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Effect of Vitamin D and Magnesium supplementation on glycemic response and insulin sensitivity in type 2 diabetic patients in wilaya of Saïda

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Dedication

I thank God who helped me and gave me the ability to reach where I am.

I dedicate a portion of his generous benevolence :

To my mother Aicha, “If my soul wandered searching for the souls of the worlds, I would not find you an atom's weight. May God prolong your life, and grant you health and well-being.

To those who make me feel that they always stand with me, in the pitfalls before successes and share my happy and sad moments.

To all those I love, those who love ,respect me from near or far and wish me well .

Finally, my deepest respect to my teachers in all

cycles of my schooling which enlightened me on the path to knowledge

Acknowledgement

First of all, I would like to thank God powerful and merciful, who gave me strength and the patience to carry out this work.

I would lik to thank

Mr Nasr-Eddine Kebir , for agreeing to supervise me , thank you for your availability, your advice and your constructive comments.

Mr Lasri Boumediene, president of the jury, for accepting the responsibility of chairing the jury and judging this work.

Mr Adli Djallal Eddine Houari, for the honor you do me by agreeing to examine this work in order to evaluate it.

Finally, we express our gratitude to all those who helped us directly or from afar.

Abreviations list

ACDP2 : A ncient conserved domain protein 2.

AC : Adenylate cyclase.

ACDP2 : Ancient conserved domain protein 2.

ATP : Adenosine triphosphate.

CNS : Central nervous system.

CYP27B1 : Cytochrome P450 Family 27 Subfamily B Member 1.

DBP : Dibutil Phathalat .

eNOS : endothelial nitric oxide synthase.

ERK : extracellular signal-regulated kinase.

GI : Glycemic index.

GLP-1: Glucagon-like peptide 1.

GLUT2 : Glucose transporter type2.

GLUT4 : Glucose transporter type 4.

GPCRs : G-protein coupled receptors.

GSIS : glucose-stimulated insulin secretion.

IDD : Insulin-dependent diabet.

IGFs : Insulin-like growth factors.

IMGU : insulin-mediated glucose uptake.

IR : Insulin receptor.

KATP : ATP-sensitive potassium channels.

MagT1 : Magnesium transporter 1.

MAPK : Mitogen-activated protein kinase.

MgD : Magnesium deficiency.

Mrs2p : Mitochondrial RNA splicing 2 protein.

NF-kB : Nuclear factor-kappa B.

NO : nitric oxide.

PFK-1 : Phosphor fructo kinase 1.

PKC : Protein kinase C.

PLCγ : Phospholipase C gamma.

reactive oxygen species (ROS).

PI3K : Phosphatidylinositol 3-kinase.

PPARδ : Peroxisome proliferator activator receptor δ.

PPARγ : Peroxisome proliferator activator receptor γ.

PTEN : phosphatase and tensin homolog.

PTH : Parathyroid hormone.

PXR : Pregnane X receptor.

RAAS : Renin-angiotensin aldosterone system.

ROS : reactive oxygen species.

RXR : Retinoid X receptor.

S6K : S6-kinase .

SLC41A1 : Solute Carrier Family 41 Member 1.

SLC41A 2 : Solute Carrier Family 41 Member 2.

SUR1 : Sulfonylurea receptor 1.

TNF-alpha : Tumour Necrosis Factor alpha.

T2D : Type 2 diabet.

TRP : Transient receptor potential.

TRPM6 : Transient Receptor Potential Cation Channel Subfamily M Member 6.

TRPM7 : Transien Receptor Potential Cation Channel Subfamily M Member 7.

UV : ultraviolet.

VDBP : Vitamin D Binding Protein .

VDD : Vitamin D deficiency .

VDR : vitamin D receptor.

VDRE : vitami n D response element .

VitD : Vitamin D.

1,25(OH)2D : 1,25-dihydroxyvitamin D.

25(OH)D : 25-hydroxyvitamin D **.**

7-DHC : 7-dehydrocholesterol .

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Résumé

Le diabète est une maladie courante dans le monde, le diabète de type 2 est le plus répandu, il représente 90 à 95 % ; Le magnésium est un cofacteur impliqué dans la glycolyse et l'activation de l'utilisation de l'insuline.

La vitamine D est une pro-hormone. La vitamine D peut jouer un rôle dans la défense contre le diabète de type 2, c'est un composant essentiel de la santé humaine et est principalement produite dans la peau sous l'influence du soleil.

La vitamine D et le magnésium sont d'importants régulateurs de l'homéostasie du glucose et jouent à leur tour un rôle crucial dans la gestion du DT2, et jouent un rôle dans l'amélioration de la sensibilité à l'insuline et dans la promotion du contrôle glycémique. Cette recherche a examiné si le magnésium et la vitamine D avaient une association avec la réponse glycémique et la sensibilité à l'insuline dans le diabète de type 2 et l'effet de leur carence et de leur supplémentation sur les patients diabétiques de type 2.

Le but de notre travail est d'évaluer le statut en vitamine D chez les patients diabétiques et la population de la wilaya de Saïda et de distinguer les différents facteurs influençant sa carence, puis d'établir des corrélations entre le statut en vitamine D et l'implication de la maladie diabétique.

Les résultats obtenus dans notre étude prospective montrent que 64% de nos patients diabétiques présentent une carence en vitamine D dont une majorité de femmes (68%) . Nos résultats ont également montré que nos patients ne consomment pas suffisamment d'aliments riches en vitamine D. Mais les données sur la relation entre les niveaux d'insuline, de vitamine D et de magnésium sont insuffisantes.

Mots clés : Diabète, Diabète de type 2 (DT2), Magnésium, Vitamine D, Sensibilité à l'insuline, Résistance à l'insuline, Contrôle glycémique.

Abstract

Diabetes is common disease occuring around the world , diabetes type 2is the most common it represent 90 to 95% ; Magnesium is a co-factor involved in glycolysis and activation of insulin use.

Vitamin D is a pro hormone Vitamin D may play a role in defense against type 2 diabetes, it is an essential component of human health and is mainly produced in the skin under the influence of sunlight.

Vitamin D and magnesium are important regulators of glucose homeostasis and, in turn, play a crucial role in the management of T2DM, and play role in improving insulin sensitivity and promoting glycemic control, This research investigated if magnesium and vitamin D had an association with glycemic response and insulin sensetivity in type 2 diabetes and effect of their dificiency and supplementation on type 2 diabetics patients .

The aim of our work is to evaluate the vitamin D status in diabetic patients and population in wilaya of Saida and distinguish the different factors influencing its deficiency, then to establish correlations between vitamin D status and the involvement of diabetes disease.

The results obtained in our prospective study show that 64% of our diabetic patients have a vitamin D deficiency, the majority of whom are women (68%). Our results also showed that our patients do not consume enough foods rich in vitamin D . But data on the relationship between levels of Insulin, vitamin D and magnesium are insufficien.

Keywords: Diabetes, Type 2 diabetes (T2D) , Magnesium, Vitamin D, Insulin sensitivity, Insulin resistance, Glycemic control.

ملخص

مرض السكري هو مرض شائع يحدث في جميع أنحاء العالم، ومرض السكري من النوع الثاني هو الأكثر شيوعاً حيث يمثل 90 إلى 95٪؛ المغنيسيوم هو عامل مساعد يشارك في تحلل السكر وتفعيل استخدام الأنسولين.

فيتامين د هو هرمون مؤيد قد يلعب فيتامين د دورًا في الدفاع ضد مرض السكري من النوع 2، فهو عنصر أساسي لصحة الإنسان ويتم إنتاجه بشكل أساسي في الجلد تحت تأثير أشعة الشمس.

