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Intestinal dysbiosis and osteoporosis: The role of vitamin D and calcium metabolism

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Dedication

I dedicate this modest work

To my dearest father, Mohamed, who was my first teacher and who instilled in me a love of learning. Thank you for your support, your advice and all the sacrifices you made for my success.

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Résumé

L'ostéoporose est une maladie osseuse multifactorielle caractérisée par une diminution de la densité minérale osseuse et une augmentation du risque de fractures. Ce travail a pour objectif d'étudier la relation entre la dysbiose intestinale, le stress oxydatif et l'ostéoporose, ainsi que l'influence des habitudes alimentaires et de certains facteurs biologiques sur la santé osseuse. Notre étude a été réalisée auprès d'une population de la wilaya de Tlemcen à l'aide d'un questionnaire portant sur les données anthropométriques, les habitudes alimentaires, les symptômes digestifs et les paramètres liés au mode de vie. Les résultats obtenus montrent une fréquence importante de troubles digestifs, ainsi qu'une faible consommation de calcium et de vitamine D, de ferritine, du GSH, de la catalase et de l'IL-10. A l'inverse, l'augmentation de la MDA, des triglycérides et de la CRP suggère l'existence d'un état inflammatoire chronique contribuant à la dégradation de la microarchitecture osseuse et à l'aggravation de la fragilité osseuse. Ces observations suggèrent l'existence d'un lien étroit entre la dysbiose intestinale, l'inflammation, le stress oxydatif et le développement de l'ostéoporose. Ainsi, une prise en charge préventive intégrant l'équilibre nutritionnel et la santé intestinale apparaît essentielle dans la prévention de cette pathologie.

Mots-clés : Dysbiose intestinale, Malabsorption intestinal, Ostéoporose, Calcium, Vitamine D, Stress oxydatif.

Abstract

Osteoporosis is a multifactorial bone disease characterised by a decrease in bone mineral density and an increased risk of fractures. The aim of this study is to investigate the relationship between gut dysbiosis, oxidative stress and osteoporosis, as well as the influence of dietary habits and certain biological factors on bone health. Our study was conducted among a population in the wilaya of Tlemcen using a questionnaire covering anthropometric data, dietary habits, digestive symptoms and lifestyle-related parameters. The results show a high prevalence of digestive disorders, as well as low levels of calcium, vitamin D, ferritin, GSH, catalase and IL-10. Conversely, the increase in MDA, triglycerides and CRP suggests the presence of a chronic inflammatory state contributing to the degradation of bone microarchitecture and the worsening of bone fragility. These observations suggest a close link between gut dysbiosis, inflammation, oxidative stress and the development of osteoporosis. Thus, a preventive approach incorporating nutritional balance and gut health appears essential in the prevention of this condition.

Keywords : Intestinal dysbiosis, Intestinal malabsorption, Osteoporosis, Calcium, Vitamin D, Oxidative stress.

المخلص

هشاشة العظام هي مرض عظمي متعدد العوامل يتميز بانخفاض الكثافة المعدنية للعظام وزيادة خطر الإصابة بالكسور. يهدف هذا البحث إلى دراسة العلاقة بين اختلال التوازن البكتيري المعوي والإجهاد التأكسدي وهشاشة العظام، بالإضافة إلى تأثير العادات الغذائية وبعض العوامل البيولوجية على صحة العظام. أجريت دراستنا على عينة من سكان ولاية تلمسان باستخدام استبيان يتناول البيانات الأنثروبومترية والعادات الغذائية والأعراض الهضمية والمعايير المرتبطة بنمط الحياة. تظهر النتائج التي تم الحصول عليها ارتفاع معدل الإصابة بالاضطرابات الهضمية، بالإضافة إلى انخفاض مستويات الكالسيوم وفيتامين د والفيريتين والجلوتاثيون والكاتالاز و IL-10 وعلى العكس، يشير ارتفاع مستوى MDA والدهون الثلاثية و CRP إلى وجود حالة التهابية مزمنة تساهم في تدهور البنية المجهرية للعظام وتفاقم هشاشة العظام. تشير هذه الملاحظات إلى وجود صلة وثيقة بين خلل التوازن البكتيري المعوي والالتهاب والإجهاد التأكسدي وتطور هشاشة العظام. وبالتالي، يبدو أن الرعاية الوقائية التي تدمج التوازن الغذائي وصحة الأمعاء أمر ضروري للوقاية من هذه الحالة المرضية.

الكلمات المفتاحية: خلل التوازن البكتيري المعوي، سوء الامتصاص المعوي، هشاشة العظام، الكالسيوم، فيتامين د، الإجهاد التأكسدي

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Liste of abbreviations

SCFA: Short-Chain Fatty Acids

Treg: Regulatory T cells

Th17: T helper 17 cells

NF- κ B: Nuclear Factor Kappa B

GPR41: G Protein-Coupled Receptor 41

TNF- α : Tumor Necrosis Factor alpha

IL-1 β : Interleukin 1 beta

IL-6: Interleukin 6

IL-17: Interleukin 17

RANK: Receptor Activator of Nuclear Factor κ B

RANKL: Receptor Activator of Nuclear Factor κ B Ligand

GALT: Gut-Associated Lymphoid Tissue

ZO: Zonula Occludens

LPS: Lipopolysaccharides

TLR: Toll-Like Receptor

CD4+: Cluster of Differentiation 4

TGF- β : Transforming Growth Factor beta

OPG: Osteoprotegerin

DKK1: Dickkopf-1

Wnt: Wingless/Integrated

1, 25(OH) $_2$ D $_3$: 1, 25-dihydroxyvitamin D3

CT: Calcitonin

IGF-1: Insulin-like Growth Factor 1

PRL: Prolactin

FGF-23: Fibroblast Growth Factor 23

TRPV6: Transient Receptor Potential Vanilloid 6

VDR: Vitamin D Receptor

UV: Ultraviolet

RXR: Retinoid X Receptor

MAPK: Mitogen-Activated Protein Kinase

PAM: Peptidoglycan-Associated Molecular Pattern

CP: Calcium Phosphate

PRR: Pattern Recognition Receptors

NCX1: Sodium-Calcium Exchanger 1

WNT- β -catenin: WNT/ β -catenin signaling pathway

BMU: Basic Multicellular Unit

PTH: Parathyroid Hormone

VEGF: Vascular Endothelial Growth Factor

NFATc1: Nuclear Factor of Activated T-cells cytoplasmic 1

M-CSF: Macrophage Colony-Stimulating Factor

BMD: Bone Mineral Density

BMI: Body Mass Index

MDA: Malondialdehyde

GSH: Reduced Glutathione

CRP: C - reactive protein

TG: Triglycerides

P<0.05: Statistical significance threshold

INTRODUCTION

We often hear that the gut is our ‘second brain’, and the gut microbiota plays a significant role in this.

The gut microbiota represents a complex microbial ecosystem comprising all the microorganisms that colonise the gastrointestinal tract. It helps maintain the host’s homeostasis and is involved in numerous essential physiological functions, particularly metabolic and immune functions (**Gomaa, 2020 ; Matthias et al., 2024**).

Gut dysbiosis refers to an alteration in the composition, diversity or activity of the gut microbiota, leading to an imbalance between beneficial and potentially pathogenic microorganisms, which can disrupt the host’s homeostasis and contribute to the development of various pathologies (**Shen et al., 2025**).

This dysbiosis can disrupt immune balance and promote the production of pro-inflammatory mediators, leading to a state of chronic inflammation that may influence various physiological processes, notably bone metabolism (**Zhao et al., 2025**).

Vitamin D is a fat-soluble secosteroid essential for calcium-phosphorus metabolism, bone health and a growing number of extra-osseous processes. It is synthesised in the skin through exposure to ultraviolet B rays or via dietary intake (**Esteban et al., 2025**). This vitamin plays a key regulatory role in calcium and bone homeostasis. Although it has multiple effects on bone and calcium metabolism, it regulates the efficiency of intestinal calcium absorption (**Fleet, 2022**).

Calcium, an essential mineral in humans, influences bone mineralisation, muscle contraction, nerve transmission, blood clotting and hormone secretion. Found primarily in skeletal tissues, it is responsible for numerous physiological functions (**Amarnath et al., 2023**).

Calcium and vitamin D are essential for maintaining bone health; low calcium intake and vitamin D deficiency appear to be positively correlated with the prevalence of osteoporosis (**Voulgaridou et al., 2023**).

Osteoporosis is a systemic skeletal disorder of multifactorial origin, characterised by a decrease in bone mineral density, a decline in bone quality and microarchitectural deterioration of bone tissue, leading to significantly increased bone fragility and an elevated risk of pathological fractures (**Aibar-Almazán et al., 2022; Ensrud and Crandall, 2024**). It has become a critical global public health challenge that severely affects quality of life (**Black and Rosen, 2016; Brown, 2021**). According to

the International Osteoporosis Foundation, approximately 250 million people worldwide are affected **(Coughlan and Dockery, 2014; Subarajan et al., 2024)**.

This study aims to investigate the role of the gut microbiota in maintaining bone homeostasis and to analyse the impact of gut dysbiosis on the development of osteoporosis. It focuses in particular on the interactions between the gut microbiota, vitamin D metabolism and calcium absorption, as well as their influence on bone remodelling. Finally, this study aims to highlight the immunological and metabolic mechanisms involved, based on recent scientific data, in order to better understand the pathophysiological implications and potential diagnostic and therapeutic prospects.

CHAPTER 1

I. The intestinal microbiota

I.1. Definition of microbiota

The human intestine functions as an anaerobic bioreactor, housing a wide variety of micro-organisms, including bacteria, fungi, archaea, protozoa, viruses, and others, living in a given environment, especially at the level of the skin and the various mucous membranes of the human body, collectively called the microbiota or flora. These microorganisms are generally commensal, participate in the balance of the organism and occupy different ecological niches on the surface of the mucosa of the gastrointestinal tract. Thus, several microbiotas exist depending on the sites of the body, including intestinal, cutaneous, oral, vaginal or nasal. In this thesis, we will pay particular attention to the intestinal microbiota **(Caitríona et al., 2020)**.

I.2. The intestinal microbiota

The gut microbiota represents the most important and complex microbial community in the human organism. Its distribution shows heterogeneity, the highest concentrations being observed in the colon. Composed of 10¹⁴ bacteria and other microorganisms such as viruses, fungi and archaea, this set is recognized as a true hidden organ of the human body in relation to its various functions in intestinal physiology and the maintenance of the overall health of the host. Its composition varies from one individual to another, and it is constantly influenced by genetic and environmental factors of the host, which affect its functional profile **(Montalto et al., 2009; Landman & Quévrain, 2016; Shen et al., 2025)**.

I.3. Composition of the intestinal microbiota

The intestinal microbiota mainly consists of bacteria belonging to various phyla, such as phyla. Firmicutes and Bacteroidetes largely dominate the human gut microbiota, followed by Actinobacteria and Proteobacteria **(Arumugam et al., et al., 2011)**.

- Firmicutes: This group includes Gram-positive bacteria with a hard or semi-hard wall, mainly belonging to the genera *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus* and *Ruminococcus*. They play a key role in fiber fermentation and the creation of short-chain fatty acids (SCFAs) **(Seong et al., 2018)**.
- Bacteroidetes: this phylum includes nearly 7,000 distinct species of Gram-negative bacteria. These small microscopic beings, represented by *Bacteroides*, *Alistipes*, *Parabacteroides*, and *Prevotella*,

play a crucial role in the decomposition of polysaccharide complexes as well as in adjusting immune reactions (**Gibiino et al., 2018**).

- **Actinobacteria:** these are Gram-positive bacteria. This group includes *Bifidobacterium*, bacteria known for their beneficial effects on gut health, mainly through their ability to strengthen the intestinal barrier.

There are few, but they play a crucial role. The phylum in question includes the genus *Bifidobacterium*, which is crucial for the fermentation of carbohydrates and is often used as a probiotic (**Essaid et al., 2015**).

- **Proteobacteria:** Even though they represent only a small part of a healthy microbiota, species such as *Escherichia coli* and *Helicobacter* can turn into pathogens when a microbial imbalance occurs.

This group includes opportunistic or pathogenic bacteria, such as *Escherichia coli*. Normally, these bacteria are present in small amounts in a healthy individual. However, excessive growth of this phylum is generally indicative of dysbiosis (microbiota imbalance) (**Cheng, 2013**).

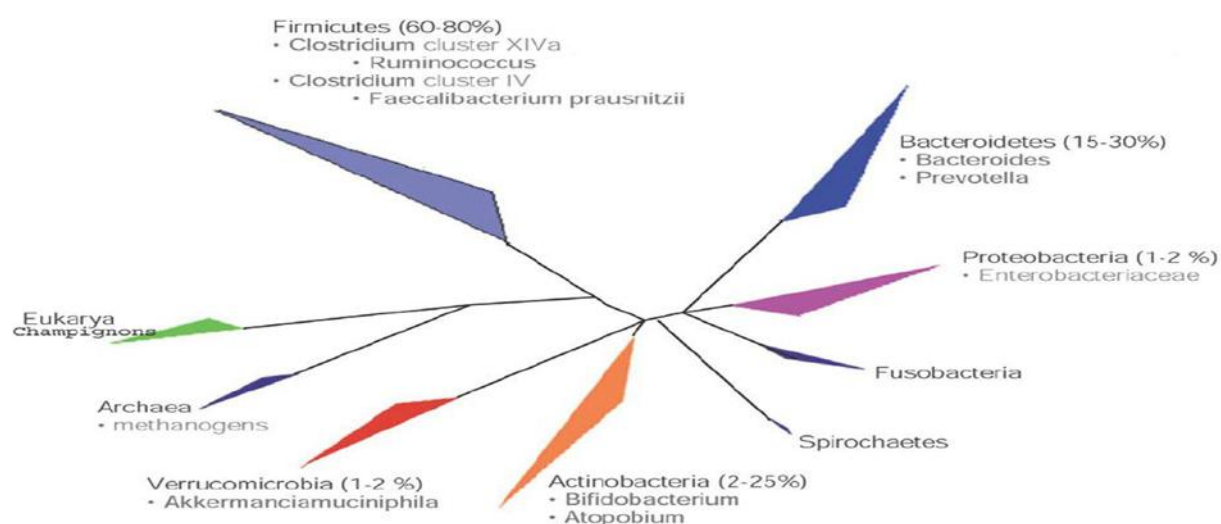


Figure 01. Schematic representation of the phylogenetic tree of bacteria residing in the colon showing the relative abundance of the majority phyla of the human intestinal microbiota (**Cheng et al., 2013**).

I.4. Factors influencing microbial balance

The balance of gut microbiota is influenced by several factors, including diet, drug use, age and environmental conditions. These factors may modulate the diversity and function of gut microbial

communities, contributing to either the maintenance of intestinal homeostasis or the development of dysbiosis **(Hasan and Yang, 2019)**.

I.4.1. Mode of delivery

The intestinal microbiota is influenced by fashion of childbirth. There is a difference in the composition of intestinal microbiota between babies born by caesarean section and those born naturally. From birth, naturally born babies are exposed to their mother's bacteria, which impacts their gut microbiota and promotes the generation of white blood cells as well as other immune system components **(Kulas et al., 2013)**.

Therefore, babies born by caesarean section have a higher risk of obesity and/or diabetes than those born naturally **(Kuhle et al., 2015)**.

Vaginal delivery promotes the early establishment of maternal bacteria such as *Lactobacillus*, *Bacteroides*, and *Prevotella*, while a caesarean section leads to an initial microbiota mainly dominated by cutaneous bacteria such as *Staphylococcus* and *Corynebacterium*, thus contributing to a less diversified microbiome.

Babies born by caesarean section show less initial microbial diversity and a delay in colonization by certain beneficial bacteria. This distinction has been linked to an increased risk of immuno-allergic diseases **(Dominguez et al., 2010)**.

The first thousand days of life represent a crucial phase for the development of the microbiota and the immune system **(Tamburini et al., 2016)**.

I.4.2. Breastfeeding

Feeding babies is another essential element for the development of gut microbiota, because breast milk is not sterile. Breast milk is considered a source of bacteria that normally live in our body and can be beneficial, which plays an important role in the development of good bacteria in the baby's gut **(Fitzstevens et al., 2016; Boix-Amorós, 2016)**.

It seems that streptococci and staphylococci are the types of bacteria most present in breast milk. These two types are also found in a lot of skin bacteria. So, breast milk can also have skin bacteria. However, *Weissella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus* and *Lactococcus* are the main bacteria found in baby colostrum. On the other hand, in milk collected at 1 and 6 months, a significant increase of *Veillonella*, *Leptotrichia* and *Prevotella* was observed **(Boix-Amorós et al., 2016)**.

The milk of obese moms has a different kind of bacteria and is less varied than that of moms who have a normal weight. This milk also has properties that increase inflammation more significantly **(Panagos et al., 2016)**.

In addition, the breast milk of mothers who had a caesarean section has a different bacterial composition from that of the milk of mothers who gave birth vaginally. The bacteria found in breast milk and on the mother's, skin are often the first micro-organisms to enter the baby's body. They could have an important role for his health **(Cabrera et al., 2012)**.

Breast milk is also an important source of IgA antibodies that protect against various germs. However, some baby formulas that contain probiotics have been proven to have a positive effect on the baby's immune system and gut microbiota.

I.4.3. Diet

Diet is very important for the proper functioning of microbiota. In fact, the way we feed has an impact on the composition of the groups of microbes in our intestines and on the creation of substances produced by these microbes, which can influence the functioning of our body. Dietary habits, such as nutrient balance and mealtimes, can change the balance of our gut by directly influencing the microbes that live in it **(Nova et al., 2022)**.

