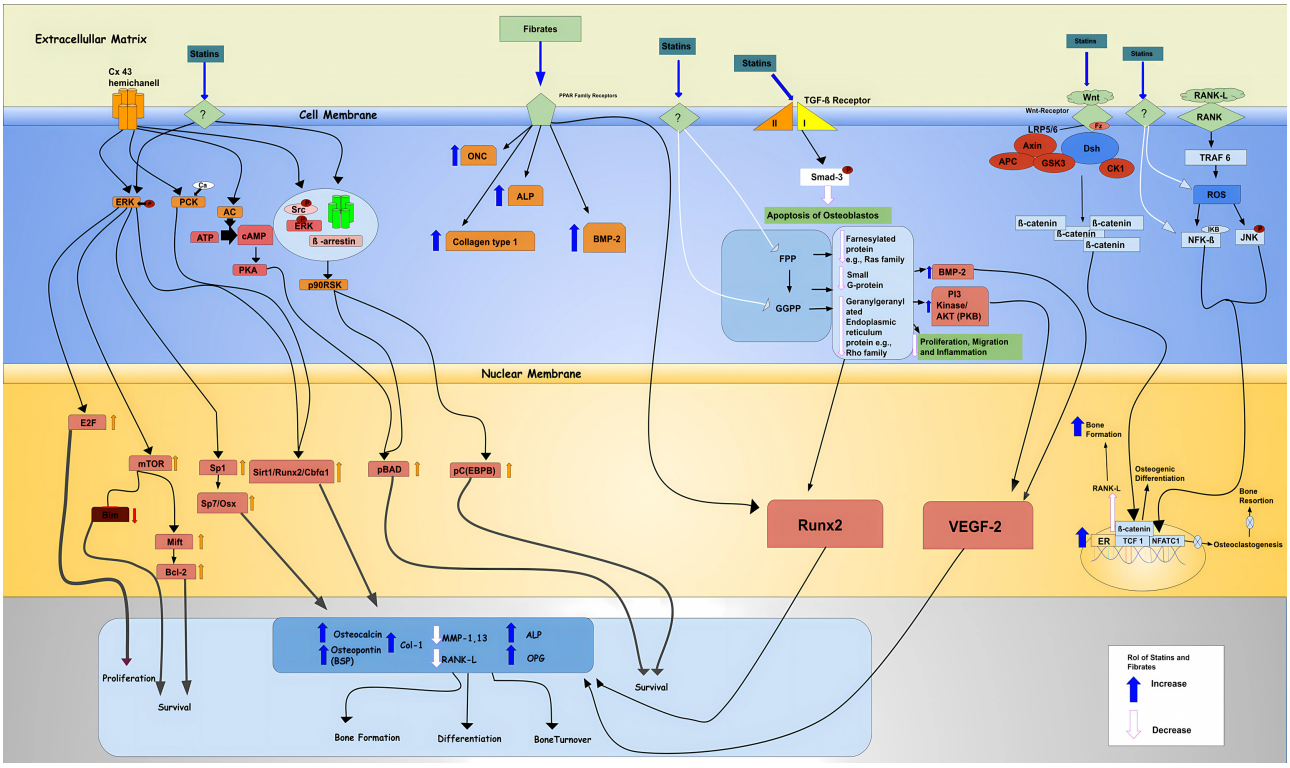

Figure 1. Schematic proposal of signaling pathways through which statins and fibrates could interact with bone metabolism. Rosuvastatin and simvastatin activates the well-known Ras/Smad/ERK pathway resulting in an overexpression of key transcription factors like VEGF-2, Sp7 and Runx2 with the subsequently expression of osteocalcin, osteopontina, collagen 1, ALP and OPG resulting in bone formation and osteoblastic differentiation. The activation of Ras/Smasd/ERK results also in an over expression of pBAD and pC generating higher cellular survival. Also due to activation of ERK signaling pathways the expression of key transcription factos like E2F, mTOR and Bcl2 increase the cellular proliferation and survival. On the other hand, the effects of fibrates are also well documented. Fibratos like fenofibrate and bezafibrates could activate due PPAR receptor the increase of intracellular concentration of PGE-2. Besides the activation of PPAR-receptors due to fenofibrate increase the concentration of BMP-2 with the posterior increasing on transcription of ALP, OPG, osteocalcin and Col1 resulting on bone formation, osteoblastic differentiation and bone turnover.

in relation to bone metabolism [87]. In order to compliment this schema of the signaling pathways, we proposed how the bone metabolism could be affected due to the pharmacodynamic effects of statins and fibrates according to the vast literature of the latest years (**Figure 1**). The effects of statins in the bone metabolism are well documented in the latest years and theses affect various crucial signaling pathway in bone. On one side statins like rosuvastatin and simvastatin activates the well-known Ras/Smad/ERK pathway resulting in an overexpression of key transcription factors like VEGF-2, Sp7 and Runx2 with the subsequently expression of osteocalcin, osteopontina, collagen 1, ALP and OPG resulting in bone formation and osteoblastic differentiation. The activation of Ras/Smasd/ERK results also in an over expression of pBAD and pC generating higher cellular survival. Also due to activation of ERK signaling pathways the expression of key transcription factos like E2F, mTOR and Bcl2 increase the cellular proliferation and survival. On the other hand, the effects of fibrates are also well documented. Vibratos like fenofibrate and bezafibrates could activate due PPAR receptor the increase of intracellular concentration of PGE-2. Besides the activation of PPAR-receptors due to fenofibrate increase the concentration of BMP-2 with the posterior increasing on transcription of ALP, OPG, osteocalcin and Col1 resulting on bone formation, osteoblastic differentiation and bone turnover [82].

However, the actual literature cannot answer all the questions regarding the whole understanding of the signaling pathways that involve the mechanism of statins and vibrates on bone metabolism. We propose in **Figure 1** a possible schematization according to the actual novelty literature.

7. Conclusion

It has been known since 1999 that statins also exert osteoanabolic properties, inhibiting osteoblast apoptosis and fostering osteoblast activity. This mechanism is mediated through increased expression of the BMP-2 gene, which promotes osteoblast differentiation and Statins may also promote embryonic stem cell differentiation towards the osteogenic lineage, through activation of increased mRNA expression of runt-related gene 2 (Runx2), osterix (OSX), and osteocalcin (OCN), as osteogenic transcription factors. While statins lower cholesterol by inhibiting cholesterol synthesis, fibrates decrease fatty acids and lower triglycerides by stimulating the peroxisomal b-oxidation pathway (80). The enhancing effects of statins on bone formation are associated with the increased expression of BMP2 via binding and activation of the gene promoter and fenofibrate acts as a PPAR*α* agonist to enhance its activity of transcriptional activator for BMP2 expression, but also it increases PPAR*α* expression via unknown mechanism yet. Although the detailed mechanism of how fenofibrate induces PPARa expression and BMP2 transcriptional activation is uncovered, we believe that fenofibrate will be a potent candidate for promoting bone formation besides statins.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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