يعد فيتامين د والمغنيسيوم منظمين مهمين لاستتباب الجلوكوز، ويلعبان بدورهما دورًا حاسمًا في إدارة مرض السكري من النوع الثاني. ويلعبان دورا في تحسين حساسية الأنسولين وتعزيز السيطرة على نسبة السكر في الدم،يـد رس هذا البحث فيما إذا كان للمغنيسيوم وفيتامين د علاقة مع استجابة نسبة السكر في الدم وحساسية الأنسولين في مرض السكري من النوع الثاني وتأثير نقصهما ومكملاتهما على مرضى السكري من النوع الثاني.

الهدف من عملنا هو تقييم حالة فيتامين د لدى مرضى السكري والسكان في ولاية سعيدة وتمييز العوامل المختلفة التي تؤثر على نقصه، ثم تحديد الارتباطات بين حالة فيتامين د والإصابة بمرض السكري.

تظهر النتائج التي تم الحصول عليها في دراستنا أن 64% من مرضى السكري لدينا يعانون من نقص فيتامين د، وغالبيتهم من النساء (68%) . وأظهرت نتائجنا أيضًا أن مرضانا لا يستهلكون ما يكفي من الأطعمة الغنية بفيتامين د. لكن البيانات المتعلقة بالعلاقة بين مستويات الأنسولين وفيتامين د والمغنيسيوم غير كافية.

الكلمات المفتاحية: مرض السكري، مرض السكري من النوع الثاني ، المغنيسيوم، فيتامين د، حساسية الأنسولين، مقاومة الأنسولين، التحكم في نسبة السكر في الدم.

INTRODUCTION

Diabetes mellitus, commonly referred to as diabetes, is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. This condition affects millions of people worldwide and poses a significant public health challenge due to its association with various severe complications, including cardiovascular disease, neuropathy, nephropathy, and retinopathy **(American Diabetes Association. 2023).** Among the different types of diabetes, Type 2 diabetes mellitus (T2DM) is the most prevalent, accounting for approximately 90-95% of all diagnosed cases **(International Diabetes Federation. 2023).**

Type 2 diabetes is primarily characterized by insulin resistance, where the body's cells fail to respond effectively to insulin, and by pancreatic beta-cell dysfunction leading to inadequate insulin production. Several factors contribute to the development of T2DM, including genetic predisposition, obesity, sedentary lifestyle, and poor dietary habits. Managing T2DM requires a multifaceted approach, including lifestyle modifications, pharmacotherapy, and sometimes insulin therapy. However, recent research has highlighted the potential role of certain micronutrients, such as vitamin D and magnesium, in modulating insulin sensitivity and glycemic control, thus offering additional avenues for improving diabetes management**(American Diabetes Association. 2023)**.

Vitamin D, a fat-soluble vitamin traditionally known for its role in calcium homeostasis and bone health, has emerged as a potential modulator of insulin sensitivity and glycemic control. The active form of vitamin D calcitriol, influences insulin secretion and sensitivity through various mechanisms, including the regulation of calcium flux within beta-cells, modulation of insulin receptor expression, and activation of insulin signaling pathways. Epidemiological studies have shown an inverse relationship between serum vitamin D levels and the risk of developing T2DM. Furthermore, vitamin D deficiency has been linked to impaired beta-cell function, increased insulin

resistance, and a higher prevalence of metabolic syndrome **(Chowdhury and al., 2013).**

Magnesium, an essential mineral involved in numerous biochemical reactions, including glucose metabolism and insulin signaling, also plays a critical role in maintaining normal insulin sensitivity. Magnesium acts as a cofactor for several enzymes involved in carbohydrate metabolism, and its deficiency can lead to impaired glucose uptake in cells and reduced insulin action. Low magnesium levels are commonly observed in individuals with T2DM, and hypomagnesemia is associated with poor glycemic control, increased insulin resistance, and higher incidence of diabetes-related complications. Supplementation with magnesium has shown promising results in improving insulin sensitivity and glycemic outcomes in individuals with T2DM **(Weng and al., 2014).**

The interplay between vitamin D and magnesium is also noteworthy, as magnesium is required for the activation of vitamin D. Therefore, deficiency in magnesium can exacerbate the effects of vitamin D deficiency **(Schwalfenberg and Genuis., 2011)**, further impairing insulin sensitivity and glycemic control. Given their interconnected roles, optimizing the levels of both vitamin D and magnesium through dietary intake or supplementation could be a beneficial strategy in the management of T2DM **(Codoñer-Franquesa and al,. 2020., Pilz and al2013).**

PARTIE II CHAPITRE I :

GENERALITIES

I.1.1. Vitamin D

I.1.1.1. Breif history

The discovery of vitamin D It was as early as the m id-1600s that W histler1and Glisson2 independently published scientific descriptions (in Latin!) o f rickets, caused, we now know, by a vitamin D deficiency. However neither treatise recognised the crucial role o f diet or exposure to sunlight on the prevention o f this disease. Around 200 years later, in 1840, a Polish physician called Sniadecki realised that cases of rickets occurred in children living in the industrial centre of Warsaw but did not occur in children living in the country outside Warsaw. He surmised that lack of exposure to sunlight in the narrow, crowded streets of the city where there was considerable pollution due to the burning of coal and wood, caused the disease. Such a view was poorly received at the time as it seemed inconceivable that the sun could have any useful benefit on the skeleton. The prevalence of rickets increased as industrial processes and labour expanded and, by the end of the nineteenth century, this bone disorder was estimated to affect more than 90% of children living in such urban polluted environments in Europe. Similarly, as Boston and New York City grew in the late 1800s, so did the number of cases until, in 1900, more than 80% of children in Boston were reported to suffer from rickets **(Norval, 2005).**

The discovery of vitamin D is associated with the prevention and cure of rickets. In 1924, several researchers demonstrated that ultraviolet (UV) radiation had the abilityto convert a substance present in certain foods and in the skin into a substanceactive curative against rickets. This was of the vitamin type and they called itvitamin D.

In 1936, Windaus and his colleagues managed to establish the exact structure of the active ingredient and its precursor. And it was in 1952 that Woodward synthesized vitamin D3.

In 1967, Norman discovered that vitamin D was converted by the body into a truesteroid hormone, calcitriol or 1,25-dihydroxyvitamin D (1,25(OH)2D).

In 1969, hediscovered a specific nuclear receptor for vitamin D (Vitamin D Receptor (VDR), present in the majority of tissues and organs. Later, receptors located oncell membranes **(Schlienger and Monnier, 2019)**.

Although it was surmised that vitamin D3 arises in skin via the irradiation of 7-dehydrocholesterol, this was not proven until 1978 **(DeLuca, 2014).**

So the history o f vitamin D is certainly not at an end, and more revelations will surely follow as further knowledge regarding this intriguing molecule emerges **(Norval, 2005).**

I.1.1.2. Definition of vitamin D :

Vitamin D, or calciferol, is a fat-soluble vitamin which can be considered a pro hormone due to its structure close to that of steroid hormones **(Holick, 2007).** It acts more like a hormone because it can not only be ingested from food and supplements but also be produced endogenously in humans **(Zhang and al., 2020).**

I.1.1.3. Types of vitamin D:

Important forms of vitamin D in this context are the following:

- **Ergocalciferol (vitamin D2):** is sparsely present in natural sources, but it is the major synthetic form of vitamin D.

- **Cholecalciferol (vitamin D3):** is widely distributed in animals, in which its provitamin D form, 7-dehydrocholesterol, is a normal metabolite, but in contrast it has an extremely limited distribution in plant.

- **Calcidiol (25-hydroxyvitamin D (25(OH)D)):** is produced in the liver from D2 or D3 and circulating calcidiol is a good indicator of the vitamin D status in humans.