A high-fiber diet promotes the growth of good bacteria like Bifidobacterium and Lactobacillus, while a Western diet rich in fats and refined sugars can harm microbial diversity and cause an imbalance called dysbiosis **(Comtet et al., 2024)**.

Bifidobacterium and Lactobacillus are bacteria that produce short-chain fatty acids, such as butyrate. These acids help keep the intestinal barrier solid and control inflammation. On the other hand, eating a lot of saturated fats and refined sugar can cause an imbalance in bacteria in the gut. This imbalance is manifested by a reduction in the diversity of microbes and an increase in bacteria that cause inflammation **(Valdes et al., 2018)**.

I.4.4 Antibiotics

Antibiotics are commonly prescribed drugs that have saved millions of lives from infections also have a profound impact on the normal gut microbiota. This effect is fast and sometimes persistent. Broad-spectrum antibiotics reduce bacterial diversity while increasing the abundance of certain bacteria that can be used by opportunistic pathogens and decreasing the number of beneficial bacteria **(Sheetal et al., 2014)**.

The use of broad-spectrum antibiotics, such as clindamycin, in infants and young children has long-lasting effects on gut microbiota composition. Early exposure to antibiotics in newborns can lead to microbial dysbiosis, which could be a predisposing factor for inflammatory bowel disease (**Souradet et al., 2010**).

I.4.5. Stress

Chronic stress in adults can reduce microbial diversity and alter the production of short-chain fatty acids, affecting immune and metabolic function. In infants and children, prenatal maternal stress is associated with impaired intestinal colonization and an increased risk of allergies and neurobehavioral disorders (**Kelly et al., 2019; Liu et al., 2020**).

I.5. Metabolic and immune functions

The metabolic and immune functions of the intestinal microbiota are very important for maintaining the balance of the body.

I.5.1. Metabolic function of the intestinal microbiota

- Fermentation of dietary fiber: intestinal bacteria degrade indigestible fibers and produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (**Rowland et al., 2018**).
- Production of vitamins: certain intestinal bacteria participate in the synthesis of vitamins, notably vitamin K and certain vitamins of group B; and in the transformation of primary bile acids into secondary bile acids, thus influencing energy homeostasis and insulin sensitivity
- Nutrient metabolism: microbiota contributes to the digestion of certain complex carbohydrates and the absorption of nutrients.
- Regulation of energy metabolism: the metabolites produced by the microbiota can influence the lipid and carbohydrate metabolism of the host (**Rowland et al., 2018**).

I.5.2. Immune function of the intestinal microbiota

The intestinal microbiota also plays an important role in the development and control of the immune system.

- Stimulation of the immune system: the microbiota helps to develop the immune system, especially in the intestine (**Belkaid & Hand, 2014**).

- Barrier and protection role: The intestinal flora acts as a barrier to protect the host when there is an increase in the number of harmful or opportunistic bacteria. Bacteria can change the permeability of

the intestinal barrier by reducing the connections between epithelial cells, which is supposed to reduce the passage of certain substances into the blood **(Delzenne and Cani, 2008)**.

- **Modulation of immunity:** Substances produced by microbes, in particular short-chain fatty acids (SCFA) and tryptophan derivatives, influence how T lymphocytes develop (Treg/Th17), control cytokine production and support maintaining the balance between tolerance and inflammation. The microbiota plays an essential role in maintaining immune balance and in the development of metabolic, inflammatory, and autoimmune diseases **(Victor et al., 2025)**.

- **Regulation of inflammation:** The intestinal microbiota is crucial for managing inflammation, both locally and throughout the body. Acids Short-chain fats (SCFAs), such as butyrate, propionate, and acetate, which are derived from the fermentation of dietary fiber, have effects that reduce inflammation. They react sensitively to the activation of pathways that cause inflammation, such as NF-K b. These metabolites also stimulate receptors that are linked to G proteins (GPR41, GPR43), which help reduce the creation of pro-inflammatory cytokines such as TNF- α , IL-6 and IL-1 β **(Belkaid & Hand, 2014)**.

- **Protection against pathogens:** the microbiota prevents colonization by pathogenic microorganisms through a phenomenon called barrier effect.

I.6. The axis between the intestine and the bones and the role of the intestinal microbiota in bone metabolism

The intestinal microbiota plays a key role in the management of bone metabolism thanks to what is called the gut-bone axis. Metabolically, the microbes in our gut keep our bones healthy by helping us to absorb minerals like calcium, making substances called short-chain fatty acids, and controlling our immune system's reactions. These mechanisms can influence the functioning of osteoclasts and osteoblasts, which helps to maintain bone balance. On the other hand, if an imbalance occurs, it can lead to health problems like osteoporosis **(Pacifici, 2018)**. At the level of the immune system, microbiota influences the production of cytokines that play a role in changing bone structure, such as TNF- α , IL-6 and IL-17. Dysbiosis can increase mild inflammation throughout the body, promote activation of osteoclasts through the RANK/RANKL pathway and cause bone loss that accumulates. In addition, changes in the intestinal barrier can make the intestines more permeable and allow bacteria to move, which can lead to continuous immune system activation and bone loss **(Lingyun et al., 2021)**.

The intestinal microbiota seems to play an important role in maintaining the balance between bone formation and resorption. Therefore, it could be a target for new treatments in the prevention and treatment of osteoporosis and other bone diseases.

II.1. Gut dysbiosis and bone health

Gut dysbiosis is a clinical condition characterised by a qualitative and/or quantitative alteration in the gut microbiota, caused by endogenous factors related to the host (genetics, immunity, underlying diseases) and exogenous factors (diet, antibiotics, environment, etc.). It is associated with a variety of digestive and extra-digestive manifestations, the symptoms of which are often broad and non-specific (Ailim et al., 2025).

II.2. Pathophysiological mechanisms of gut dysbiosis

Gut dysbiosis is defined as a qualitative and quantitative imbalance in the gut microbiota, characterised by a reduction in microbial diversity and an alteration in beneficial commensal bacterial communities, often to the benefit of pathogenic or opportunistic species. This imbalance has been associated with a variety of pathophysiological processes involving the disruption of intestinal homeostasis, aberrant stimulation of the immune system and the onset of persistent systemic inflammation, with consequences for distant tissues, including bone tissue.

II.2.1. Alteration of the intestinal barrier

The intestinal barrier constitutes a dynamic interface between the intestinal lumen and the internal environment. It consists of a layer of mucus, a single-layered epithelium formed of enterocytes connected by tight junctions, and an underlying immune compartment (GALT). This structure serves a dual function: to enable the selective absorption of nutrients and to prevent the translocation of luminal microorganisms, toxins and antigens into the systemic circulation (Celebi et al., 2022).

The intestinal barrier consists of four layers: microbiological, chemical, physical and immunological. Under the influence of exogenous or endogenous factors, this barrier can be compromised or destroyed. This phenomenon is known as ‘leaky gut’ (Barbara et al., 2024).

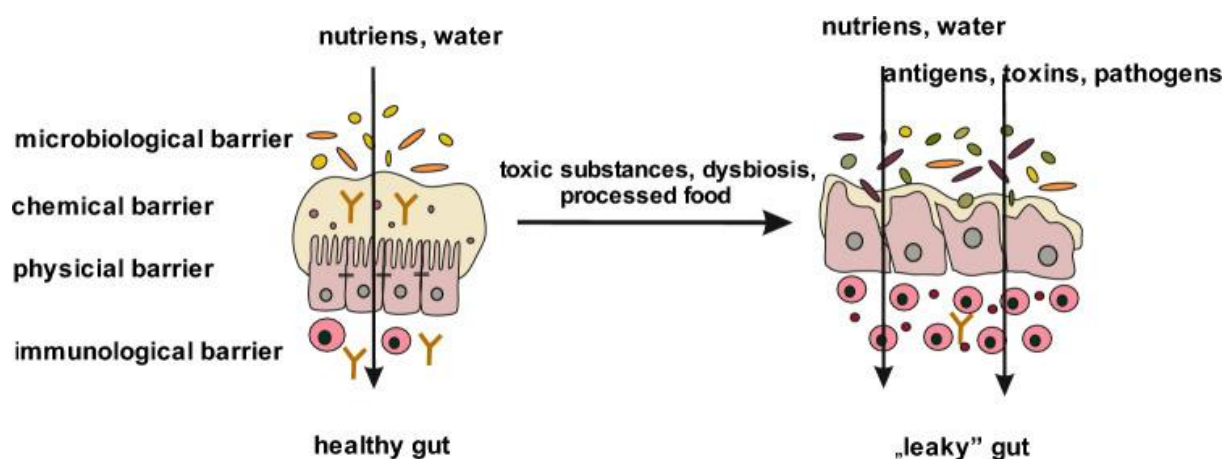


Figure 02. Intestinal barrier. The intestinal barrier consists of four layers: microbiological, chemical, physical and immunological. Under the influence of exogenous or endogenous factors, the intestinal barrier is compromised. This phenomenon is known as ‘leaky gut’ (Barbara et al., 2024).

The integrity of the intestinal barrier depends on tight junctions, including protein complexes such as claudins, occludin and ZO proteins. These structures control the permeability of the paracellular space via two distinct pathways: the ‘pore pathway’, which is highly selective, and the ‘leak pathway’, which is less selective and increased under inflammatory conditions. Any disruption to these mechanisms leads to increased intestinal permeability (Turner et al., 2009).

In a state of dysbiosis, the production of short-chain fatty acids (particularly butyrate) by commensal bacteria is reduced, leading to a decrease in the expression of tight junction proteins such as ZO-1 and occludin. This reduction in junction proteins increases intestinal permeability, promoting the translocation of lipopolysaccharide (LPS) into the systemic circulation and contributing to immune activation and chronic inflammation (Parada et al., 2019; Tilg et al., 2022).

This translocation stimulates innate immune receptors, particularly TLRs, leading to immune activation and increased production of pro-inflammatory cytokines (Clara et al., 2021).

II.2.2. LPS translocation and immune activation

Increased intestinal permeability facilitates the diffusion of bacterial compounds, notably lipopolysaccharide (LPS), into the circulatory system. LPS plays a key role as a ligand for Toll-like receptors (TLRs), particularly TLR4, which are present on innate immune cells. The NF- κ B signalling pathway is stimulated by the LPS-TLR4 interaction, leading to the overproduction of pro-inflammatory cytokines such as IL-6, TNF- α and IL-1 β . This low-grade systemic inflammatory

response, which persists over time, plays a key role in the chronic immune activation observed in states of intestinal dysbiosis and is associated with numerous metabolic and inflammatory conditions (Shen et al., 2025).

II.2.3. Th17/Treg immune imbalance

Chronic inflammation induced by intestinal dysbiosis promotes the polarisation of CD4⁺ T lymphocytes towards the Th17 phenotype at the expense of regulatory T lymphocytes (Tregs). The persistent presence of pro-inflammatory cytokines, notably IL-6 and IL-1 β , stimulates the differentiation of Th17 cells, which secrete IL-17, a key mediator of chronic inflammation. At the same time, the reduction in Tregs, which produce IL-10 and TGF- β , impairs immune regulatory mechanisms.

This Th17/Treg imbalance contributes to the maintenance of a persistent systemic inflammatory state and plays a central role in inflammatory conditions associated with dysbiosis (Yuyuan et al., 2025).

II.2.4. Role of the RANKL/OPG ratio in osteoclast activation and bone loss

The immune imbalance favouring Th17 cells amplifies the expression of RANKL (Receptor Activator of Nuclear Factor κ B Ligand) in stromal and osteoblastic cells. IL-17, produced by Th17 cells, directly stimulates RANKL production, whilst chronic inflammation reduces the expression of OPG (Osteoprotegerin), a soluble receptor acting as a natural antagonist of RANKL. An increase in the RANKL/OPG ratio promotes osteoclast differentiation and activation, leading to excessive bone resorption and a decrease in bone mineral density. This mechanism constitutes a fundamental axis of osteoimmunology and explains the link between chronic inflammation and bone loss (Pengcheng et al., 2022).

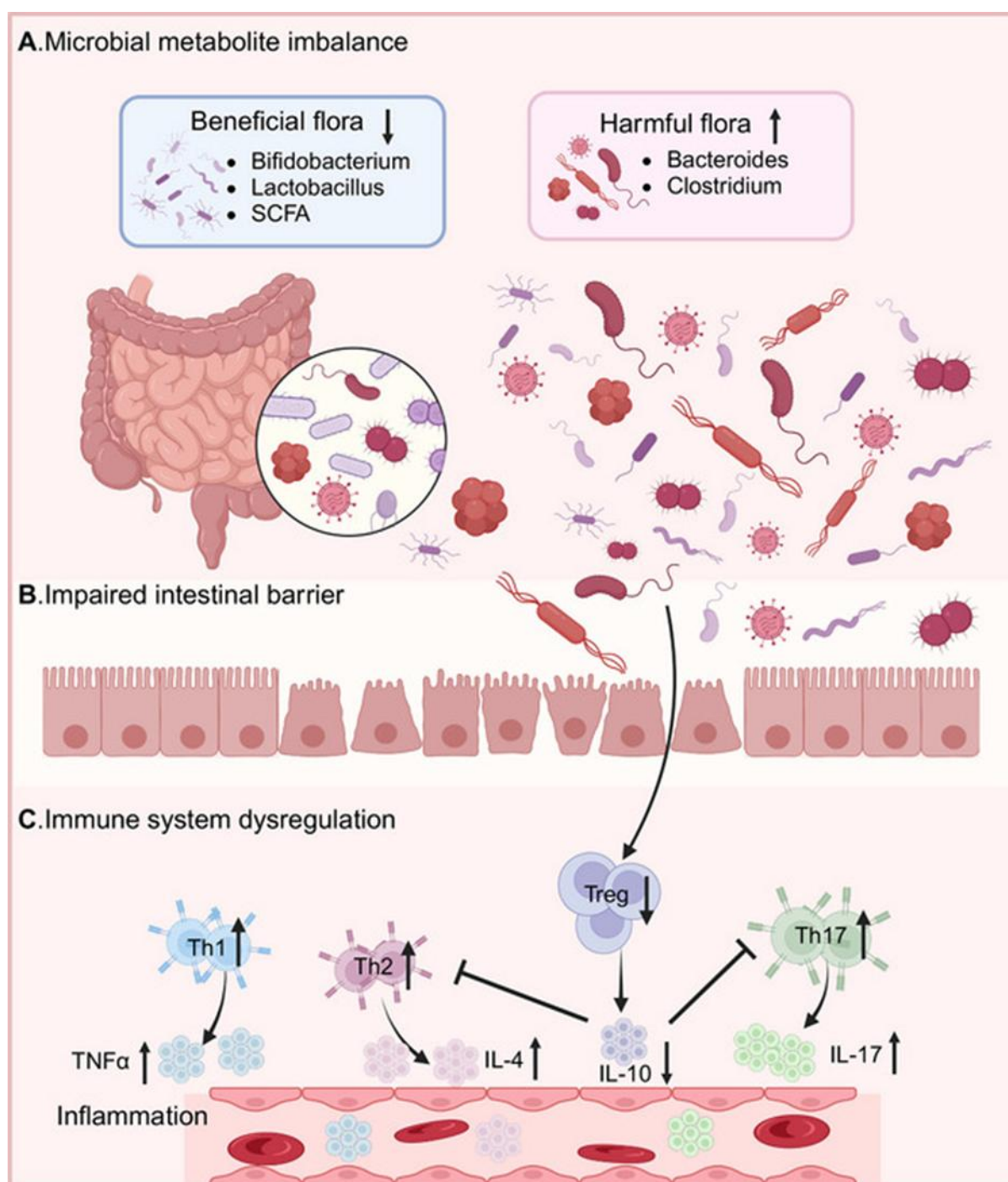


Figure 03. Mechanism by which gut microbiota dysbiosis triggers disease (Yao et al., 2025).

II.3. Chronic inflammation and bone resorption

Chronic inflammation is defined as a prolonged immune response characterised by sustained production of pro-inflammatory mediators, including TNF- α , IL-1 β , IL-6 and IL-17, which disrupt bone homeostasis by promoting bone resorption rather than bone formation (Ayadi et al., 2025).

Bone remodelling is a dynamic process governed by two main cell populations: osteoblasts, responsible for bone formation, and osteoclasts, responsible for bone resorption. Under physiological conditions, these populations operate in balance to maintain bone mineral density (**Jung et al., 2020**).

The RANK/RANKL/OPG system constitutes the central mechanism regulating osteoclastogenesis: the RANKL ligand, expressed by osteoblasts, osteocytes and certain immune cells, binds to its RANK receptor on osteoclast precursors, inducing their differentiation and activation. Osteoprotegerin (OPG) acts as a decoy receptor that neutralises RANKL and thus inhibits osteoclast formation (**Nakashima et al., 2011; Hofbauer et al., 2004**).

In a state of chronic inflammation, the production of pro-inflammatory cytokines such as IL-17 and TNF- α is increased, leading to an upgrade of RANKL and a relative reduction in OPG, which significantly increases the RANKL/OPG ratio. This imbalance promotes excessive osteoclastogenesis and bone resorption.

Immune system cells play a crucial role in this process: CD4⁺ T lymphocytes (particularly Th17 subtypes) produce IL-17, which directly stimulates RANKL expression and promotes osteoclast activation, whilst certain regulatory populations (Tregs) can conversely downregulate inflammation and osteoclast activation (**Takayanagi et al., 2007**).

The activation of the NF- κ B and MAPK pathways, induced by mediators such as TNF- α and IL-1 β , also promotes the expression of pro-osteoclastogenic genes and the activation of mature osteoclasts, thereby enhancing bone resorption in chronic conditions (**Ohlsson et al., 2017**).

It has also been observed that chronic inflammation can inhibit osteoblast activity, notably by inducing mediators such as DKK1, which suppress Wnt signaling—essential for osteoblast differentiation—thereby contributing not only to increased resorption but also to reduced bone formation.

II.4. Impaired calcium absorption

Calcium (Ca²⁺) plays an essential role in maintaining the skeleton and is involved in key physiological processes. Its homeostasis is controlled by the intestine, kidneys, bones and parathyroid glands.

Intestinal absorption of Ca²⁺ varies according to different physiological conditions. It is stimulated when there is a high demand for Ca²⁺, particularly during growth, pregnancy, breastfeeding, dietary

Ca²⁺ deficiency and intense physical activity. Conversely, intestinal transport of Ca²⁺ decreases with age (**Vanessa et al., 2020**).

Calcium deficiency, or hypocalcaemia, is associated with the development of bone disorders such as osteopenia and osteoporosis, as well as with hypertension, insulin resistance, obesity and metabolic syndrome (**Areco et al., 2015**).

- It is also recommended to ensure an adequate calcium intake (> 1000 mg per day) to reduce the risk of falls and fractures (**Carmeliet et al., 2015**).

II.4.1 Pathways of calcium absorption

Intestinal absorption is the sole route by which Ca²⁺ enters the body from dietary nutrients. It occurs via two distinct mechanisms. When the concentration of Ca²⁺ in the intestinal lumen is higher than that in plasma, Ca²⁺ enters the intestine mainly via the paracellular route, through the tight junctions between neighbouring enterocytes. This non-saturable passive transport occurs throughout the small intestine, without significant variations. Conversely, when the concentration of Ca²⁺ in the intestinal lumen is lower than that in plasma, the cation is actively absorbed via the transcellular pathway. This active, saturable system predominates in the duodenum. These two mechanisms are regulated by various endocrine and non-endocrine factors. Both pathways are regulated by calcitriol and other hormones, as well as by dietary factors. Part of the intestinal transport of Ca²⁺ appears to be independent of vitamin D. Intestinal absorption of Ca²⁺ varies depending on physiological conditions. It is stimulated when there is a high demand for Ca²⁺, such as during growth, pregnancy, breastfeeding, dietary Ca²⁺ deficiency and intense physical activity. Conversely, intestinal transport of Ca²⁺ declines with age. Oxidative stress inhibits intestinal absorption of Ca²⁺, whilst antioxidants neutralise the effects of pro-oxidants, leading to the normalisation of this physiological process. Several conditions, such as coeliac disease, inflammatory bowel disease, Turner syndrome and others, are associated with impaired intestinal absorption of calcium (Ca²⁺). Certain forms of hypercalciuria are associated with hyperabsorption of Ca²⁺. Most of these abnormalities are linked to the vitamin D endocrine system (**Vanessa et al., 2020**).

II.4.2. Physiological importance of calcium and regulation of its homeostasis

Calcium plays a role in bone mineralisation; this divalent cation is an important intracellular messenger and is actively involved in numerous physiological functions such as nerve excitation and transmission, muscle and cardiac contraction, blood clotting, glandular secretion and enzyme

activation. It is also involved in cell differentiation and apoptosis, as well as in the immune response, thereby playing a significant role in tumourigenesis and cancer development (**Liao et al., 2019**).

This wide range of functions highlights the need for a homeostatic regulatory system. The homeostasis of extracellular and metabolic Ca^{2+} is maintained by the concerted action of several Ca^{2+} regulatory hormones: parathyroid hormone (PTH), 1,25(OH) $_2$ D $_3$ or calcitriol, and calcitonin (CT). Oestrogens, insulin-like growth factor (IGF-1), prolactin (PRL) and fibroblast growth factor (FGF-23) also play a role in Ca^{2+} regulation (**Wongdee et al., 2015**).

II.4.3. Intestinal dysbiosis and impaired calcium absorption: impact on bone metabolism

Transcellular transport involves, in sequence, the apical entry of Ca^{2+} via the TRPV6 channel, its intracellular binding to calbindin-D9k facilitating its diffusion into the cytosol, and then its basolateral efflux via the Ca^{2+} -ATPase pump (PMCA1b) or the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX1). The expression of these proteins is directly regulated by the activation of the vitamin D receptor (VDR), highlighting the central role of vitamin D signalling in the efficiency of calcium absorption (**Christakos et al., 2016; Fleet, 2017**).

Impaired intestinal calcium absorption corresponds to a quantitative and/or functional reduction in these transport mechanisms, resulting from vitamin D deficiency, chronic intestinal inflammation or gut microbiota dysbiosis. Indeed, dysbiosis can disrupt the integrity of the intestinal barrier, reduce the production of short-chain fatty acids (SCFAs) and negatively modulate the expression of VDR and calcium transporters, thereby compromising the efficiency of transcellular transport (**Xu et al., 2023; Ohlsson & Sjögren, 2018**).

Furthermore, pro-inflammatory cytokines such as TNF- α and IL-6, which are frequently elevated in chronic inflammatory conditions, impair the differentiation and function of enterocytes and may inhibit the expression of proteins involved in calcium transport. This situation leads to reduced calcium absorption, resulting in relative hypocalcaemia and compensatory activation of parathyroid hormone secretion (secondary hyperparathyroidism). Chronic elevation of PTH stimulates bone resorption via an increase in the RANKL/OPG ratio, thereby contributing to bone mass loss and the development of osteoporosis (**Daniel et al., 2020; Pacifici, 2018**).

Maintaining calcium homeostasis is essential for the proper functioning of the skeletal system; however, episodes of hypocalcaemia promote bone resorption and may ultimately lead to osteoporotic

syndromes. In older adults, reduced intestinal calcium absorption is observed compared to younger individuals (**Nordin et al., 2004**).

This decrease is due, in part, to a progressive decline in vitamin D levels in the body, but also to resistance to its action in the intestine. The loss of oestrogen at the menopause or the progressive decline in testosterone may also explain these phenomena (**Pérez et al., 2008**).

All these factors lead to disturbances in the intestinal absorption of calcium, resulting in an imbalance in calcium homeostasis, which creates conditions conducive to bone loss.

CHAPTER 2

I. Vitamin D: Metabolism and role in bone mineralisation

I.1. Synthesis and activation of vitamin D

Vitamin D, a fat-soluble vitamin, occurs naturally in certain foods, is added to others, and can be consumed as a nutritional supplement. A unique feature of vitamin D is that it is produced internally by the human body, where it is synthesised in the skin following exposure to ultraviolet (UV) rays from the sun. There are two main types of vitamin D: vitamin D2 (ergocalciferol), which is plant-based and frequently used to fortify foods, and vitamin D3 (cholecalciferol), which is produced in human skin from 7-dehydrocholesterol and obtained from animal food sources (NIH, 2025 ; Ross et al., 2011).

Vitamin D is metabolised into its biologically active forms through two hydroxylation processes. The liver is the first site where hydroxylation occurs, thanks to the action of the enzyme 25-hydroxylase, which converts vitamin D, whether from food or sun exposure, into 25-hydroxyvitamin D. The second hydroxylation takes place in the kidneys, where the enzyme 1-alpha-hydroxylase converts 25-hydroxyvitamin D into its biologically active form, 1,25-dihydroxyvitamin D (or calcitriol) (Bikle, 2014).

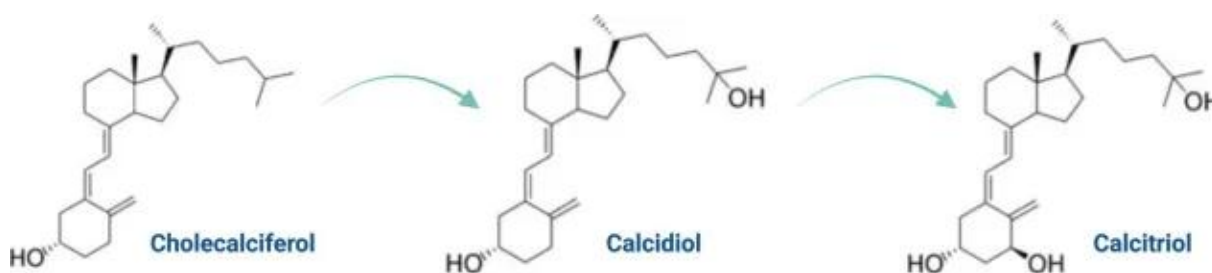


Figure 04.Chemical structure of cholecalciferol, calcifediol and calcitriol (Ortiz-Prado et al., 2025).

Various cells, including cardiomyocytes and immune cells, express nuclear vitamin D receptors (VDRs) and are sensitive to 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) (Amrein et al., 2011). While renal hydroxylation dominates systemic calcitriol production, extrarenal activation (e.g., in macrophages) contributes to local immunomodulation (Small et al., 2021).

I.2. Vitamin D receptor (VDR)

The VDR, a nuclear receptor, is essential for mediating the biological effects of vitamin D. It is found in various cells, particularly those of the immune, cardiovascular and metabolic tissues. Once bound

to calcitriol, the VDR forms a complex with the retinoid X receptor (RXR). It then moves to the nucleus where it binds to vitamin D response elements (VDRE) located in the promoter regions of target genes. This complex controls the transcription of genes associated with inflammation, oxidative stress, calcium absorption and cell differentiation **(Fenercioglu, 2024)**.

Calcitriol reduces the production of pro-inflammatory cytokines such as TNF- α and IL-6 by inhibiting the NF- κ B and MAPK pathways, while promoting the production of anti-inflammatory cytokines such as IL-10. In immune cells, vitamin D enhances the expression of antimicrobial peptides (such as cathelicidin and defensins) and stimulates the differentiation of regulatory T cells (Tregs), thereby influencing immune tolerance **(Fenercioglu, 2024; Ghosh et al., 2024)**.

I.3. Role of vitamin D in intestinal homeostasis

Vitamin D is essential for regulating the gut microbiota, and its receptor (VDR) is found in intestinal epithelial cells, immune cells and the gut microbiota. Vitamin D regulates antimicrobial peptides and immune responses, thereby influencing the composition and diversity of the gut microbiota **(Fakhoury et al., 2020)**.

Vitamin D stimulates the secretion of defensins by Paneth cells, thereby reducing microbial growth in the small intestine. A notable aspect of innate intestinal immunity is the generation of antimicrobial peptides (AMPs), which have the ability to directly eradicate or destroy microorganisms present in the intestinal lumen. Within the small intestine, Paneth cells (PCs), located at the bottom of Lieberkühn's crypts, are the major source of antimicrobial elements released into the intestinal lumen, such as α -defensins, lysozyme and phospholipase A2 **(Ayabe et al., 2002)**.

Vitamin D deficiency is associated with an imbalance in the intestinal microbiota, a change in intestinal protection mechanisms and an increase in intestinal permeability **(Singh et al., 2020; Yamamoto & Jørgensen, 2020)**.

The intestinal epithelium has a vitamin D receptor (VDR) that is essential for maintaining the integrity of the mucosal barrier and regulating innate intestinal immunity **(Martens et al., 2020)**. VDR plays a crucial role in regulating intestinal homeostasis by preventing pathogenic bacterial invasion, inhibiting inflammation and maintaining barrier functions **(Gombart et al., 2005 ; Kamen & Tangpricha, 2010 ; Waterhouse et al., 2009 ; Liu et al., 2007 ; Kong et al., 2008 ; Lagishetty et al., 2010)**. In addition, VDR is a transcription factor that regulates the expression of numerous target

genes, including those encoding the tight junction proteins claudin-2, -5, -12 and -15 (**June & Zhang, 2022**).

The intestinal mucosal barrier, through its physical, biochemical and immune mechanisms, constitutes the first barrier of the intestine. Mucus first separates the hydrolysed luminal contents from the intestinal epithelium (**Turner, 2009**).

The expression of mucin genes is regulated by vitamin D, and vitamin D deficiency due to poor diet leads to a change in mucus composition and increased permeability. Tight junctions located on the lateral edges of intestinal epithelial cells play a crucial role in innate immunity. Research in animal models has shown that vitamin D deficiency can cause a significant reduction in the expression of mucin genes, as well as genes related to tight junctions, such as ZO1, occludin and claudin (**Su et al., 2016**).

Given the multiple roles of vitamin D, a deficiency can disrupt intestinal homeostasis and barrier functions. Indeed, vitamin D, through its interaction with its receptor, has the ability to influence bacterial colonisation, impact the architecture of tight junctions and trigger anti-inflammatory responses (**Akimbekov et al., 2020**).

Vitamin D regulates innate intestinal immunity through pattern recognition receptors (PRRs). PRRs play a crucial role in the innate immune system. They are cellular sensors capable of identifying molecules specific to various types of pathogens (**Dionne et al., 2014**).

The active form of vitamin D ($1,25(\text{OH})_2\text{D}$) selectively eliminates pathogenic microorganisms, thereby creating an environment conducive to colonisation by beneficial bacteria (**Hewison, 2011**).

Vitamin D also regulates genes essential for calcium and phosphate homeostasis, such as TRPV6 and calbindin, which facilitate intestinal calcium absorption (**Dawivedi et al., 2025**).

I.4. Vitamin D as a regulator of the immune axis and bone metabolism

The relationship between the immune system and the skeletal system has long been studied. In 2000, Arron et al, proposed the concept of ‘osteimmunology’ and emphasised that there is a complex interaction between the immune system and the skeletal system (**Ma et al., 2025**).

Immune cells contribute primarily to the maintenance of bone homeostasis through the release of pro- and anti-inflammatory factors. Subsequently, another study revealed that intestinal inflammation promotes OC differentiation and bone loss by promoting cytokine changes **(Ma et al., 2025)**.

Calcitriol can modulate the activity of all immune system cells expressing the vitamin D receptor (VDR) **(Vieth, 2007)** and plays an immunoregulatory and tolerogenic role by influencing the maturation and migration of different dendritic cell (DC) subtypes as well as their production of cytokines and chemokines through binding to the VDR **(De Martinis et al., 2021)**.

This binding induces the arrest of DC differentiation and maturation and, consequently, the production of pro-inflammatory cytokines (IL-6, IL-12 and IL-23) and tumour necrosis factor α (TNF- α); it increases the production of anti-inflammatory cytokines (IL-8 and IL-10); it decreases the expression of major histocompatibility complexes class I and II and surface costimulatory molecules (CD40, CD80, CD83 and CD86) **(Zittermann et al., 2013)**; and it inhibits the differentiation of B lymphocytes into plasma cells and their production of antibodies. Vitamin D may also act on activated T lymphocytes expressing VDR by reducing Th1 and Th17 activity and promoting the differentiation of Th2 into IL-4-producing CD4⁺ cells **(De Martinis et al., 2021)**.

Although 25(OH)D deficiency is a key factor in the development of osteoporosis, it also appears to be involved in the pathogenesis of many conditions characterised by impaired bone metabolism, osteoporosis or bone damage **(De Martinis et al., 2021)**.

I.5. Intestinal absorption of calcium

The intestine plays a major role in the absorption and control of calcium concentration in the body. In fact, approximately 90% of calcium is absorbed in the small intestine **(Areco et al., 2015)**. Calcium is supplied by food and is found in the form of Ca²⁺ ions in the intestinal lumen. Two distinct pathways are responsible for the absorption of these ions within the intestinal mucosa **(Figure 02)**.

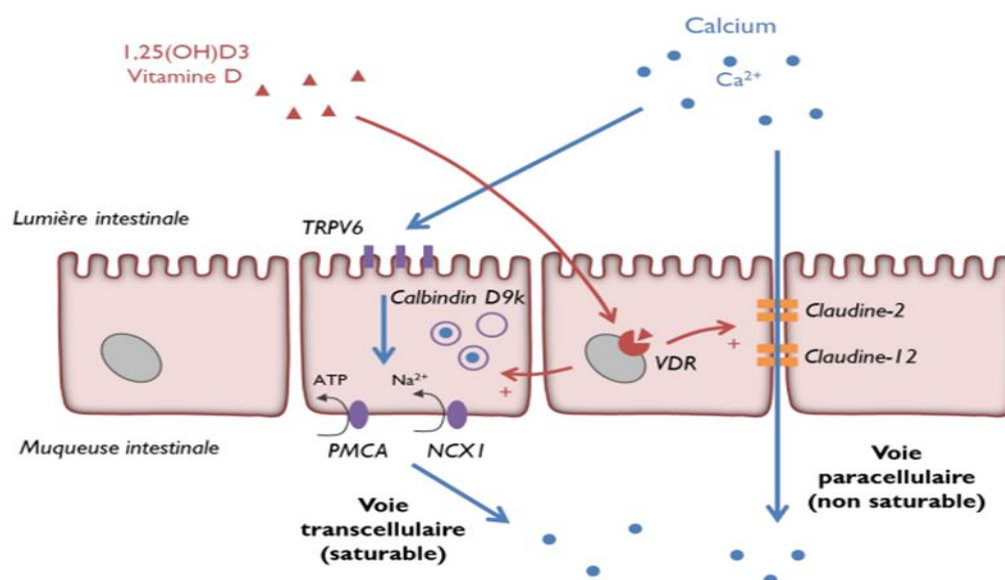


Figure 05. Schematic representation of intestinal calcium absorption. Two absorption pathways are distinguished: the paracellular pathway and the transcellular pathway. PMCA: Plasma Membrane Ca²⁺ ATPase, NCX1: Sodium-Calcium Exchanger 1 (**Raveschot, 2018**).