- **Calcitriol (1,25-dihydroxyvitamin D (1,25(OH)2D)):** is the result of further hydroxylation of calcidiol in the kidney; it is the most active form of vitamin D **(van Staveren and De Groot, 2011) .**

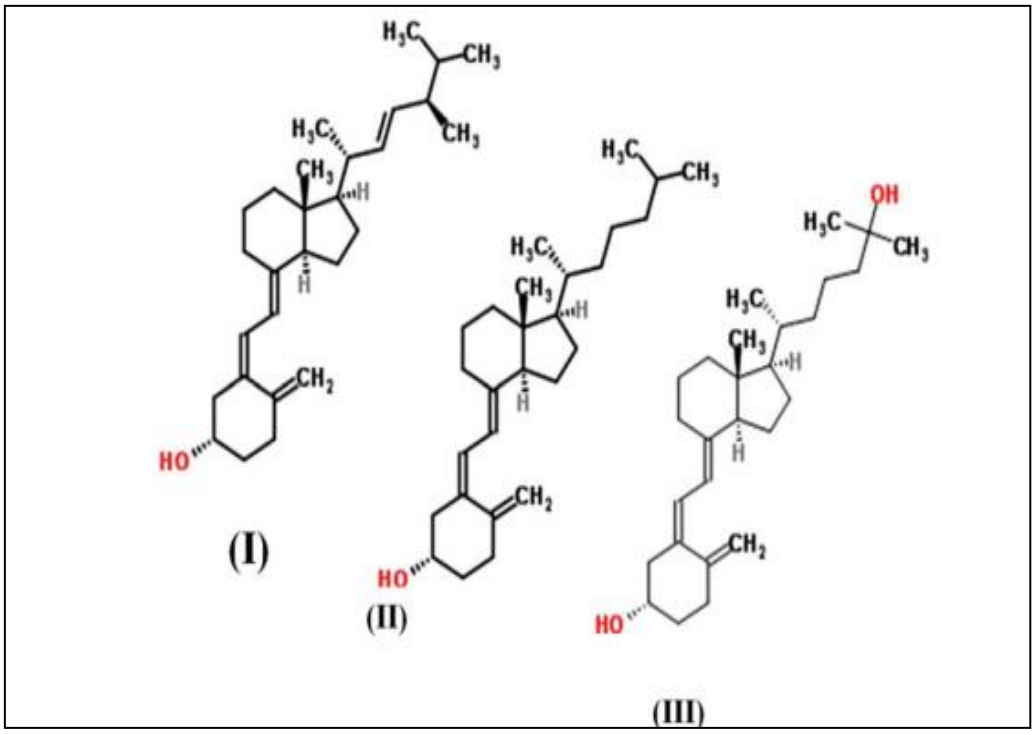

Figure 1 : chemical structures of natural dietary forms of vitamin D : (I) ergocalciferol (vitamin D2) , (II) cholecalciferol (vitamin D3) , and (III) 25- hydroxycholecalciferol**.(Borel and al., 2015).**

I.1.1.4. Chemical and physical characteristics of vitamin D:

Vitamin D is the generic descriptor for all steroids exhibiting qualitatively the biological activity of cholecalciferol. These compounds contain the intact 'A', 'C', and 'D' steroid rings being ultimately derived in vivo by photolysis of the 'B' ring of 7-dehydrocholesterol. These compounds have either of two types of isoprenoid side chains attached to the steroid nucleus at C-17 of the 'D' ring.

- Ergocalciferol and derivatives have one side chain containing nine carbons and a double bond.

- Cholecalciferol and derivatives have a side chain with eight carbons and no double bond.

- Cholecalciferol (D3) and ergocalciferol (D2) are white to yellowish powders and are insoluble in water, moderately soluble in fats, oils, and ethanol, and freely soluble in acetone, ether, and petroleum ether. Each shows a strong UV absorption, with a maximum at **264.5 nm**.

Table 1: physical and chemical Data of Vitamin D (Calciferol) **(Bockisch, M , 1998).**

Vitamin D		D_2 (Ergocalciferol)	D_3 (Cholecalciferol)
Chemical formula		$C_{28}H_{44}O$	$C_{29}H_{44}O$
Molecular weight		396.63	384.62
Form		Prisms	Needles
Soluble in		fats and oils (slightly), alcohol ether chloroform	
Insoluble in		water	
Melting point	(°C)	115–118	84–85
	(°F)	239–244	183–185
Sublimation at		0.0006 hPa	
Density (20/4)	(g/mL)	$1.399^{20}{}_4$	
λ_{max}	(nm)	264.5	264.5

[a]One international unit (1 iu) corresponds to 0.025 µg of crystalline vitamin D_2 or D_2.
Sources: Roche (1970), CRC (1976), Geigy (1968), Neumüller (1987), Wollard (1993).

-Vitamin D is sensitive to oxygen, light, and iodine. Heating and mild acidity can convert it to an inactive form. Whereas the vitamin is stable in dry form, in organic solvents and most plant oils (due to the presence of -tocopherol, which serves as a protective antioxidant), its thermal and photolability can result in losses during such processes as saponification with refluxing. However, storage and processing of foods in general do not affect vitamin D activity **(van Staveren and De Groot, 2011).**

I.1.1.5. Vitamin D dosage:

To quantify vitamin D in medicine or food, we use the unitinternational (UI) or the microgram (µg) but in biology the serum concentration or Plasma is expressed in nmol/L or (ng/mL).

These units are linked by the relationship: 100 IU=2.5 µg; 1 nmol/l=0.4 ng/ml **(Guilland, 2015).**

People can measure vitamin D intake in micrograms (mcg) or international units (IU). One mcg of vitamin D is equal to 40 IU **(Adam, 2024**).

People can measure vitamin D intake in micrograms (mcg) or international units (IU). One mcg of vitamin D is equal to 40 IU **(Adam, F. 2024**).

The recommended daily intakes of vitamin D are as follows:

Table 2: Recommendation daily intakes of Vitamin D **(Adam, 2024).**

Demographic	Recommended daily intake
Infants 0-12 months	400 IU (10 mcg)
Children 1-18 years	600 IU (15 mcg)
Adults up to 70 years	600 IU (15 mcg)
Adults over 70 years	800 IU (20 mcg)
Pregnant or lactating women	600 IU (15 mcg)

I.1.1.6. Vitamin D genetics:

Vitamin D status is hereditary in 28.8% **(Ahn and al., 2010) .** Four genes contribute tovariability of serum 25OHD concentrations. They are involved in coding enzymes keys: 7-DHC reductase, VDBP **(Shea and al., 2009).**

The human VDR gene, consisting of 14 exons, spans more than 60 kb on chromosome 12.97,98 The major VDR transcript is a 4.8 kb mRNA species, but multiple promoters and alternative splicing give rise to a multitude of less abundant transcripts that mostly vary in their 5′ untranslated region but encode the same 427-amino-acid protein. However, two of these mRNAs are translated into VDR proteins that contain an additional 23 or 50 amino acids at the N terminus. **(Bouillon and al., 2001).**

I.1.1.7. Vitamin D receptor (VDR):

VDR is a member of the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor as well as its non-genomic actions outside of the nucleus **(Cui and al., 2017., Lorenzen and al., 2017).**

VDRs are present mostly in body organs such as the colon, small intestine, bone, breast, brain, pancreas, pituitary, and muscles. The

widespread distribution of VDRs and production of calcitriol may interpret the increasing number of diseases related to vitamin D deficiency **(Narula and al.,2012)**.

Binding of calcitriol to VDR prompts the transcription of vitamin D-responsive genes (at least 913 genes) involved in cell proliferation, differentiation, function, and the reninangiotensinsystem **(Bokhari and Albaik, 2019).**

VDR forms a heterodimer complex with the retinoid X receptor (RXR) capable of binding to a vitamin D response element (VDRE) in the promoter region of a target gene and thereby regulates transcription of more than thousand genes (**Lorenzen and al., 2017**)

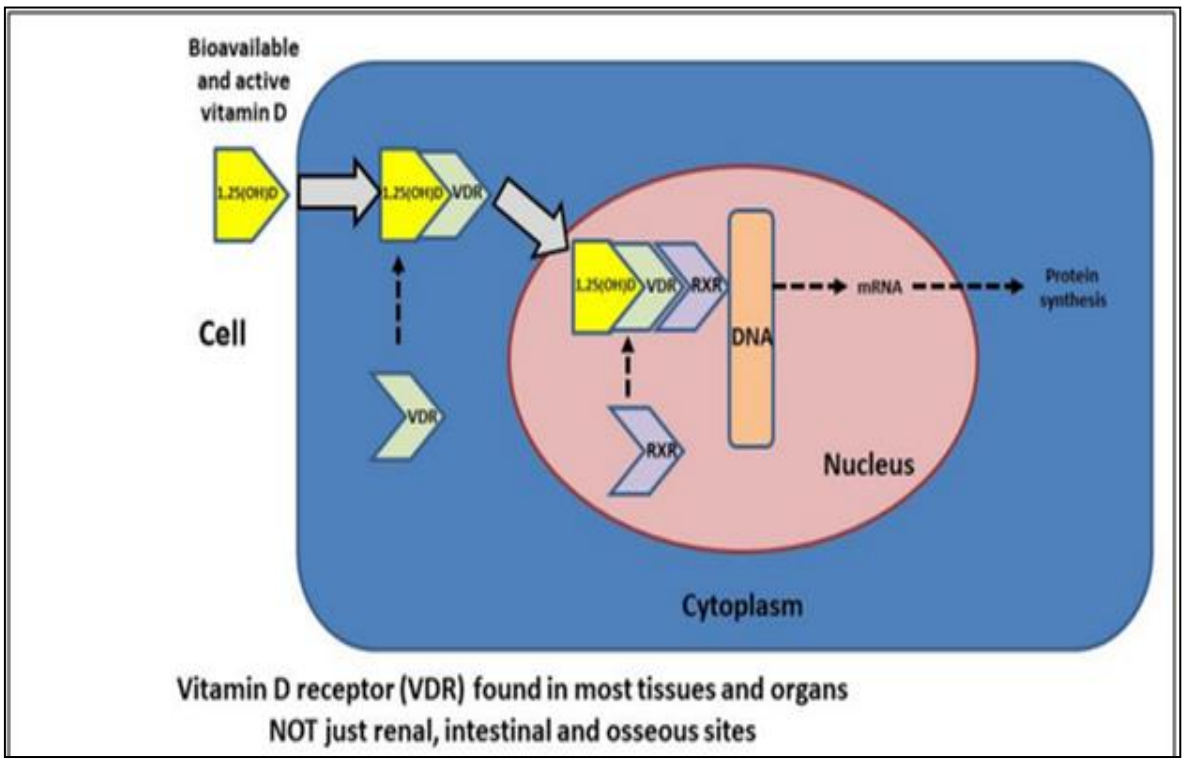

Figure 2: The importance of VDR activity. RXR,retinoid X ; VDR,Vitamin D receptor **(Ribbans and al., 2021).**

I.1.1.8. Metabolism of vitamin D:

I.1.1.8.1. Vitamin D Synthesis:

Vitamin D (D3) production in the skin is not an enzymatic process. It made from 7-dehydrocholesterol (7-DHC) by a two-step process, UVB intensity and skin pigmentation **(Aguilar-Shea ,2021).**

In humans, Vitamin D3 synthesis from 7-dehydrocholesterol occurs mostly in the two basal layers of the epidermis, the stratum basale and the stratum spinosum **(Fraser, 2021)**. Vitamin D defuses into the blood from the skin by Vitamin D binding protein (DBP) and is transported to the liver.

While Vitamin D from the diet is absorbed in the intestine and reached the liver via chylomicrons and DBP. 25-hydroxy vitamin D in the liver is transported to the kidneys and bound to DBP. When 25(OH)D is passing through the kidneys it is hydroxylated at the α-position of C-1 by the 1α-hydroxylase enzyme to generate 1,25-dihydroxy Vitamin D. It is the most important, hydroxylation of Vitamin D occurs in the kidney through the actions of mitochondrial CYP27B1 and results in the synthesis of the active hormone 1,25(OH)2D3 or calcitriol (**Bikle, 2014**).

The bioconversion of 25(OH)D to 1,25(OH)2D is strictly regulated by serum calcium and phosphorus levels (**Saponaro and al., 2020., Zhu and al ., 2013**). Vitamin D production and metabolism systematic representation has been summarised in figure 3**.**