Calcium absorption via the paracellular pathway occurs through passive diffusion of ions across the intestinal epithelium, representing a non-saturable process that depends on an electrical gradient formed between the intestinal lumen and the intestinal mucosa (**Pérez et al., 2008**). This absorption pathway is still poorly characterised, but recent work has shown that absorption is controlled by tight junction proteins, namely claudin-2 and claudin-12, which act as calcium-selective channels (**Fujita et al., 2008**).

The transcellular pathway is a calcium absorption pathway that can become saturated and involves several phases. First, the calcium transporter (Transient Receptor Potential Vanilloid 6, TRPV6), also known as CaT1, integrates calcium ions into intestinal cells. The calbindin D9k protein plays a key role in intracellular calcium transport, while calcium elimination is accomplished by two transporters: an ATPase (plasma membrane ATPase Ca²⁺) and a sodium-calcium exchanger (Sodium-Calcium Exchanger 1, NCX1) (**Fleet & Schoch, 2010; Pu et al., 2016**).

These two absorption pathways are regulated by vitamin D (**Figure 02**). For example, the TRPV6 transporter, calbindin D9k and claudins-2 and 12 are regulated by vitamin D, demonstrating its involvement in both calcium absorption pathways (**Bronner, 2003; Fleet, 2017; Fujita et al., 2008; Haussler et al., 2010**).

I.6. Bone cells

➤ Osteoclasts :

Osteoclasts are multinucleated cells responsible for bone resorption and derived from myeloid precursor cells (**Park-min, 2018**). Traditionally considered to be terminally differentiated cells, osteoclasts are in fact long-lived cells that are constantly renewed by circulating precursors (**Tsai et al., 2023**). RANKL is a major inducer of osteoclast differentiation and has been used as a therapeutic target for bone loss (**Miller, 2009; Kendler et al., 2022**).

➤ Osteoblasts :

Osteoblasts promote mineralisation and bone formation by producing osteoid and releasing bone matrix. Cells derived from the skeletal lineage contribute to osteogenesis and undergo three specific stages of differentiation: osteoprogenitors, preosteoblasts and osteoblasts (**Long, 2011**). During the maturation of preosteoblasts, Osterix, induced by the WNT- β -catenin signalling pathway, directs their differentiation into osteoblasts (**Nakashima et al., 2002**). Mature osteoblasts differentiate into border cells (osteocytes) or undergo apoptosis (**Choi et al., 2024**).

➤ Osteocytes :

Osteocytes, derived from a subset of osteoblasts, are terminally differentiated cells embedded in the mineralised bone matrix (**Wilson, 2011; Bellido, 2014**). Osteocytes, which are the predominant cells in bone, play an essential role in the bone remodelling process. They respond to hormonal and mechanical signals and regulate bone balance by supervising osteoblasts and osteoclasts. Adult osteocytes selectively release molecules such as Dickkopf-1 (DKK1) protein, osteoprotegerin (OPG) and sclerostin (**Robling & Bonewald, 2020**).

I.7. Bone remodelling

Bone remodelling is a process in which old or damaged bone is removed by osteoclasts and replaced by new bone formed by osteoblasts (**Kim et al., 2020**). Bone mass is thus physiologically maintained through the renewal of bone tissue. This is a continuous process over time (**Solal & Vernejoul, 2003**).

Bone remodelling takes place jointly in multicellular basic units (BMUs), alternating between a resorption phase carried out by osteoclasts and a formation phase orchestrated by osteoblasts. The

time required to completely replace cancellous bone can vary from five years to approximately ten years for cortical bone (Desoutter et al., 2012; Meunier, 2003).

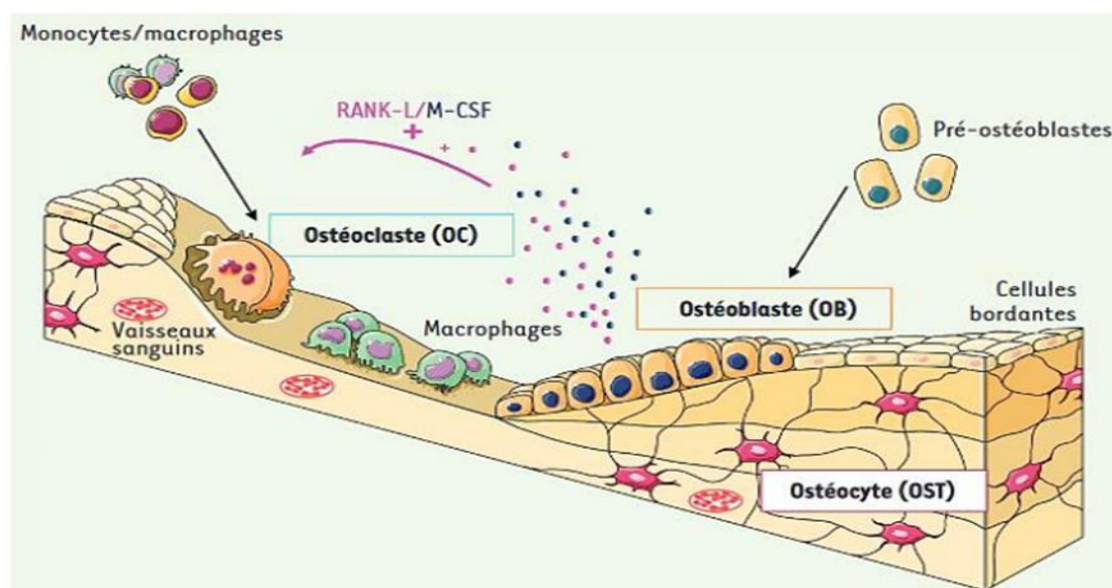


Figure 06. Bone remodelling (Camuzard et al., 2017).

The bone remodelling cycle comprises four successive phases: the activation phase, the resorption phase, the reversal phase and the bone tissue formation phase.

- The activation phase

The activation of osteoclasts is triggered by the interaction between haematopoietic stem cells and osteoblastic cells. The role of osteoclasts is closely associated with their interaction with bone matrix proteins, such as osteopontin and bone sialoprotein, which were released by osteoblasts during the last bone formation cycle (Neve et al., 2011).

- The resorption phase

Mature osteoclasts resorb bone that has reached the end of its functional life. This resorption process begins with the dissolution of the mineral component through acidification and ends with the decomposition of the organic matrix. This phase takes approximately two weeks.

- The reversal phase

When resorption is complete, the reversal phase begins. Osteoclasts undergo apoptosis and osteoblast precursors multiply in situ, developing into mature osteoblasts that move into the resorption cavity generated by the osteoclasts **(Neve et al., 2011)**. This stage lasts approximately four to five weeks.

- The bone tissue formation phase

Mature osteoblasts synthesise a new non-mineralised bone matrix (osteoid) that fills the resorption lacuna and mineralises during the resting phase **(Neve et al., 2011)**. This phase lasts approximately four months.

I.8. Metabolic disruption in osteoporosis

Bone formation is a highly dynamic and finely regulated physiological process centred on complex interactions between osteoblasts and osteoclasts **(Zhang et al., 2019 ; Zhang et al., 2016)**.

In human osteoblasts, PTH regulates osteoclast formation by controlling the expression of the receptor activator of nuclear factor κ B (RANK), receptor activator of nuclear factor κ B ligand (RANKL) and osteoprotegerin **(Sun et al., 2020)**. Dysregulation in osteoclast activation leads to excessive bone resorption **(Vu & Bose, 2019)**.

The RANKL/RANK/OPG axis is a regulatory system essential for bone homeostasis, being primarily managed by osteoblasts. These cells produce RANKL to stimulate osteoclast differentiation and OPG to inhibit it. The key process is based on the interaction of RANKL with RANK on osteoclast precursors, which triggers their differentiation. OPG therefore acts as a decoy receptor, competitively inhibiting this interaction **(Xiong et al., 2025)**.

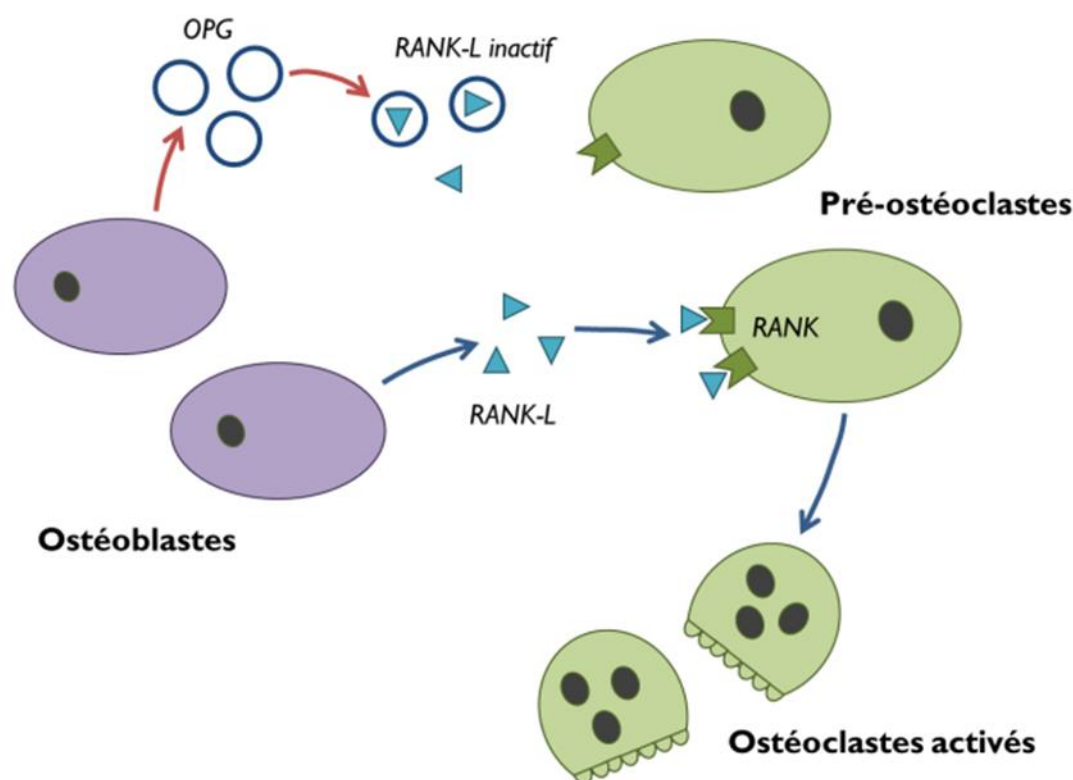


Figure 07. Schematic diagram of local factors contributing to bone remodelling. These factors include the RANK receptor and its ligand RANK-L, which activate osteoclasts. OPG compensates for this activation by capturing RANK-L. OPG: Osteoprotegerin, RANK: Receptor Activator of Nuclear Factor KB, L: Ligand (**Raveschot, 2018**).

In addition, osteocytes are an essential source of RANKL. During active remodelling, they express this protein to maintain metabolic balance. Furthermore, there is a link between RANK and angiogenesis: VEGF activates RANK expression in endothelial cells, enhancing their pro-angiogenic response to RANKL. An imbalance in this axis is a determining factor in osteoporosis (**Xiong et al., 2025**).

II. Impact of intestinal dysbiosis on bone homeostasis

II.1. Metabolic mechanisms linking intestinal dysbiosis to the genesis of osteoporosis:

The human intestine is recognised as a multi-constituent and multifunctional digestive organ (**Cani, 2018**). Intrinsic structures such as the intestinal epithelium, the intestinal microbiota and the metabolites derived from them, as well as their interactions, collectively constitute the intestinal microecology (**Lu et al., 2020**).

Numerous studies highlight complex bidirectional communication networks between the gut microbiota and the skeletal system, referred to as the ‘gut-bone axis’ **(Lu et al., 2021)**.

Microbial communities and their metabolic derivatives regulate bone homeostasis through direct and indirect mechanisms, modulating osteoblast-osteoclast dynamics via metabolic, inflammatory and immune pathways **(Li et al., 2022)**.

The gut microbiota primarily regulates the immune system, produces beneficial metabolites such as short-chain fatty acids (SCFAs), regulates the absorption of minerals such as calcium, and modulates bile acid metabolism through multiple mechanisms that affect bone metabolism and contribute to the maintenance of bone health **(Mingyue et al., 2025)**.

The intestinal barrier is composed of a layer of mucus, the gut microbiota, immune cells, and a monolayer of intestinal epithelial cells **(Cardoso-Silva et al., 2019)**. Lipopolysaccharides (LPS), the main component of the bacterial cell wall of the gut microbiota, induce chronic inflammatory responses, leading to bone loss. In addition, LPS promote the formation and activation of osteoclasts, thereby contributing to bone loss **(Smith et al., 2006)**.

Barrier damage is consistently observed in all models of osteoporosis, mainly manifesting as altered tight junction proteins, changes in intestinal villi morphology, and increased intestinal permeability **(Ma et al., 2021; Xiao et al., 2021)**. This alteration of the intestinal barrier facilitates the translocation of microbes or potential antigens to the subepithelial mucosa, thereby activating immune cells and triggering aberrant intestinal and systemic immune responses, leading to bone loss **(Li et al., 2016)**.

The gut microbiota influences the bone marrow by modulating mucosal barrier function; increased membrane expression of Toll-like receptor 4 (TLR4) is observed in primary cells treated with RANKL or LPS. TNF- α is secreted via the endotoxin/TLR4 signalling pathway and modulates RANKL-induced osteoclastogenesis **(AlQranei et al., 2021)**. The integrity of the gut microbiota prevents contact between bacterial LPS and TLR4 on macrophages in the chorion, thereby preventing osteoporosis **(Yuan & Shen, 2021)**.

The physiological stability of microbiota promotes immune balance, supporting osteocyte function and balanced bone renewal **(Indrio & Salatto, 2025 ; Novince et al., 2017)**. Conversely, microbial dysbiosis disrupts skeletal homeostasis through several mechanisms: disruption of the immune system alters the ratios of CD4⁺ T cell subsets and cytokine profiles, in particular receptor activation of nuclear factor kappa-B ligand (RANKL) expression, thereby promoting osteogenesis **(Indrio &**

Salatto, 2025); direct regulation mediated by metabolites of osteoblast and osteoclast differentiation via short-chain fatty acids (SCFA) and bile acid signalling (**Kondo et al., 2022**); and dysbiosis-induced inflammatory cascades releasing bone catabolic mediators that disrupt the formation-resorption coupling (**Uchida et al., 2018**).

Bone remodelling is often influenced by the activation of B and T lymphocytes and by an increase in osteoclastogenic factors (interleukin (IL)-17, IL-6, RANKL, TNF- α). These elements lead to increased osteoclast activity while hindering osteoblast formation and function, exacerbating the imbalance in bone structure (**Cline-Smith et al., 2020**).

Vitamin D also plays a crucial role in maintaining bone balance. Fat-soluble vitamin D is absorbed by the small intestine and then activated into 1,25-(OH) $_2$ -D by the liver and kidneys, which stimulates calbindin in intestinal epithelial cells to maintain adequate blood calcium levels (**Burt et al., 2019**).

In addition, vitamin D facilitates the active transport of calcium across the intestinal mucosa, thereby enabling the body to adapt to low calcium intake. Vitamin D deficiency can have a negative effect on bone mineral density (**Marchiset, 2010**).

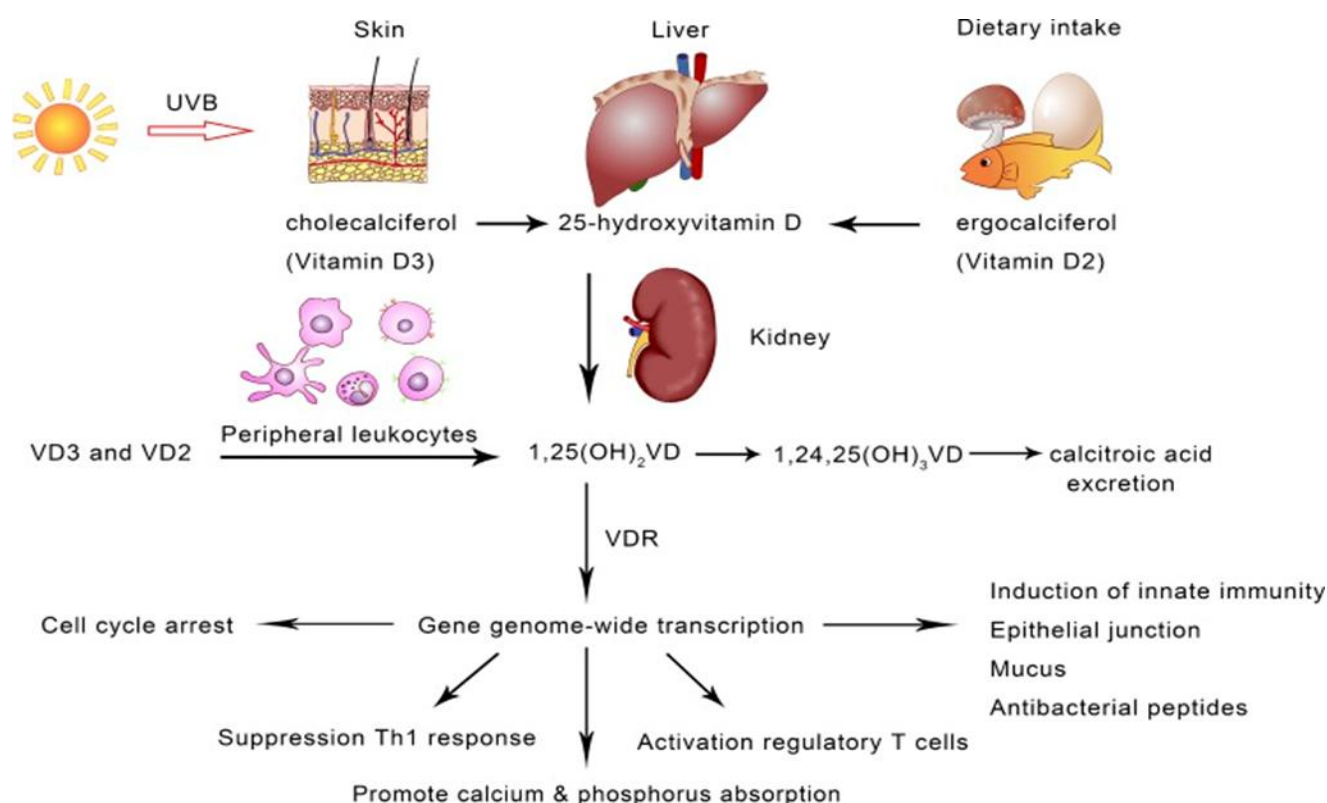


Figure 08. Main biological functions of vitamin D (**Zeng et al., 2020**).