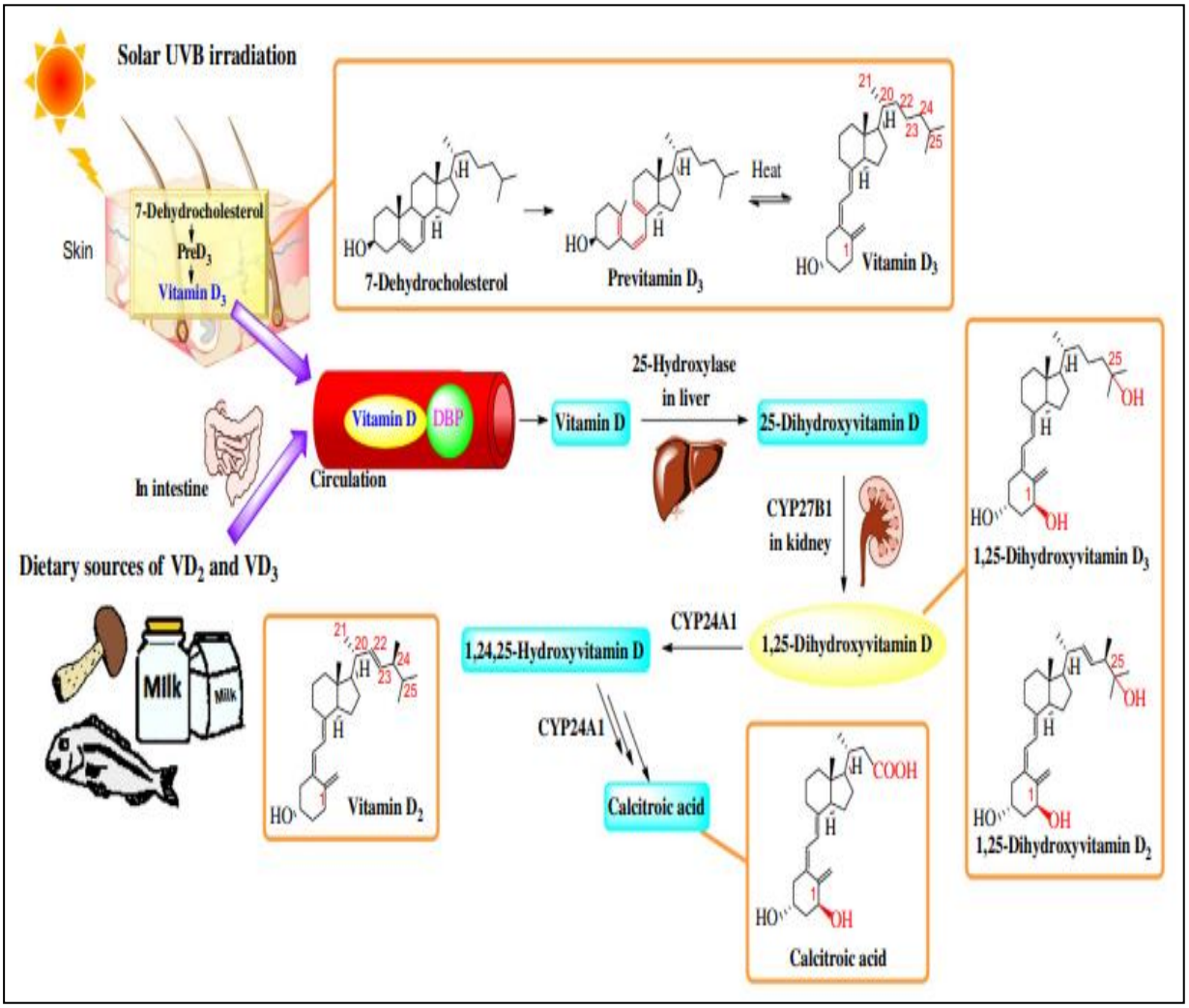

Figure 3: A systematic representation of Vitamin D production through sun light and foods and its metabolism through vital organs such as liver kidney, etc **(Zhang, S and al., 2020).**

I.1.1.8.1.1. Factors affecting vitamin D synthesis :

Many factors affect vitamin D synthesis and its concentration: aging (age decreases the capacity of the skin to produce vitamin D due to lower

availability of 7-DHC), season of the year (autumn and winter), weather conditions (cloudiness), geographical locations (higher latitude), sun exposure, sunscreen (with a protection factor of 30 reduces above 95% of vitamin D synthesis in the skin), skin pigmentation (darker skin needs 3–5 times longer sun exposure to synthesize the same amount of vitamin D than light skin since melanin absorbs UVB radiation), genetic factors (SNPs and mutations), skin damage (burns decrease its production), adiposity (obesity has reduced vitamin D levels), workplace (indoor vs. outdoor), lifestyle, physical activity, clothing habits, air pollution, smoking, diet and calcium intake, vitamin D supplements, and individual height**(Bokhari and Albaik., 2019).**

I.1.1.8.2. Carriage of vitamin D:

The free hormone hypothesis theorises that only unbound 1,25(OH)D can enter cells and exert influence **(Ribbans and al., 2021).**

Vitamin D, both in its inactive and active forms, needs special carriers for transport through the bloodstream because it's a fat-soluble molecule.

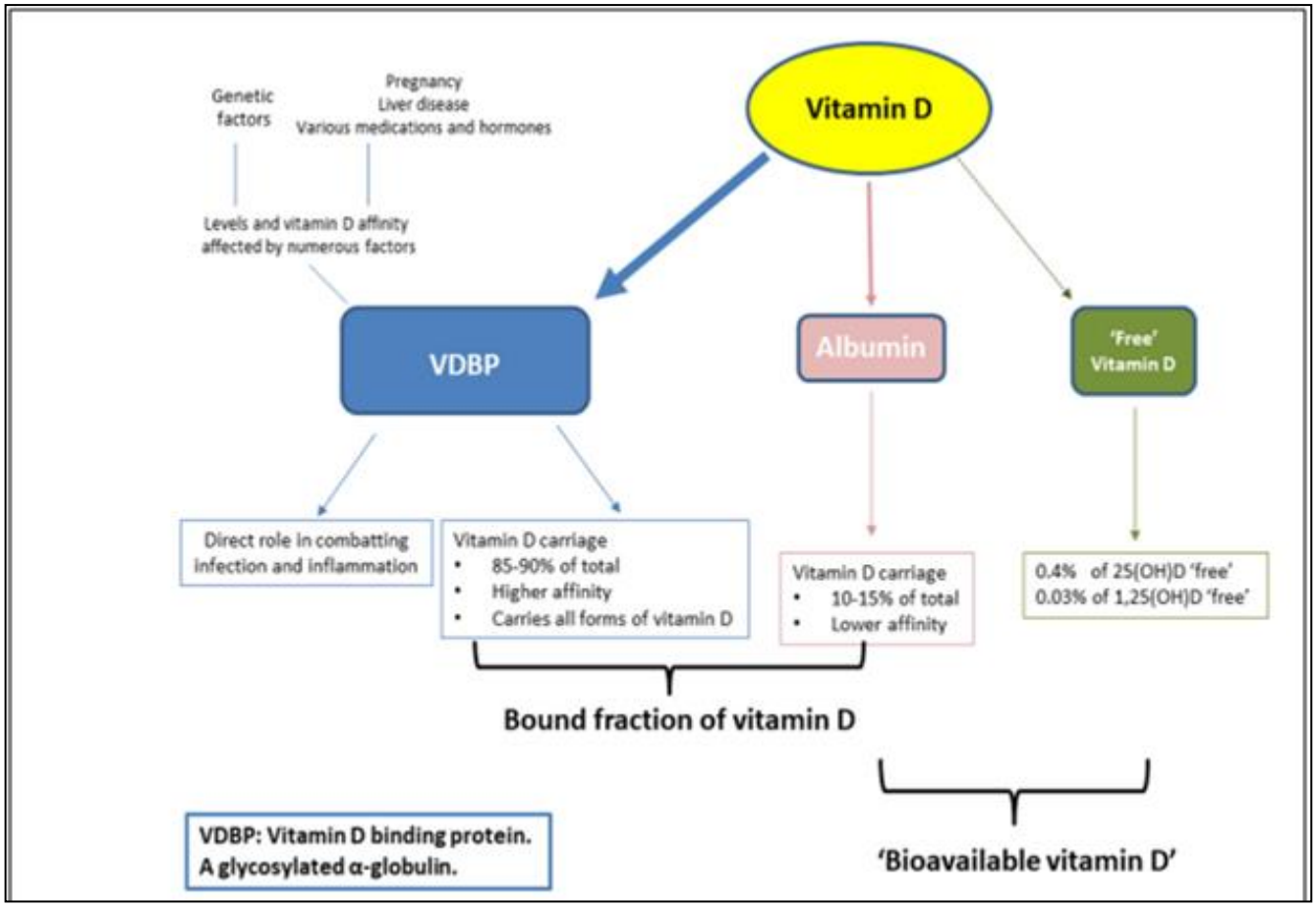

Figure 4: Vitamin D carrige in circulation . VDBP, Vitamin D binding protein **(Ribbans and al., 2021).**

I.1.1.8.2.1. vitamin D Carriers:

- **Vitamin D Binding Protein (DBP):** This is the major carrier protein, responsible for transporting about 85-90% of circulating vitamin D metabolites. It has a high affinity for both 25-hydroxyvitamin D (25OHD) and 1,25-dihydroxyvitamin D (1,25(OH)2D). DBP isproduced by the liver.

- **Albumin:** This is another protein found in the blood plasma that can bind a smaller portion (around 10-15%) of vitamin D metabolites, especially when DBP levels are low. **(Ribbans and al., 2021).**

I.1.1.8.2.2. Carriage process:

- **Vitamin D Absorption:** After obtaining vitamin D from sun exposure or diet, it gets absorbed in the intestines with the help of fat.
- **Binding to Carriers:** In the intestinal cells, vitamin D binds to DBP or albumin.
- **Circulation:** DBP-vitamin D complex or albumin-vitamin D complex travels through the bloodstream to target tissues **(Ribbans and al., 2021).**

I.1.1.8.3. Vitamin D mechanisms of actions :

Similar to other steroid hormones, vitamin D functions according to two modes of action: a mechanism mediating gene transcription (genomic action) and a rapid non-transcriptional action, mediated by the activation of secondary messengers and phosphokinase activation (non-genomic action) (**Haussler and al., 2011., Fleet, 2004**).