Active vitamin D3 increases blood calcium levels by stimulating intestinal calcium absorption (**Bora et al., 2018**).

A certain proportion of calcium is absorbed passively through the intestinal wall by a diffusion gradient mechanism, but it is also actively absorbed by the action of 1,25-dihydroxycalciferol (1,25[OH]₂D) through calcium channels in the intestinal endothelium (**Gavin & Richard, 2008**). Insufficient calcium intake reduces the amount of calcium absorbed, increases serum parathyroid hormone levels and can increase bone loss (**Marchiset, 2010**).

Maintaining calcium homeostasis is essential for the proper functioning of the skeletal system, but hypocalcemia promotes bone resorption and can ultimately lead to osteoporotic syndromes (**Nordin et al., 2004**).

This decrease is due, in part, to a gradual decline in vitamin D concentration in the body, but also to resistance to its action in the intestine (**Pérez et al., 2008**).

Calcium balance is regulated by complex interactions between the intestines, kidneys and bones, and is mainly influenced by parathyroid hormone (PTH) and calcitriol. The intestine must absorb dietary calcium in order to make it available for the body's physiological functions (**Beggs et al., 2022**).

CasR can control the production of parathyroid hormone (PTH). Along with vitamin D, PTH is one of the hormones that plays an important role in calcium balance, as it acts directly on the kidneys or bones to regulate calcium concentration in the body.

Activation of CasR stimulates PTH secretion by the parathyroid gland, which has a direct impact on bone remodeling by stimulating bone resorption in order to mobilise calcium reserves in the bones (**Raveschot, 2018**).

II.2. Pathophysiology of osteoporosis

Osteoporosis, a systemic degenerative bone disease characterised by a reduction in bone mass and a disruption of bone microstructure, has become a major public health problem that hinders the care of ageing populations. It affects more than 200 million people worldwide (**Priya et al., 2023**).

This disease involves an imbalance between bone resorption and formation, leading to a progressive decrease in bone mass and density, both trabecular and cortical. In most cases, osteoporosis is mainly caused by an increase in osteoclastic resorption or a decrease in osteoblastic bone formation.

However, this imbalance can be caused by an increase in osteoclastic activity (**Lane, 2006**). As the resorption phase of bone remodeling is shorter than the formation phase, even a slight increase in resorption can lead to bone loss (**Munoz et al., 2020; Raisz, 2005**), indicating that any increase in bone resorption is likely to result in bone mass loss (**Raisz, 2005**). A decrease in bone density leads to a reduction in the bone's ability to bear weight, which increases the risk of fractures, particularly vertebral fractures, especially in affected individuals.

Osteoclasts are mainly induced by the receptor activator of nuclear factor κ B ligand (RANKL). RANKL binds to its receptor, RANK, which is found on osteoclast precursors and activates downstream signaling cascades, such as nuclear factor kappa B (NF- κ B) and nuclear factor of activated T cells 1 (NFATc1), which promote the differentiation of precursors into mature osteoclasts, which are responsible for bone resorption, and increase their resorptive capacity. The stimulation of osteoclastogenesis is promoted by inflammatory mediators, such as interleukin-6 (IL-6) and tumour necrosis factor α (TNF- α), but their action is mainly modulatory and is generally mediated by the RANK-RANKL axis. For example, these mediators increase RANKL activity in stromal/osteoblastic cells or sensitise precursors to RANKL stimulation (**Munoz et al., 2020; Raisz, 2005**).

RANKL, produced by osteoblasts, osteocytes, bone marrow stromal cells, and activated T and B lymphocytes, binds to RANK on osteoclast precursors to promote their differentiation into mature osteoclasts and stimulate osteoclastic activity via the NF- κ B signalling pathway (**Murusuri et al., 2026**). Circulating monocytes release cytokines, such as interleukin 1 (IL-1) and TNF α . The release of the lymphokine IL-7 is stimulated by these cytokines, which target mesenchymal cells. By stimulating the production of pro-osteoclastogenic cytokines, macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κ B ligand (RANKL), IL-7 suppresses the function of osteoblasts and T lymphocytes. In addition, interleukin 7 (IL-7) reduces the activity of osteoprotegerin (OPG), the decoy receptor for RANKL. These changes lead to an increase in net osteoclastic activity. RANKL acts in collaboration with IL-1 and tumor necrosis factor α (TNF α) from monocytes to increase bone loss (**Ross, 2003**).

The RANK/RANKL/OPG axis is now considered essential in several other diseases characterised by increased bone remodeling.

II.3. Preventive strategies for osteoporosis: Modulation of the microbiota and optimisation of vitamin D and calcium status

- **Calcium and vitamin D supplementation:**

Bone mineralisation and remodelling require adequate calcium and vitamin D intake. It is important to consume calcium-rich foods, such as dairy products, green leafy vegetables and fortified foods, as part of a balanced diet. Foods containing vitamin D include oily fish, fortified foods and exposure to sunlight. People with insufficient dietary intake or limited exposure to sunlight may need to take supplements (**Hao et al., 2024**).

- **Protein intake:**

Protein is essential for bone formation and maintenance, as it provides the amino acids necessary for collagen synthesis and bone matrix deposition (**Devignes et al., 2022; Selvaraj et al., 2024**). Bone health is promoted by an adequate intake of protein from sources such as lean meats, poultry, fish, eggs, legumes and dairy products. However, excessive protein consumption, particularly from animal sources, can have adverse effects on bone health and should be moderated (**Hao et al., 2024**).

- **Nutrient-rich diet:**

A balanced diet, including fruit, vegetables, whole grains and lean protein, provides the vitamins, minerals and antioxidants necessary for bone health. The phytochemicals found in plant foods may have positive effects on bone metabolism and reduce inflammation associated with osteoporosis (**Hao et al., 2024**).

- **Physical activity:**

Bone loss is caused by a lack of mechanical stimulation, particularly by an increase in bone resorption without an increase in bone formation. The best physical activity is weight-bearing exercise, i.e. activities involving running and jumping, such as football, basketball, volleyball or racket sports (tennis, squash ...). Not all activities strengthen bones in the same way: walking is less effective than running or activities that involve jumping. However, non-weight-bearing exercise, such as weight training, can also strengthen the skeleton, provided that intense muscle contractions are performed (**Meunier, 2020**).

Exercises involving weight-bearing and resistance, such as walking, jogging, weight training and yoga, help stimulate bone formation and improve bone mineral density. Regular physical activity is essential (**Hao et al., 2024**).

Bone mass acquisition is optimised when physical activity is combined with good nutritional intake (calcium, vitamin D, protein) (**Meunier, 2020**).

- **Discontinuation of non-essential medication:**

Oral glucocorticoids should be avoided. If they cannot be discontinued, the minimum effective dose should be sought (**Briot et al., 2018**).

- **Limit sodium and caffeine:**

High sodium intake and excessive caffeine consumption have been associated with calcium excretion and bone loss (**Heaney, 2002; Park et al., 2014; Reuter et al., 2021**). Limiting sodium intake and moderating caffeine consumption from sources such as coffee, tea and soft drinks can help preserve bone mineral density and reduce the risk of osteoporosis (**Hao et al., 2024**).

Materials and Methods

1. Aim of the study

The main aim of this study is to assess vitamin D status, calcium metabolism, oxidative stress and inflammation in patients with osteoporosis.

This study was conducted at the PPABIONUT laboratory at the University of Tlemcen, in collaboration with a hospital.

To achieve this objective, the following parameters were studied:

- The participants' anthropometric characteristics (age, weight, height and body mass index (BMI))
- Dietary habits and lifestyle factors.
- Medical and family history.
- Serum concentrations of vitamin D and calcium.
- Markers of oxidative stress and the antioxidant system.
- C-reactive protein (CRP) as a marker of inflammation.

This study aims to determine the role of disturbances in vitamin D status, calcium metabolism, oxidative stress and inflammation in the pathophysiology of osteoporosis.

2. Study design

This is a cross-sectional epidemiological study with descriptive and analytical objectives, conducted at Tlemcen University Hospital in collaboration with the PPABIONUT laboratory at the University of Tlemcen.

Data were collected using a questionnaire covering the participants' sociodemographic characteristics, dietary habits, lifestyle and medical history. Blood samples were also taken for biological and biochemical analyses.

3. Study population

The study involved 30 women recruited from Tlemcen University Hospital and divided into two groups:

- Osteoporotic group: 15 women with osteoporosis.
- Control group: 15 women without osteoporosis.

Participants were selected according to predefined inclusion and exclusion criteria.

3.1. Inclusion criteria

Women who volunteered to take part in the study, gave their consent to participate, and provided all the required information via the questionnaire and the biological tests carried out were included in this study.

3.2. Exclusion criteria

Women with conditions likely to affect bone metabolism (renal, endocrine or metabolic diseases), pregnant women, those undergoing long-term corticosteroid treatment, and participants whose data were incomplete or unusable were excluded from the study.

4. Data collection tool

Data was collected using a structured questionnaire designed to gather information on the participants' sociodemographic characteristics (age, educational attainment, BMI), dietary habits, sun exposure, lifestyle (physical activity, smoking), as well as their medical history, including osteoporosis, fractures and metabolic diseases.

5. Study procedure

The study was conducted between 24 February 2026 and 1 May 2026. Data were collected using a structured questionnaire administered to participants recruited at Tlemcen University Hospital, particularly in the trauma and physical medicine and rehabilitation departments. Blood samples were also taken to carry out the necessary biological and biochemical analyses.

6. Parameters studied

The parameters studied included anthropometric characteristics, dietary habits, vitamin D and calcium intake, medical history, and biological parameters related to vitamin D status, calcium metabolism, oxidative stress and inflammation.

7. Blood samples

Blood samples were taken from the antecubital vein after applying a tourniquet. A volume of 5 to 10 mL of blood was collected from each patient and distributed into heparinised tubes.

Biochemical analyses, including a complete blood count (CBC), vitamin D levels and serum calcium, were carried out at the central laboratory of the Haemobiology Department at Tlemcen University Hospital.

In addition, other biological parameters were assessed to obtain an overall picture of the subjects' metabolic and inflammatory status, notably:

- Ferritin
- C-reactive protein (CRP), a marker of inflammation
- Lipid profile (total cholesterol and triglycerides)

7.1. MDA Assay

Plasma and erythrocyte malondialdehyde (MDA) are determined using the method described by Draper and Hadley (1990), based on the reaction with thiobarbituric acid (TBA). This method is widely used for the assessment of lipid peroxidation, due to its simplicity and high sensitivity.

Following acid treatment and heating, the aldehydes resulting from lipid peroxidation react with thiobarbituric acid to form a chromogenic MDA-TBA complex, consisting of two TBA molecules and one MDA molecule. The absorbance of this complex is measured by spectrophotometry at 532 nm.

The MDA concentration is then calculated using the molar extinction coefficient of the MDA-TBA complex ($\epsilon = 1.56 \times 10^5 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$ at 532 nm).

7.2. Determination of reduced glutathione

The measurement of erythrocyte and plasma reduced glutathione (GSH) is carried out using the colorimetric method with Ellman's reagent (DTNB) (ELLMAN, 1959). The reaction involves the cleavage of the 5,5-dithiodis-2-nitrobenzoic acid (DTNB) molecule by GSH, which releases thionitrobenzoic acid (TNB) according to the following reaction

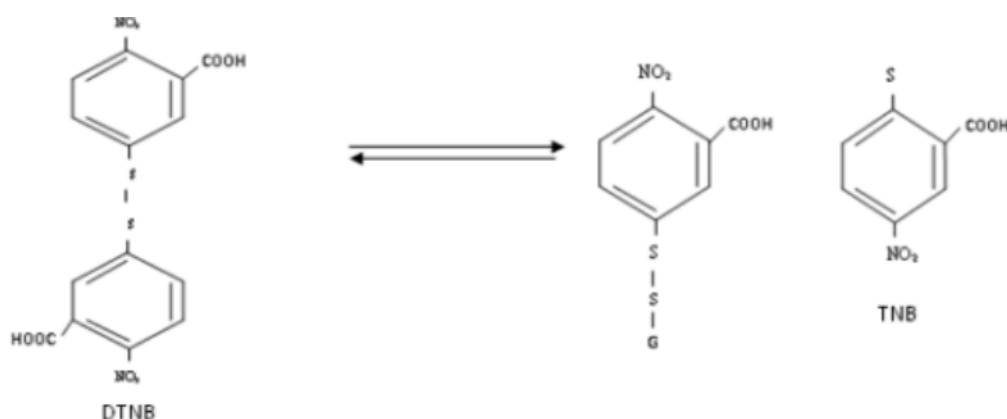


Figure 09. Thionitrobenzoic acid (TNB) at an alkaline pH (8–9) exhibits an absorbance at 412 nm with an extinction coefficient of $13.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

7.3. Determination of catalase activity

This enzymatic activity is measured by spectrophotometric analysis of the rate of hydrogen peroxide decomposition (AEBI, 1974). In the presence of catalase, the decomposition of hydrogen peroxide leads to a decrease in the absorbance of the H_2O_2 solution over time. The reaction medium contains 1 ml of supernatant (lysate diluted 1 :50 or plasma), 1 ml of H_2O_2 and 1 ml of phosphate buffer (50 mmol/l, pH 7.0). After a 5-minute incubation, 1 ml of titanium sulphate (TiOSO_4) reagent (1.7 g in 500 ml of 2N H_2SO_4) is added. The reading is taken at 420 nm. The concentrations of the remaining H_2O_2 are determined using a standard series of H_2O_2 with phosphate buffer and the TiOSO_4 reagent, so as to obtain concentrations of 0.5 to 2 mmol/l in the reaction medium.

The calculation of one unit of enzymatic activity is:

$$A = \log A_1 - \log A_2$$

A_1 is the initial H_2O_2 concentration

A₂ is the H₂O₂ concentration after incubation (after 5 min)

Specific activity is expressed in U/g Hb or in U/ml.

7.4. Creatinine Assay

Kinetic determination of creatine kinase in accordance with the recommendations of the IFCC and the DGKC. Creatine kinase (CK) catalyses the reversible transfer of a phosphate group from phosphocreatine to ADP. This reaction is in addition to others catalysed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P DH): The rate of NADPH formation, determined by photometry, is proportional to the catalytic concentration of CK in the sample tested.

7.5. Urea Assay

Urease catalyses the hydrolysis of urea, present in the sample, into ammonia (NH₃) and carbon dioxide (CO₂). The ammonia ions react with salicylate and hypochlorite (ClONa), in the presence of the nitroprusside catalyst, to form a green indophenol: The intensity of the colour formed is proportional to the urea concentration in the test; the decrease in NAD⁺ concentration in the method is proportional to the urea concentration in the test sample.

7.6. Vitamin D Assay

The vitamin D assay was performed by determining 25-hydroxyvitamin D [25(OH)D] in serum, which is the best indicator of vitamin status.

After blood collection, the samples were centrifuged at 3000 rpm for 10 minutes to recover the serum. This was then stored at -20°C until analysis. The assay is performed using an ELISA (Enzyme-Linked Immunosorbent Assay) method, based on a specific antigen–antibody reaction.

The principle of the method relies on the binding of vitamin D present in the sample to specific antibodies attached to an ELISA plate. After several washing steps to remove unbound substances, an enzyme conjugate is added. The enzymatic reaction produces a colour, the intensity of which is proportional to the concentration of vitamin D in the sample.

The optical density is measured using a spectrophotometer at a wavelength of 450 nm. The vitamin D concentration is then determined from a calibration curve created using standards of known concentrations.

Results are expressed in **ng/mL**.

7.7. Complete Blood Count (CBC)

The CBC is performed using an automated analyser capable of:

- Measuring hemoglobin
- Measuring hematocrit
- Measuring mean corpuscular volume (MCV)
- Measuring means corpuscular hemoglobin (MCH)

These parameters enable the diagnosis and characterisation of anaemia in women.

The hemactological analyses were carried out using an automated analyser, a device capable of counting blood cells (red blood cells, white blood cells, platelets), calculating the hematocrit, and measuring hemoglobin levels. The use of this automated analyser offers the advantage of considerable time savings and greater accuracy compared to manual techniques. It works by separating the blood cells to provide a specific analysis for each patient.

The results of the complete blood count (CBC) are an essential tool for diagnosing anemia in children, complementing clinical signs such as pallor, fatigue or skin and nail abnormalities. The parameters analysed include hemoglobin, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), which help determine the type of anemia.

The complete blood count is performed using a blood sample obtained by venipuncture and collected in a tube containing an EDTA (ethylenediaminetetraacetic acid) anticoagulant.