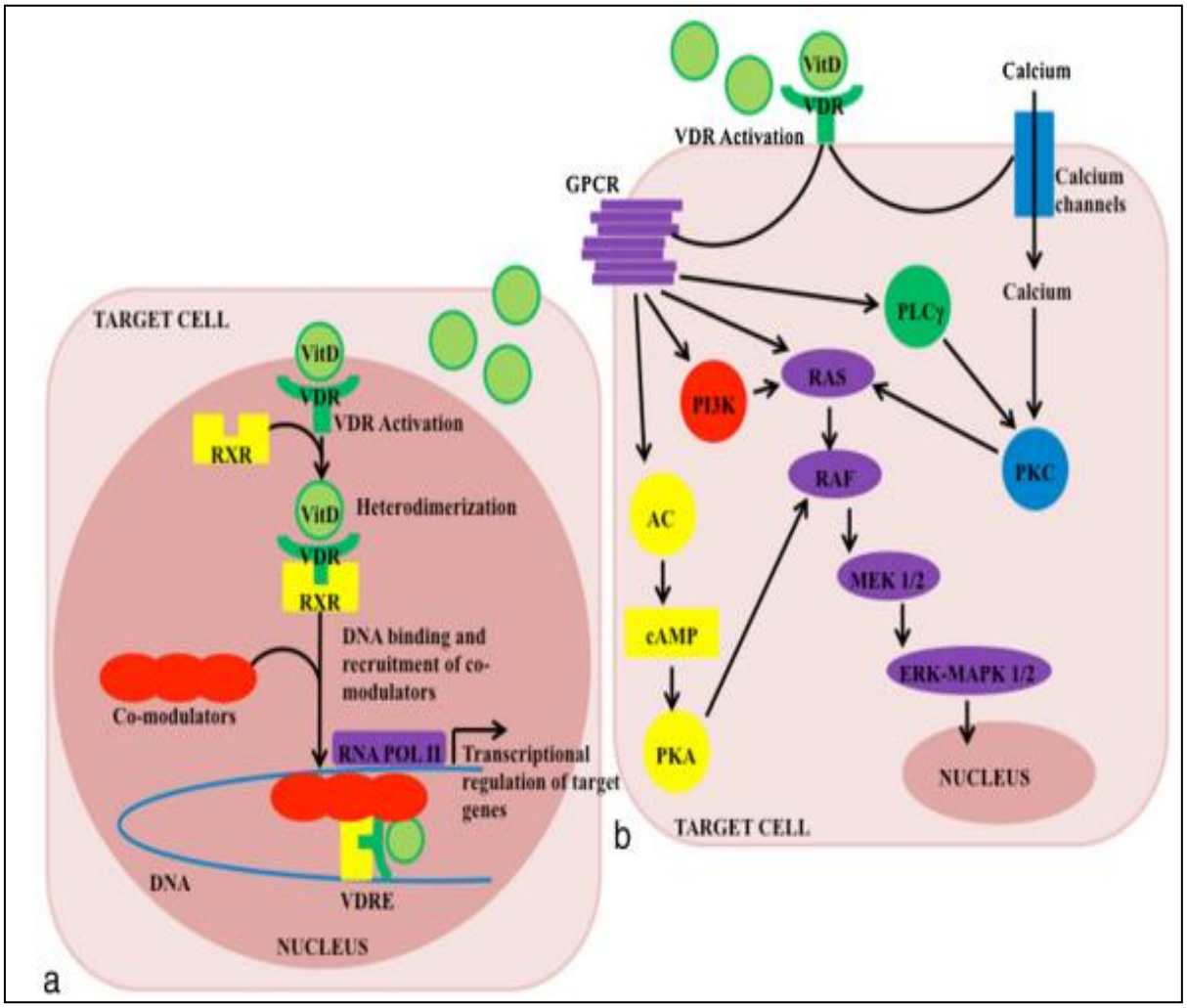

Figure 5: Vitamin D Genomic and Non-genomic Actions. (a) Genomic actions. (b) Non genomic action **(Angelis, 2017).**

I.1.1.8.3.1. genomic action:

Is mediated by the binding of 1,25(OH)2D with a high affinity vitamin D receptor (VDR). When activated, the VDR acts as a transcriptional factor (**Garcia Layana and al ., 2017**), and may directly or indirectly control 200 to 2000 genes in various tissues and cells(**Hossein-nezhad and Holick, 2013**). This includes genes involved in mineral and bone homeostasis, but also genes controlling cell proliferation, differentiation, and apoptosis(**Garcia Layana, A**

and., 2017). The VDR is ubiquitously expressed throughout the human body, including in immune cells, endothelial cells and vascular smooth muscle cells (**Kassi and al., 2013**)**,** but also in eye tissues, including the retina**.** It was recently demonstrated that vitamin D3 supplementation (400 to 2000 IU/day for 8 weeks) is associated with related alterations of 291 genes, including 17 genes known to play important roles in transcriptional regulation, immune function, apoptosis, and responses to stress(**Garcia Layana and al., 2017**).

Genomicaction of 1α,25-dihydroxy-vitamin D3 (VitD) determines a VitD receptor (VDR)-mediated modification of target genes expression. The classical nuclear VDR belongs to the superfamily of nuclear receptors and is a DNA-binding transcription factor. In target cells, VDR, which is activated upon ligand binding, translocates to the nucleus and forms a heterodimer with retinoid X receptor (RXR). The VDR-RXR heterodimer recognizes a VitD response element (VDRE) in the promoter region of target genes, and modulates gene transcription. Sequence variations in VDRE of different VitD target genes induce unique conformations in the VDR-RXR complex, by promoting the recruitment of distinct subsets of co-modulators, which results in either gene transcription stimulation or repression(**Angelis, 2017**).

I.1.1.8.3.2. Non genomic action:

The non-genomic pathway involves the interaction of 1,25(OH)2D with a specific receptor localized to the plasma membrane of target cells(**Fleet, J. C. 2004**)**.** Based on cell type, signal transduction may involve different secondary messengers and cytosolic kinase systems, leading to biological effects that include the regulation of cell proliferation, differentiation, or apoptosis(**Campbell and al., 2010**)**.**

Non-genomic action of Vitamin D is hypothesized to ultimately activate the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) 1 and 2 cascade by means of several intermediate effectors, which become activated upon VitD binding to VDR. Activated VDR stimulates calcium influx, which, in turn, activates calcium-driven intracellular pathways, such as protein kinase C (PKC). Moreover, VitD might activate G-protein coupled receptors (GPCRs), which, in turn, stimulate several downstream pathways, including phosphatidylinositol 3-kinase (PI3K), adenylate cyclase (AC), Ras, and phospholipase C gamma (PLCγ). Each of these pathways might converge by different signaling on the activation of ERK-MAPK 1/2, which might engage in cross-talk with the classical genomic VDR-driven pathway, to modulate gene expression**.** (**Angelis, C., 2017**).

I.1.1.8.4. Vitamin D activity :

1,25(OH)D can be considered to have both endocrine activity and autocrine/paracrine activities **(Morris and Anderson., 2010).** The substrate should not be regarded as a vitamin but as a secosteroid hormone. The different pathways and location of various endocrine and autocrine/paracrine activities are shown in figure 6 (**Ribbans and al., 2021**)**.**

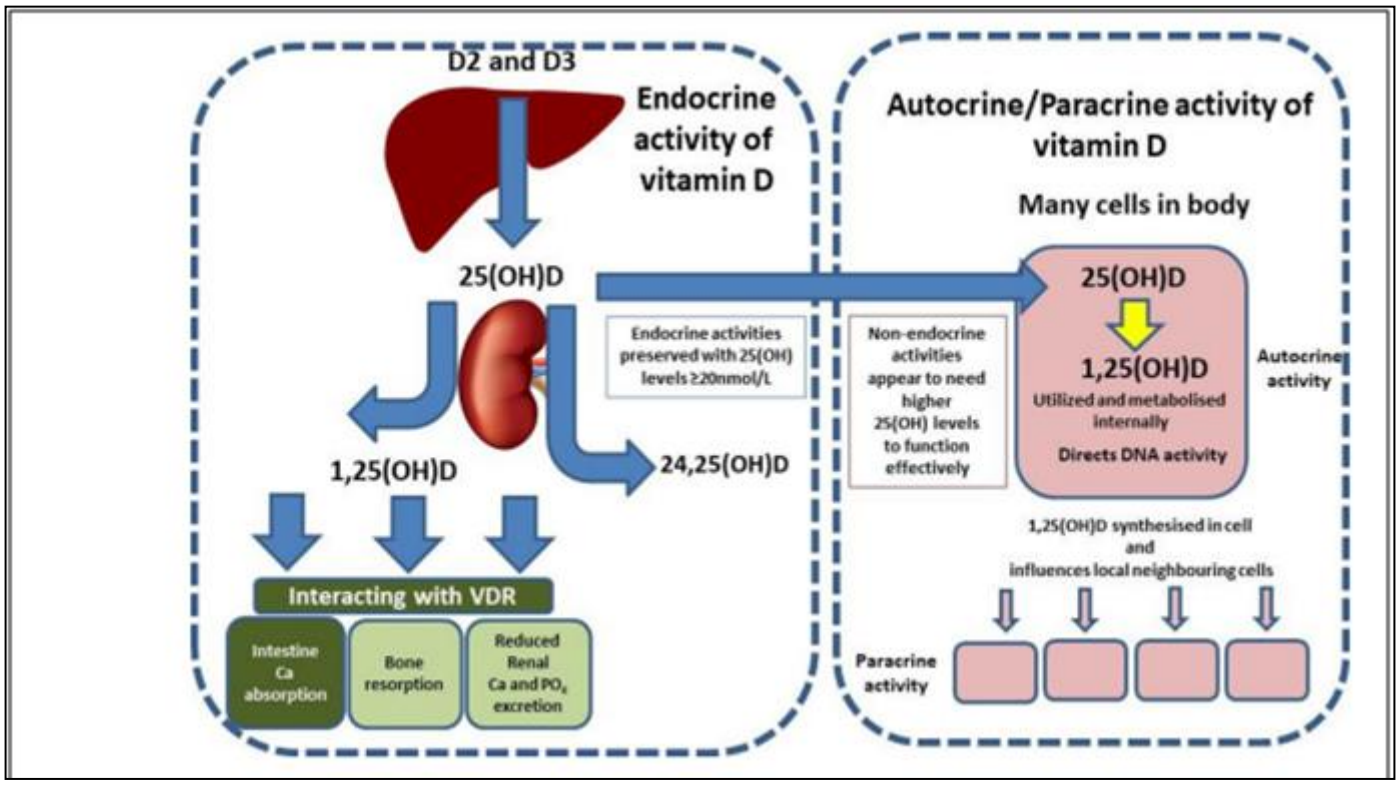

Figure 6: Endocrine and autocrine / paracrine activity of Vitamin D. VDR, Vitamin D receptor **(Ribbans and al., 2021).**

I.1.1.8.5. Distribution of vitamin D:

In tissues depends on the chemical form of the molecule. The cholecalciferol (D3) which represents 65% of all vitamin D in the body is essentially stored in adipose tissue 75% while [25(OH) D] which represents 35% of vitamin D is distributed in different tissues (35% in adipose tissue, 30% inserum, 20% in muscles, and 15% in other tissues). The blood concentration of both forms of [25(OH) D] , ([25(OH) D2] + [25(OH) D3]) makes it possible to

characterize the reserves invitamin D of each individual, and must therefore be determined to assess vitamin D statusof the organism **(Souberbielle, 2013).**

I.1.1.8.6. Storage of vitamin D:

During the summer, the high endogenous synthesis allows a quantity of vitamin D to be stored in reserve to cope with seasons where endogenous synthesis is less or even zero (**Souberbielle, 2013**)

Vitamin D is known to be stored extensively in the liver. These stores sustain normal vitamin D-dependent functions during the winter at high latitudes even in the absence of significant dietary intakes. However, quantitative data on amounts stored alternative storage sites, or the precise mechanisms for deposition and release, are not available. Smaller amounts of vitamin D are stored in extrahepatic tissues. The cartilage oligomeric matrix protein may provide a local storage mechanism that supports rapid delivery to nearby target structures(**Guo and al., 1998**).

Although it is well-established that vitamin D is primarily stored in adipose tissue, it is also stored in smaller amounts in muscles and other tissues**.** Vitamin D is gradually released when the levels of circulating vitamin D and serum 25(OH)D declineamount of vitamin D stored in adipose tissue is too little to maintain adequate serum 25(OH)D levels**,**. However, in so-called vitamin D winter, when there is no UVB radiation effective for vitamin D synthesis, serum 25(OH)D levels fell by only 20–40% (when vitamin D intake was known**),** and it is logical to assume that gradual release from the accumulated storage in adipose tissuemoderates serum 25(OH)D levels(**Martinaityte, 2018**).

The prolonged calculated half-life of vitamin D metabolites, for example serum 25(OH)D has a traced half-life of approximately 15–25 day, while the calculated half-life of vitamin D is around 82 days(**Martinaityte, 2018**).

I.1.1.8.7. Vitamin D catabolism and catabolism regulation:

Vtamin D catabolism is the process by which vitamin D is broken down in the body. It's an important process for regulating vitamin D levels and preventing toxicity**.**

Control of serum 1,25(OH)2D generally involves a balance between synthesis and degradation rates. The latter is carried out in the target cells and involves numerous steps. The major catabolic enzyme is 24-α-hydroxylase which oxidizes the side chain of 25-hydroxyvitamin D (25OHD) this is the major circulating and storage form of vitamin D and 1,25-dihydroxyvitamin D (1,25(OH)2D) this is the active form of vitamin D, which has hormonal effects on the body on the C-24 carbon followed by oxidation of C-23 and subsequent oxidative cleavage of the side chain. Each oxidation step results in a progressive loss of biological activity . Thus, the final cleavage product 1,24,25(OH)3D or calcitroic acid is biologically inert.(**Brown AJ and al. 1999**). However, 24,25(OH)2D, an oxidation product of 25OHD, could have a physiological role in skeletal mineralization (**Garabedian, M. 2000**)**.**