7.8. Measurement of triglycerides and total cholesterol

➤ Triglycerides measurement

This was carried out using the GPO-PAP (Glycerol Phosphate Oxidase–Peroxidase–Aminophenazone) enzymatic method. This technique is based on the hydrolysis of triglycerides into glycerol and fatty acids, followed by the oxidation of glycerol, resulting in the formation of hydrogen peroxide. This then reacts with a chromogen in the presence of peroxidase to produce a color that can

be measured by spectrophotometry. The intensity of this color is directly proportional to the concentration of triglycerides present in the serum.

➤ **The total cholesterol assay**

Was performed using the CHOD-PAP (Cholesterol Oxidase–Peroxidase Aminophenazone) enzymatic method. This method is based on the hydrolysis of cholesterol esters followed by the oxidation of free cholesterol by cholesterol oxidase, producing hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide reacts with a chromogen to form a colored compound, the intensity of which is proportional to the concentration of total cholesterol in the sample. Absorbance was measured by spectrophotometry at the appropriate wavelength.

The results were expressed in **g/L**. These parameters were selected due to their involvement in lipid metabolism and their potential relationship with the inflammatory and metabolic mechanisms associated with osteoporosis.

7.9. Measurement of serum calcium

Serum calcium was measured using venous blood samples collected in dry tubes without anticoagulant. After clotting, the samples were centrifuged at 3000 rpm for 10 minutes to obtain the serum. Calcium concentration was determined by using a colorimetric method with Arsenazo III reagent, which forms a colored complex with calcium ions. The intensity of the color, which is proportional to the calcium concentration, was measured at a wavelength of 650 nm using an automated biochemistry analyser (such as the Cobas or Mindray). Calibration was performed using standard solutions, and internal quality controls were employed to ensure the reliability of the results. Calcium concentrations were expressed in mmol/L and interpreted in relation to physiological reference values. This parameter was studied as part of the assessment of calcium metabolism, in relation to intestinal dysbiosis and the risk of osteoporosis (Gindler and King, 1972 ; Burtis and Ashwood, 2008).

7.10. Ferritin Assay

Quantitative measurement of serum ferritin is used as an indicator of iron status and aids in the diagnosis of iron deficiency or iron overload in children. It is performed using the IMMULITE 2000 analyser.

➤ **Principle :**

The measurement of ferritin using the IMMULITE 2000 system is a two-step immunoenzymatic method performed in a solid-phase format. The sample is mixed with reagent R1 (buffer), then with reagent R2 (anti-ferritin antibodies bound to latex particles), resulting in the formation of antigen–antibody complexes. The analyser is equipped with a computer system that controls the reagents, dilutions, the progress of the analyses and the identification of samples. Results are expressed in **ng/mL**.

Serum iron (serum ferritin) is measured using a colorimetric method.

7.11. Inflammatory markers

7.11.1. CRP measurement

Inflammatory markers were assessed by measuring C-reactive protein (CRP), considered a major biological indicator of systemic inflammation. This parameter was measured in serum obtained from a venous blood sample taken whilst fasting, followed by centrifugation to separate the blood components.

CRP measurement was performed using the immunoturbidimetric method, widely used in clinical biochemistry for its sensitivity and analytical reliability. This technique is based on the antigen–antibody reaction between the CRP present in the sample and specific anti-CRP antibodies contained in the reagent. The formation of immune complexes leads to an increase in the turbidity of the solution, the intensity of which is directly proportional to the concentration of serum CRP.

This turbidity was measured using automated spectrophotometry at a suitable wavelength, and the results were expressed in **mg/L**.

7.11.2. IL-10 Assay

The interleukin-10 (IL-10) assay was performed using serum samples obtained following blood collection into dry tubes. After clotting, the samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, which was then aliquoted and stored at -20°C until analysis.

The serum concentration of IL-10 was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) technique in accordance with the manufacturer's recommendations for the kit used. This immunoenzymatic method is based on the 'sandwich' assay principle, in which IL-10-specific antibodies are pre-immobilised on the bottom of the wells of a microplate. The IL-10 present in the

samples and standards binds to these antibodies during incubation. After several washing steps to remove unbound molecules, an enzyme-conjugated detection antibody is added to form an antigen–antibody complex. The addition of the chromogenic substrate tetramethylbenzidine (TMB) results in the development of a colour whose intensity is proportional to the concentration of IL-10 in the sample. The reaction is stopped by adding a stop solution, after which the optical density is measured using a microplate reader at a wavelength of 450 nm.

IL-10 concentrations were calculated from a calibration curve obtained using standards of known concentrations and expressed in picograms per milliliter (**pg/mL**). IL-10 is a major anti-inflammatory cytokine involved in the modulation of the immune response and bone remodeling, its measurement was included in this study to assess the role of immuno-inflammatory mechanisms in the pathophysiology of osteoporosis.

The choice of this marker is explained by its role in assessing low-grade chronic inflammation, which is often implicated in metabolic imbalances, alterations in the gut microbiota, and the pathophysiology of osteoporosis.

8. Ethical considerations

- Respect for anonymity and confidentiality
- Voluntary participation
- Implied consent via questionnaire
- Use of data for scientific purposes only

9. Statistical analysis

The results are presented as mean $\pm$ standard deviation. Following verification of the normal distribution,

The comparison of means between the two groups studied (the population with osteoporosis)

Is carried out using Student's t-test. Differences between the means are considered significant when P is,

Less than 0.05. All statistical calculations are performed using STATISTICA software.

Results and interpretations

I. Analysis and interpretation of the questionnaire results

I.1. Anthropometric parameters

I.1.1. Age and BMI

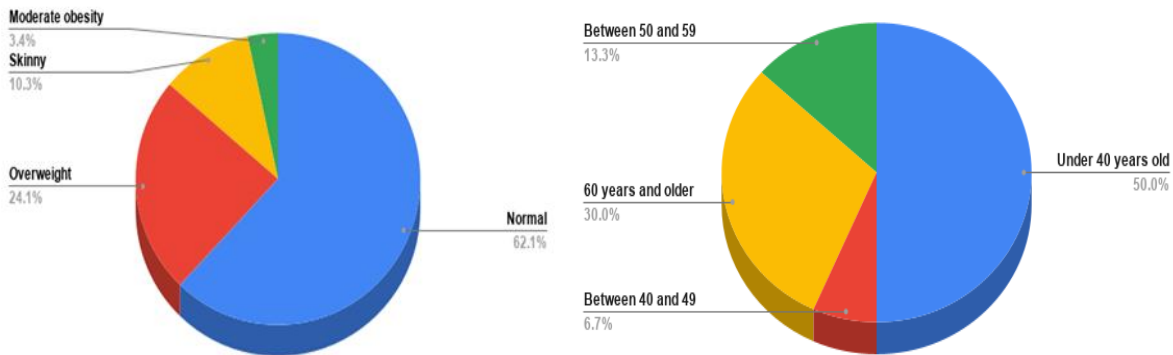


Figure 10. Distribution of the study population by age and body mass index (BMI).

The distribution of participants by age shows a predominance of women under 40 (50%), followed by women aged 60 and over (30%). The 50–59 and 40–49 age groups account for 13.3% and 6.7% respectively. The significant presence of older participants is a key factor, as advancing age is a major risk factor for osteoporosis due to the progressive decline in bone density.

The majority of participants have a normal BMI (62.1%), whilst 24.1% are overweight, 10.3% are underweight and 3.4% are moderately obese. These results suggest an overall satisfactory nutritional status in the study population. However, being underweight may contribute to a reduction in bone mass and increase the risk of osteoporosis.

I.1.2. Physical activity

Regular physical activity.

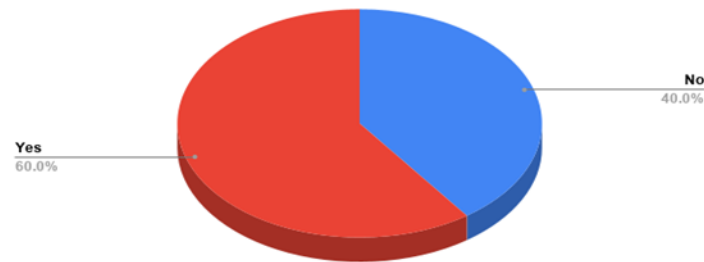


Figure 11. Distribution of the study population according to regular physical activity.

Physical activity is more common among non-osteoporotic women (~55%) than among osteoporotic women (~45%).

I.1.3. Duration of osteoporosis

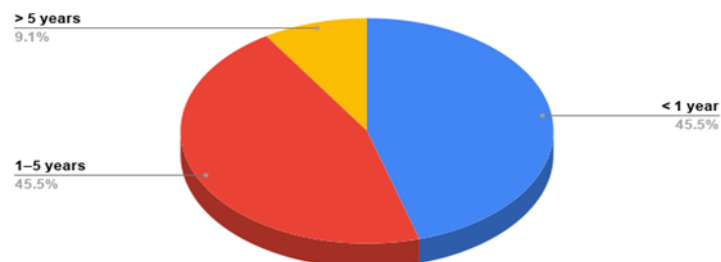


Figure 12. Breakdown of the study population by duration of osteoporosis.

With regard to the duration of osteoporosis, the majority of cases are recent, with ~45% having developed within the last year and ~45% between 1 and 5 years ago, whilst long-standing cases (>5 years) remain in the minority (~10%).

I.1.4. Fractures

Number of patients who have already experienced a

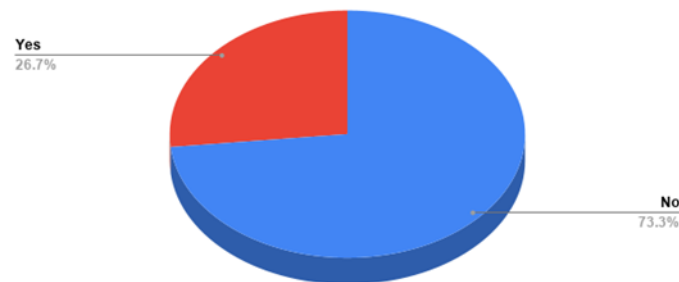


Figure 13. Distribution of patients according to history of fractures.

The results show that the majority of patients (73.3%) had never suffered a fracture, whilst 26.7% had already suffered a fracture. These data suggest that fractures remain present in a proportion of osteoporotic patients, confirming the impact of bone fragility associated with osteoporosis.

I.1.5. Family history

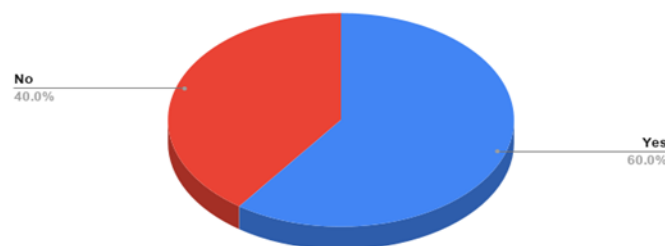


Figure 14. Distribution of patients according to family history of osteoporosis.

The results indicate that 60% of patients have a family history of osteoporosis, compared with 40% reporting no family history. This predominance suggests a possible role for genetic factors in the development of osteoporosis within the study population.

I.1.6. Antibiotic use

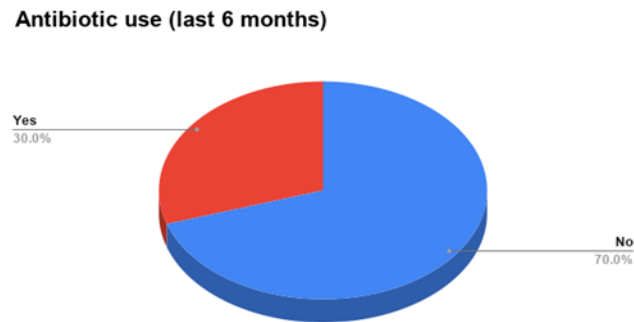


Figure 15. Breakdown of participants by antibiotic use over the past six months.

The results show that 70% of participants had not used antibiotics in the past six months, whilst 30% reported having taken them. These data indicate a low frequency of antibiotic use within the study population.

I.1.7. Digestive disorders



Figure 16. Distribution of participants suffering from digestive disorders.

This graph shows the distribution of patients suffering from digestive disorders in the study. The majority of participants have digestive disorders, at a rate of 60%, whilst 40% of patients do not suffer from any digestive disorders. These results suggest a relatively high prevalence of digestive disorders among the participants studied.

I.1.8. Digestive symptoms (bloating, diarrhoea, etc.)

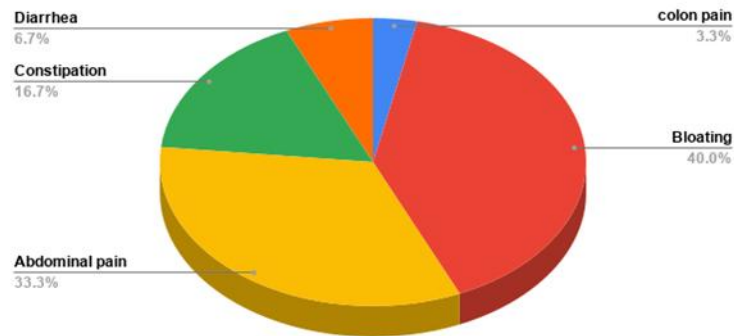


Figure 17. Distribution of various digestive symptoms among study participants

This graph shows the distribution of various digestive symptoms among the study participants. Bloating is the most common digestive symptom at 40%, followed by abdominal pain at 33.3%. Constipation is moderately common at 16.7%, whilst diarrhoea is less common at 6.7%. In contrast, colon pain is the least frequently reported symptom at just 3.3%.

I.1.9. Frequency of symptoms

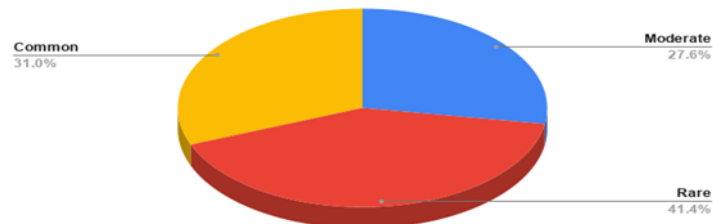


Figure 18. Breakdown of participants by frequency of digestive symptoms.

This graph illustrates the frequency of digestive problems among the study participants. Rare digestive problems were the most common category, accounting for 41.4%, followed by frequent problems at 31%. Moderately frequent digestive disorders are the least common, at 27.6%. These results show that the majority of participants experience occasional digestive disorders rather than regular or moderate symptoms.

I.1.10. Vitamin D testing carried out

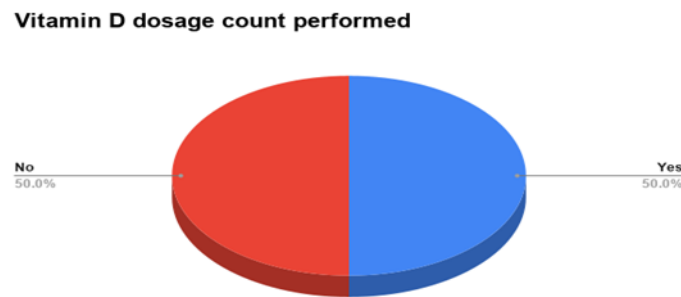


Figure 19. Distribution of participants according to whether they underwent vitamin D testing

This graph shows the distribution of participants who underwent a vitamin D test.

The results indicate that 50% of participants underwent a vitamin D test, whilst the remaining 50% did not. The distribution is therefore perfectly balanced between the two groups, suggesting the absence of a dominant trend regarding the use of this test in the study population. This may also reflect the fact that vitamin D testing is not yet carried out systematically enough among participants.

I.1.11. Medical treatments

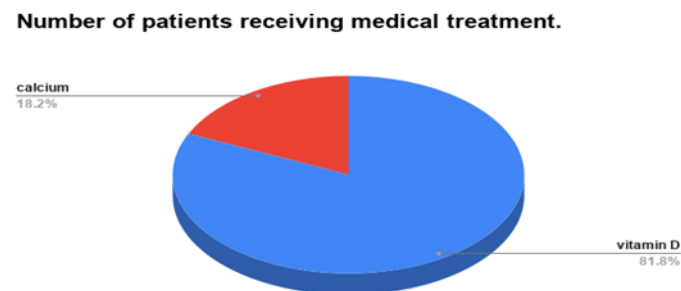


Figure 20. Distribution of participants by medical treatment received

The vast majority of patients are taking vitamin supplements (81.8%), suggesting that this is the primary treatment in this population. In contrast, calcium is prescribed or taken much less frequently (18.2%), and probiotics are not used at all (0%), which may indicate that they are not part of the treatment strategy adopted.

I.1.12. Improvement

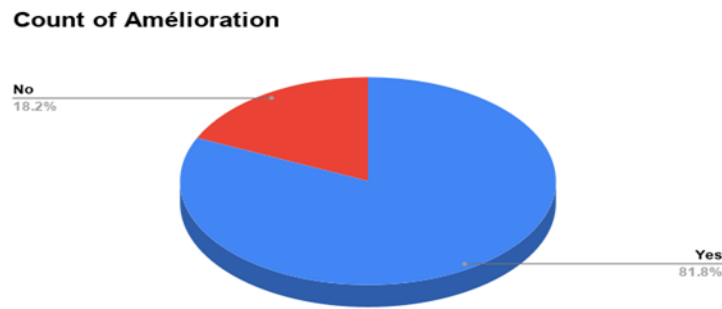


Figure 21. Distribution of patients showing improvement following vitamin D and/or calcium supplementation

The findings show that 81.8% of patients show improvement after taking vitamin D and/or calcium. In contrast, 18.2% of subjects showed no improvement. These results suggest an overall beneficial effect of supplementation in the management of the patients studied.

1.1.13. Use of dietary supplements by participants

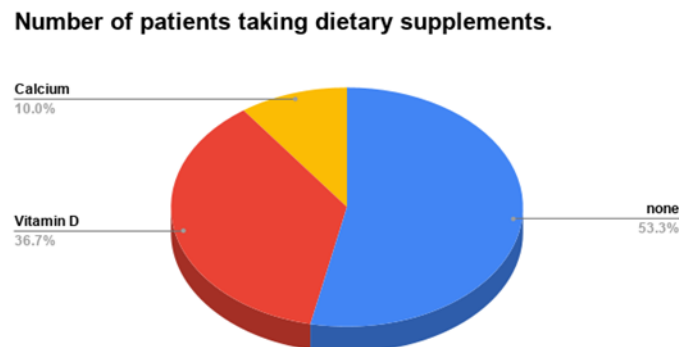


Figure 22. Distribution of participants according to the use of dietary supplements (vitamin D and calcium)

The results indicate that more than half of the participants (53.3%) do not take any dietary supplements. In contrast, 36.7% of subjects consume vitamin D, whilst only 10% use calcium supplements. These data suggest a low frequency of supplementation, particularly for calcium, within the study population.

II. Markers of oxidative/antioxidant status in cases and controls (Figure 23, Table A1 in the Annexe)

Erythrocyte malondialdehyde (MDA) levels in control women and women with osteoporosis (Figure 23)

Erythrocyte malondialdehyde (MDA) levels are elevated in women with osteoporosis compared with control women. This increase is significant ($p < 0.05$), reflecting a difference in the level of lipid peroxidation between the two groups.

Reduced glutathione (GSH) levels in erythrocytes in control women and women with osteoporosis (Figure 23)

The erythrocyte reduced glutathione (GSH) content is reduced in women with osteoporosis compared to control women. This reduction suggests an impairment of antioxidant defenses in osteoporotic patients.

Erythrocyte catalase content in control women and women with osteoporosis (Figure 23)

The erythrocyte catalase content is also reduced in women with osteoporosis compared to control women. This reduction indicates a decrease in enzymatic antioxidant activity in women with osteoporosis.

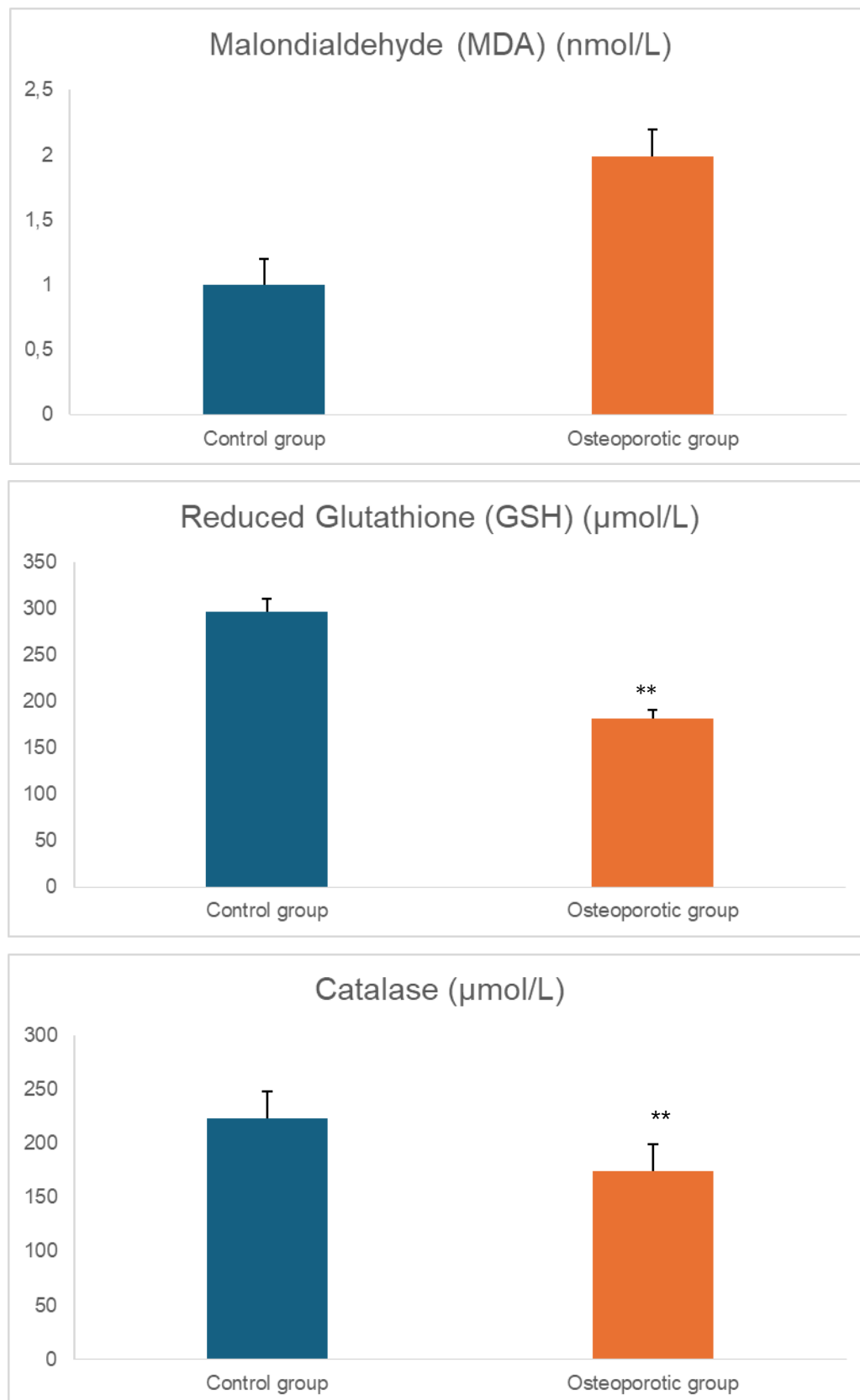


Figure 23. Comparison of markers of oxidative stress between the control group and the osteoporotic group: malondialdehyde (MDA), reduced glutathione (GSH) and catalase.

Values are expressed as mean $\pm$ standard deviation.

Comparisons between groups were performed using Student's t-test. A difference is considered statistically significant when $p < 0.05$.

III. Biochemical parameters in the study population:

III.1. Plasma triglyceride and cholesterol levels:

Plasma triglyceride and cholesterol levels are shown in Figure 24 and Table A1 in the Annexe.

The results show a non-significant decrease in triglycerides in the osteoporotic group compared with the control group. Furthermore, there is no significant difference in plasma cholesterol levels between the osteoporotic group and the control group.

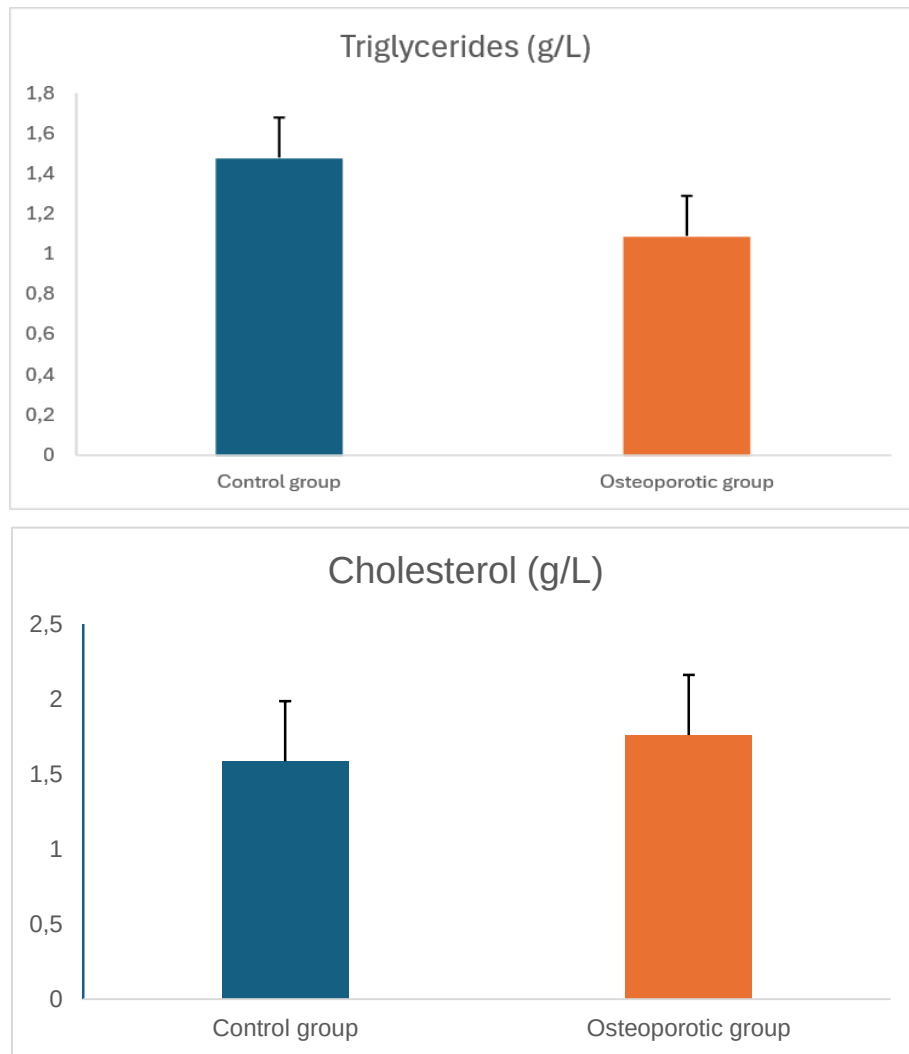


Figure 24. Plasma triglyceride and cholesterol levels in the study population.

Values are expressed as mean $\pm$ standard deviation.

Comparisons between groups were performed using Student's t-test. A difference is considered statistically significant when $p < 0.05$.

III.2. Serum vitamin D levels:

Serum vitamin D levels are presented in Figure 25 and Table A1 in the Annexe. The results show a highly significant decrease in vitamin D levels in the osteoporotic population compared with the control population.

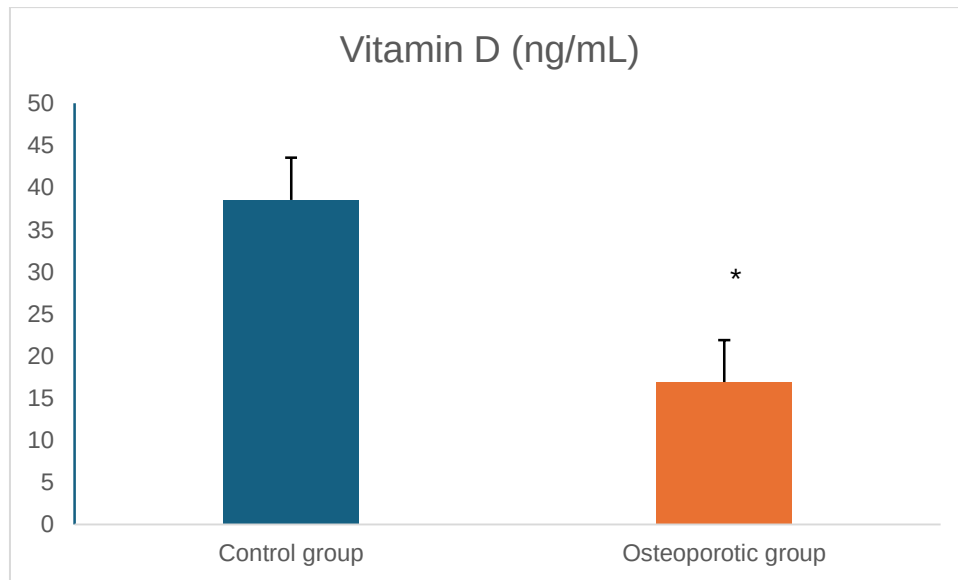


Figure 25. Serum vitamin D levels in the study population.

Values are expressed as mean $\pm$ standard deviation.

Comparisons between groups were performed using Student's t-test. A difference is considered statistically significant when $p < 0.05$.

III.3. Plasma calcium levels:

Plasma calcium levels are presented in Figure 26 and Table A1 in the Annexe.

The results show that plasma calcium levels are lower in the osteoporotic group than in the control group.

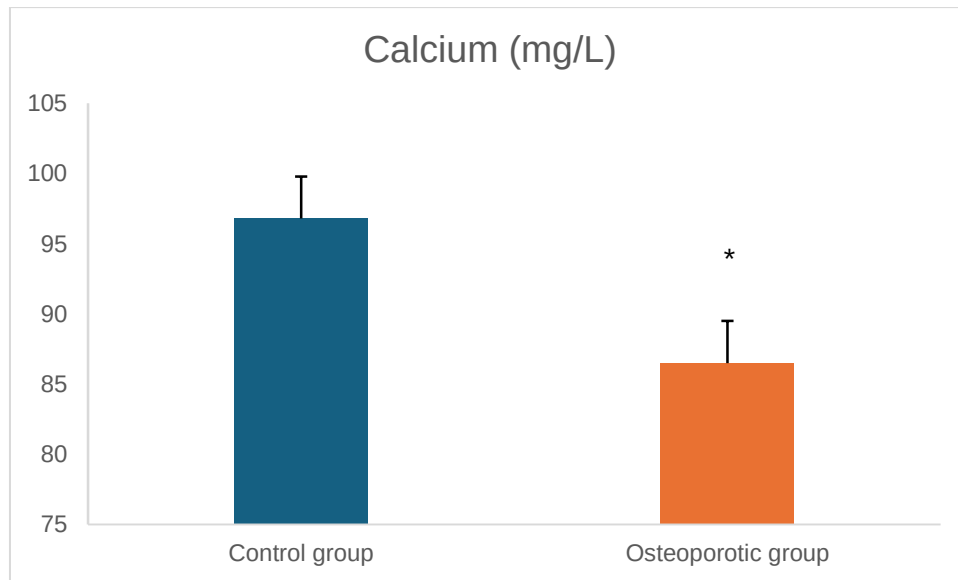


Figure 26. Plasma calcium levels

Values are expressed as mean $\pm$ standard deviation.

Comparisons between groups were performed using Student's t-test. A difference is considered statistically significant when $p < 0.05$.

III.4. Serum ferritin levels:

Serum ferritin levels are shown in Figure 27 and Table A1 in the Annexe.

Our results indicate a highly significant reduction in ferritin levels in the osteoporotic group compared with the control group.

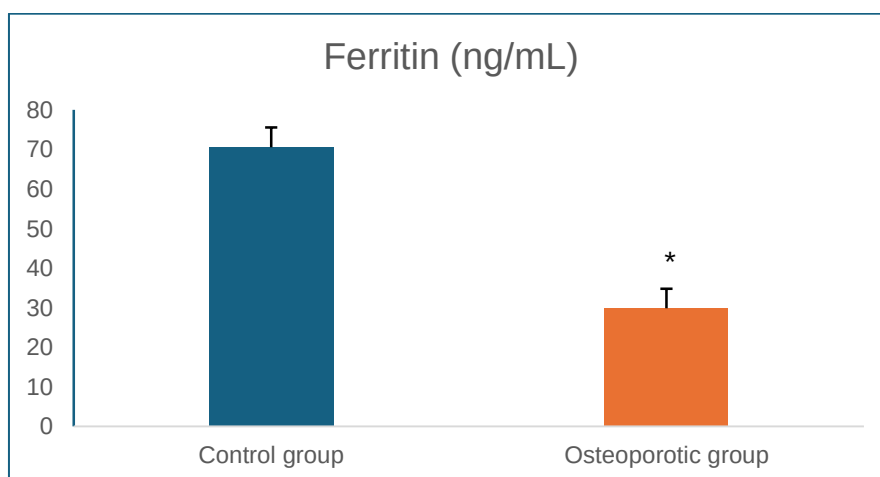


Figure 27. Serum ferritin levels in the study population.

Values are expressed as mean $\pm$ standard deviation.

Comparisons between groups were performed using Student's t-test. A difference is considered statistically significant when $p < 0.05$.

III.5. Serum C-reactive protein levels:

Serum CRP levels are presented in Figure 28 and Table A1 in the Annexe.

Our results show a significant increase in CRP concentrations in the osteoporotic population compared with the control population.

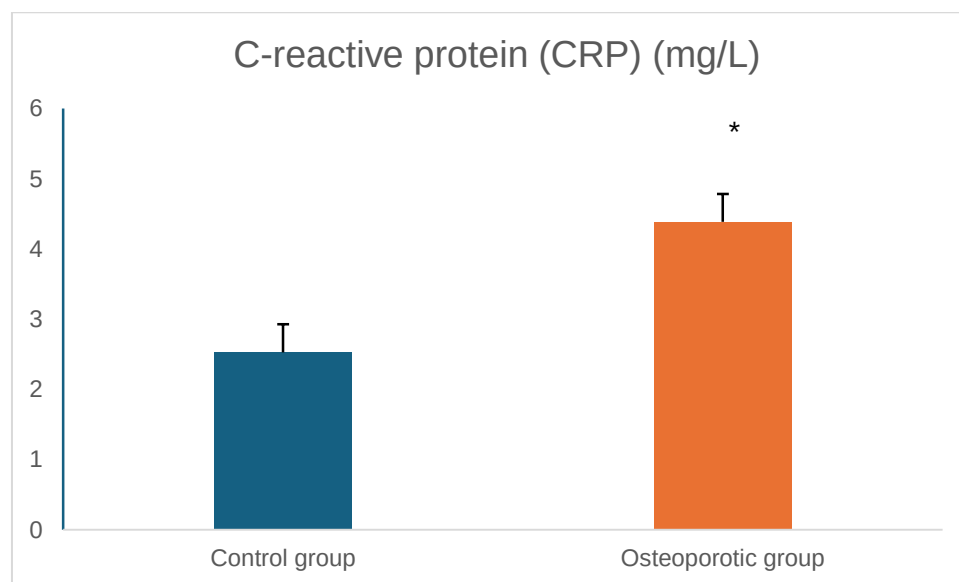


Figure 28. Serum CRP levels in the study population.

Values are expressed as mean $\pm$ standard deviation.

Comparison between groups was performed using Student's t-test. A difference is considered statistically significant when $p < 0.05$.

III.6. Plasma IL-10 levels:

Plasma IL-10 levels are presented in Figure 29 and Table A1 in the Annexe.

The results indicate that plasma IL-10 levels are reduced in the osteoporotic population compared with the control population.

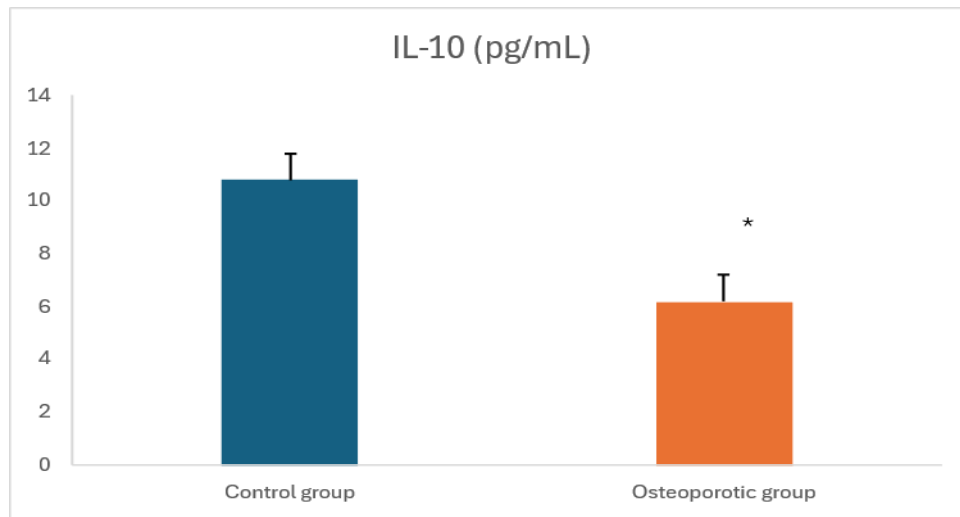


Figure 29. Plasma IL-10 levels.

Values are expressed as mean $\pm$ standard deviation.

Comparisons between groups were performed using Student's t-test. A difference is considered statistically significant when $p < 0.05$.

Discussion

This study, conducted among participants from the wilaya of Tlemcen—including both women and men, as well as control subjects and those with osteoporosis—yielded several important findings regarding the factors associated with osteoporosis. This study is based on both a survey and biological analyses, as well as findings on oxidative stress, incorporating an investigation into the link between gut dysbiosis and osteoporosis ; it may serve as a preliminary basis for future nutritional and biological studies among individuals affected by this condition. This work has also helped to update existing data and better document the current state of the issue in the wilaya of Tlemcen, highlighting the value of an integrated approach combining clinical, biological and nutritional data to better understand the interaction between gut microbiota imbalance and bone fragility.

- In our study, the majority of participants had a normal BMI. These findings are consistent with those of **(Jianping et al., 2026)**, who reported that a low BMI increases the risk of osteoporosis. This relationship may be explained by the fact that being underweight reduces the mechanical stress exerted on the bone as well as the production of oestrogen by adipose tissue, which promotes bone resorption. However, being overweight may also contribute to an imbalance in the microbiota and to a state of inflammation that is detrimental to bone quality.

- Several participants had a family history of osteoporosis, suggesting a genetic predisposition, in line with the findings of **(Kanis et al., 2004)**. This susceptibility could be explained by disturbances in bone metabolism affecting bone mineral density and bone remodelling, thereby promoting bone fragility.

- In our study, some participants reported having taken antibiotics in the past six months. These findings are consistent with those of **(Zaura et al., 2015)**, who demonstrated that antibiotic use can disrupt the gut microbiota and indirectly affect bone health. This mechanism could promote inflammation and increase bone resorption, thereby contributing to bone fragility.

- Regarding management, half of the participants had had their vitamin D levels tested, despite its essential role in bone mineralisation. Our results are consistent with those of **(Rizzoli et al., 2014)** and **(Migliorini et al., 2025)**, who demonstrated the benefits of vitamin D and calcium supplementation in postmenopausal osteoporosis. This mechanism could be explained by the role of vitamin D in the intestinal absorption of calcium and the maintenance of bone metabolism, thereby contributing to improved bone mineralisation. However, the low frequency of supplementation observed in our population could promote bone demineralisation.

- According to our findings, digestive disorders were more common among participants with osteoporosis than among the control group. These results are consistent with those of **(Xu et al., 2023)**, who demonstrated that gastrointestinal diseases are a factor influencing osteoporotic fractures in Chinese postmenopausal women. This association could be explained by reduced intestinal absorption of calcium and vitamin D, impaired bone metabolism, and a chronic inflammatory state promoting bone resorption and bone fragility.
- We also noted that, according to our results, a decrease in the use of vitamin D testing was observed in our population despite its importance in the management of osteoporosis. These findings are consistent with those of **(Robert et al., 2014)**, who demonstrated that vitamin D deficiency leads to reduced intestinal absorption of calcium, which is responsible for impaired bone mineralisation. This imbalance is accompanied by increased bone resorption, contributing to bone fragility and the risk of fractures. The authors concluded that maintaining adequate levels of vitamin D is essential for preserving the balance of bone remodelling and reducing the risk of osteoporotic complications.
- The results of our study showed a significant increase in MDA levels in patients with osteoporosis compared with the control group. These findings are consistent with those reported by **(Riegger et al., 2023)**, who also observed elevated MDA levels in patients with osteoporosis, reflecting an imbalance in the oxidant-antioxidant balance and increased lipid peroxidation. This increase could be explained by excessive production of reactive oxygen species, promoting oxidative stress and contributing to disturbances in bone remodelling. Thus, MDA appears to be an important marker involved in the physiology of osteoporosis and in the worsening of bone fragility.
- We also observed a significant decrease in catalase and GSH activity in osteoporotic patients compared to controls, where these parameters remained within normal ranges. These results are consistent with several studies, notably those by **(Iantomasi et al., 2023)** and **(Marcucci et al., 2023)**, which also observed a decline in antioxidant defences associated with high oxidative stress in osteoporosis. This reduction in antioxidant systems may be explained by an increase in reactive oxygen species, leading to an imbalance in cellular oxidative status. In this context, bone cells become more sensitive, with increased osteoclast activity and reduced bone formation by osteoblasts, which promotes the progression of osteoporosis.
- Our results show that plasma triglyceride levels are non-significantly reduced in the osteoporotic population compared with controls. However, other studies conducted in subjects aged ≥ 50 years have observed a positive correlation between TG levels and the onset of this bone disease. Furthermore, the majority of participants (82.5%) had chronic conditions that could influence both

lipid metabolism and osteoporosis (**Kan et al., 2021**). The low TG levels observed in our study may be linked to a low-fat diet among patients with osteoporosis.

- In our study, no significant difference in plasma cholesterol levels was observed between the osteoporosis group and the control group, suggesting no association between the lipid profile and osteoporosis in our population. In contrast, the cross-sectional study by (**Cao et al. 2023**), conducted in adults with a control group, showed that an increase in total cholesterol is associated with a significant decrease in bone mineral density ($p < 0.001$) in both sexes. These results suggest that hypercholesterolaemia may contribute to bone fragility via mechanisms involving chronic inflammation.

- One of the key parameters in our study is vitamin D. The results show a very significant reduction in vitamin D levels in the osteoporotic population compared with the control group, suggesting that vitamin D deficiency may be associated with intestinal malabsorption linked to gut microbiota dysbiosis. These findings are consistent with those of (**Chen et al., 2024**), who also observed a significant decrease in serum 25(OH)D levels in postmenopausal women associated with a significant increase in PTH, indicating hyperparathyroidism. This elevation in PTH is correlated with a decrease in bone mineral density (BMD), indicating a worsening of bone fragility.

- In addition to vitamin D, calcium is a key parameter involved in bone metabolism and was also assessed in our study. (**Liu et al., 2019**) found a positive association between calcium concentrations and bone mineral density in elderly subjects. The research team emphasises that any disruption to calcium homeostasis may contribute to impaired bone structure and the development of osteoporosis, which aligns with our analysis revealing lower plasma calcium levels in subjects with osteoporosis compared to control subjects, highlighting the essential role of calcium in maintaining bone health.

- Our results show a highly significant reduction in ferritin levels in the osteoporotic group compared with the control group, which may reflect an impairment in iron metabolism involved in bone remodelling, particularly osteoblast activity and bone collagen synthesis. Meanwhile, the study by (**Peng et al., 2022**) shows that iron overload may promote oxidative stress, inhibit osteoblast activity and stimulate bone resorption, thereby contributing to the development of osteoporosis in women over 45 years of age.

- Analysis of our CRP results highlights a significant increase in serum levels in the osteoporotic population, suggesting the involvement of a chronic inflammatory state in bone metabolism, promoting bone resorption by stimulating osteoclastic activity, which is consistent with (**Dong et al.,**

2024), who report that an increase in biomarkers of systemic inflammation in participants with osteoporosis is associated with activation of the osteoclastic pathway via pro-inflammatory cytokines, promoting bone resorption and the development of osteoporosis.

- Our results highlight a reduction in plasma IL-10 levels in the osteoporosis patient group compared with controls, suggesting an impairment in the immune-inflammatory regulation of bone remodelling, with reduced inhibition of osteoclast differentiation via the RANKL/OPG pathway, These findings are consistent with those reported in the study by **(Zhang et al., 2024)**, which demonstrated that disruption of the circadian clock leads to reduced activity of regulatory T cells and decreased IL-10 production, associated with significant bone loss.

Conclusion and Outlook

Osteoporosis is now a major multifactorial condition that poses a significant public health problem due to its increasing prevalence, functional complications and considerable impact on quality of life. Through this study, which examines the relationship between gut dysbiosis, oxidative stress and osteoporosis, it has been possible to highlight the involvement of several nutritional, metabolic, hormonal and environmental factors in the development of bone fragility.

Among these factors, gut dysbiosis, defined as an imbalance in the gut microbiota, can disrupt the body's homeostasis. This disruption can notably affect the absorption of nutrients essential for bone health, such as vitamin D and calcium.

This study, which aimed to determine lipid metabolic changes, inflammatory status and the oxidant/antioxidant balance through the evaluation of several biological parameters in a population with osteoporosis in the wilaya of Tlemcen, supports the theory linking these disorders to this condition.

The results clearly highlighted the presence of biological disturbances in the population with osteoporosis, notably a decrease in plasma concentrations of vitamin D and calcium, two essential elements involved in bone metabolism and mineral homeostasis.

At the same time, a significant increase in CRP was observed, indicating the presence of a low-grade systemic inflammatory state. Furthermore, the decrease in levels of IL-10, a cytokine with anti-inflammatory activity, suggests an imbalance in immune-inflammatory regulation.

Furthermore, an analysis of the digestive symptoms reported by participants highlights the frequency of gastrointestinal complaints such as bloating, constipation, abdominal pain and bowel dysfunction, suggesting a possible disruption of the balance of the gut microbiota. These observations support the hypothesis that gut dysbiosis may play an important role in the pathophysiology of osteoporosis by disrupting the absorption of nutrients essential for bone metabolism and by promoting a chronic low-grade inflammatory state.

Finally, despite certain limitations notably the relatively small sample size, the reliance on self-reported data from participants, methodological constraints, and the absence of in-depth immunological and microbiological analyses that would allow for a precise characterisation of the gut microbiota this study nevertheless opens up promising avenues for research in the field of osteoimmunology and the relationship between the gut microbiota and bone health. Building on this, future research into osteoporosis should focus on the in-depth and ongoing exploration of the

underlying mechanisms of the disease. To this end, our findings may help to establish awareness and education programmes to inform people exposed to various risk factors about the importance of diet and physical activity in maintaining bone health.

Annexe

1. Survey Questionnaire:**1. Current age**

- ☐ Under 40 years
- ☐ Between 40 and 49 years
- ☐ Between 50 and 59 years
- ☐ 60 years and above

2. Hormonal status

- ☐ Menopausal
- ☐ Non-menopausal

3. Marital status

- ☐ Single
- ☐ Married
- ☐ Divorced
- ☐ Widow

4. Number of children

.....

5. Weight (kg)

.....

6. Height (cm)

.....

7. Current BMI (Body Mass Index)

- ☐ Underweight
- ☐ Normal

- ☐ Overweight
- ☐ Moderate obesity
- ☐ Severe obesity

8. Place of residence

.....

9. Occupation

.....

10. Have you been diagnosed with osteoporosis?

- ☐ Yes
- ☐ No

11. Was the result in favor of osteoporosis?

- ☐ Yes
- ☐ No

12. If yes, for how long?

- ☐ < 1 year
- ☐ 1–5 years
- ☐ > 5 years

13. Have you ever had a fracture?

- ☐ Yes
- ☐ No

14. Family history of osteoporosis

- ☐ Yes
- ☐ No

15. Antibiotic use during the last 6 months

☐ Yes

☐ No

16. Long-term treatment (corticosteroids, etc...)

☐ Yes

☐ No

17. Do you suffer from digestive diseases?

☐ Yes

☐ No

18. Digestive symptoms

☐ Bloating

☐ Diarrhea

☐ Constipation

☐ Abdominal pain

☐ Frequent gas

19. Frequency of digestive symptoms

☐ Rare

☐ Moderate

☐ Frequent

20. Fiber consumption

☐ Low

☐ Moderate

☐ High

21. Consumption of fermented foods

- ☐ Never
- ☐ Sometimes
- ☐ Regularly

22. Probiotic consumption

- ☐ Yes
- ☐ No

23. Bowel transit disorders

- ☐ Constipation
- ☐ Diarrhea
- ☐ None

24. Abdominal pain

- ☐ Yes
- ☐ No

25. Recent antibiotic use

- ☐ Yes
- ☐ No

26. Dairy product consumption

- ☐ Low
- ☐ Moderate
- ☐ High

27. Other calcium-rich food sources

- ☐ Sardines
- ☐ Almonds
- ☐ Green vegetables

28. Do you consume foods rich in vitamin D (fish, liver, etc...)?

- ☐ Yes
- ☐ No

29. Protein intake

- ☐ Low
- ☐ Normal
- ☐ High

30. Tea or coffee after meals

- ☐ Yes
- ☐ No

31. High-salt diet

- ☐ Yes
- ☐ No

32. Do you follow a specific diet?

- ☐ Yes
- ☐ No

33. Have you ever had a vitamin D test?

- ☐ Yes
- ☐ No

34. 25-OH Vitamin D level (ng/mL)

.....

35. Do you take supplements?

☐ Yes

☐ No

36. Frequency of supplementation

☐ Daily

☐ Weekly

☐ Monthly

37. Do you take dietary supplements?

☐ Vitamin D

☐ Calcium

☐ None

38. Daily sun exposure duration

☐ < 10 min

☐ 10–30 min

☐ > 30 min

39. Time of sun exposure

☐ Morning

☐ Midday

☐ Afternoon

40. Do you use sunscreen?

☐ Yes

☐ No

41. Do you use natural remedies?

☐ Yes

☐ No

42. Purpose of use

☐ Bone health

☐ Digestive disorders

☐ Calcium/Vitamin D deficiency

43. Mode of use

☐ Food

☐ Herbal infusion

☐ Supplement

44. Frequency of use

☐ Daily

☐ Weekly

☐ Occasional

45. Duration of use

☐ < 1 month

☐ 1–3 months

☐ > 3 months

46. Perceived effectiveness

☐ Very effective

☐ Moderate

☐ Low

☐ None

47. Medical treatments used

- ☐ Calcium
- ☐ Vitamin D
- ☐ Probiotics

48. Have you noticed any improvement?

- ☐ Yes
- ☐ No

49. Regular physical activity

- ☐ Yes
- ☐ No

50. Type of physical activity

.....

51. Stress level

- ☐ Low
- ☐ Moderate
- ☐ High

52. Sleep duration

- ☐ < 6 h
- ☐ 6–8 h
- ☐ > 8 h

53. Do you frequently feel fatigued?

- ☐ Yes
- ☐ No

54. Do you experience bone or muscle pain?☐ Yes☐ No**Table A1: Summary table of oxidative stress parameters and biochemical markers (MDA, GSH, calcium, triglycerides and inflammatory markers).**

Parameter	Unit	Osteoporotic group (M ± SD)	Control group (M ± SD)
MDA	nmol/mL	1,992 ± 0,912	0,999 ± 0,215
GSH	μmol/L	181,80 ± 34,74	296,33 ± 59,62
Catalase	μmol/L	173,75 ± 10,24	223,50 ± 18,27
Cholesterol	g/L	1,76 ± 0,50	1,59 ± 0,98
Triglycerides	g/L	1,48 ± 0,37	1,09 ± 0,25
Ferritin	ng/mL	29,84 ± 41,13	70,57 ± 7,85
Vitamin D	ng/mL	16,91 ± 3,26	38,56 ± 17,14
CRP	mg/L	4,39 ± 1,11	2,53 ± 0,97
IL-10	pg/mL	6,20 ± 1,50	10,80 ± 2,90
Calcium	mg/L	86,5 ± 8,4	96,8 ± 6,92

Values are expressed as mean ± standard deviation.

The comparison between the two groups was performed using Student's T-test. The difference is considered statistically significant when $p < 0.05$.

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