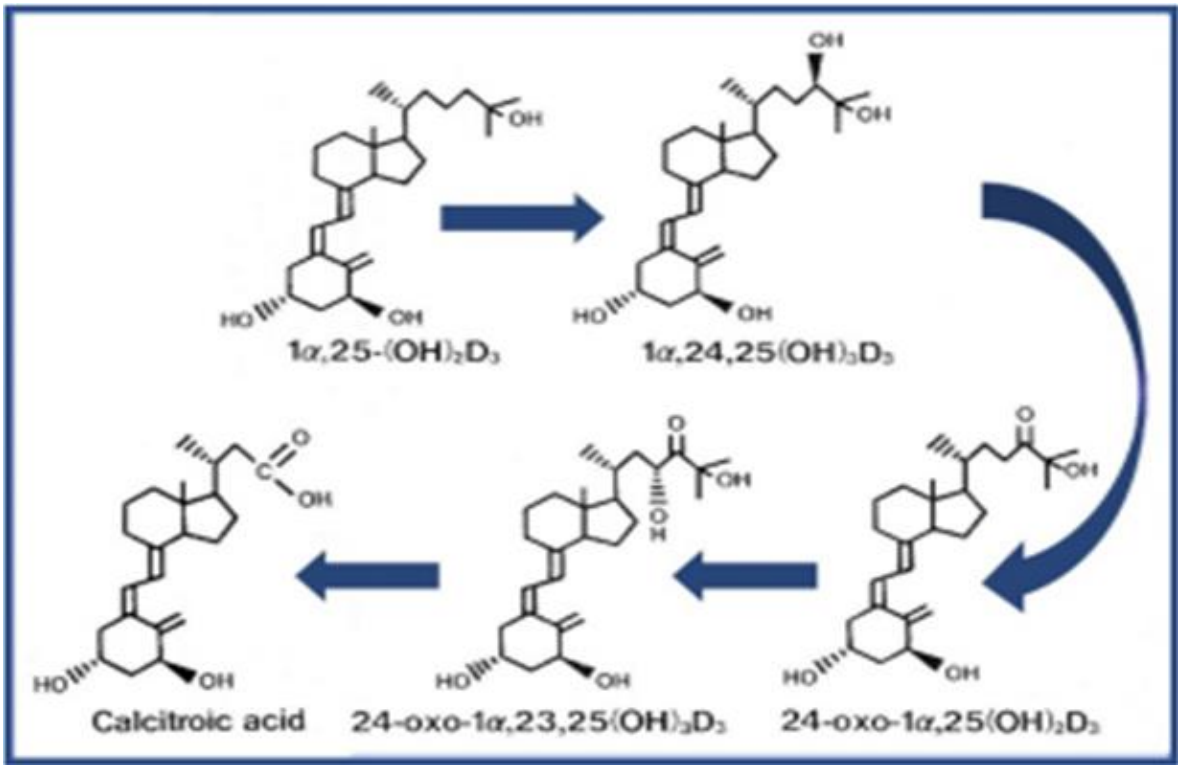

Figure 7: Catabilism of Vitamin D (**Wimalwansa. 2019**).

Regulation of catabolism The degradation of vitamin D depends, in the kidneys, on the regulation of CYP24A1, which is the opposite of that of CYP27B1(**Bikle, 2014**). Thus, PTH negatively modulates the expression of the 24-α-hydroxylase gene by suppressing its mRNA through a cAMP/PKA signaling pathway. Unlike PTH, FGF-23 induces the expression of this mRNA in the kidney, however, the regulatory mechanism remains unclear**.** In addition, calcitonin also regulates its expression(**Bikle, 2014**). However, the main transcription factor involved in the regulation of the gene encoding 24-α-hydroxylase is VDR. Indeed, its promoter contains two vitamin D response elements (VDRE) to which VDR/RXR (retinoid X receptor) bind(**Bikle, 2014**). In this way, 1,25(OH)2D stimulates the transcription of CYP24A1. In addition, the

PXR (pregnane X receptor), a nuclear receptor involved in the regulation of xenobiotic and drug metabolism, also allows the induction of the gene encoding CYP24A1 **(Garabedian, 2000).**

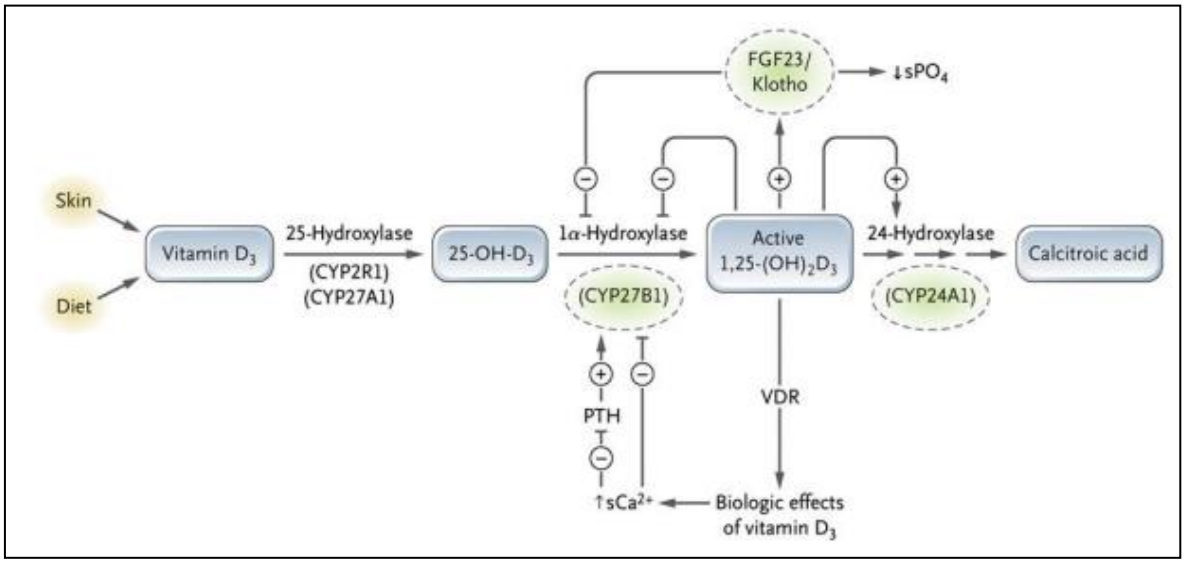

Figure 8: Metabolism and regulation of vitamin D metabolism **(Jones and al., 2012).**

vitamin D catabolism is also regulated by other factors including:

- **Vitamin D levels:** When vitamin D levels are high, CYP24A1 activity increases to break down the excess vitamin D.
- **Calcium levels:** Low calcium levels stimulate CYP24A1 activity, while high calcium levels inhibit it. **(Garabédian, 2000).**

I.1.1.8.8. Vitamin D excretion:

Calcitroic acid the 3- and 24-glucuronides of 24,25-D, and additional vitamin D metabolites are excreted with bile. Since intestinal absorption of vitamin D is very efficient, losses of active vitamin D via this route are likely to

be minor. Quantitative information in this regard is limited however_ 25-D in plasma is bound to DBP (group-specific component, Gc), a single peptide chain with molecular weight of 52 000**.** A small percentage of this complex is filtered in the renal glomeruli. DBP binds with high affinity to cubilin at the brush border membrane of the proximal renal tubule, as described above. Megalin assists with the endocytosis and intracellular trafficking of cubilin and all its captured ligands (which include retinol-binding protein and transferrin among others). Due to the high efficiency of the process very little of the filtered vitamin D escapes with urine. Calcitroic acid is a major catabolite of both vitamin D2 and D3 in urine **(Zimmerman and al., 2000).**

I.1.1.9. Vitamin D sources:

I.1.1.9.1. Endogenoussources:

Exposure of skin to ultraviolet light with wavelengths between 290 and 315 nm (UV-B) converts some of the cholesterol precursor 7-dehydrocholesterol to previtamin D3, which rearranges spontaneously to vitamin D **(Martinaityte, I. 2018**).

Suberythemal (a dose that does not cause sunburn) irradiation of skin with UV-B (0.5 J/cm2) was found to convert about one-third of endogenous 7-dehydrocholesterol (2.3IJ.g/cm2) into previtamin D3, and another third into the precursor lumisterol and the inactive metabolite tachystero **(Obi-Tabot and al., 2000).** UV-B inactivates some of the newly generated vitamin D and its unstable precursors. Vitamin D synthesis rapidly becomes maximal upon continued exposure. because light-induced production and destruction of vitamin D reach an equilibrium. It has been estimated that exposure of the entire body to summer sun for less than 20 minutes is sufficient to generate vitamin D in skin equivalent to an oral dose of 250 IJ.g or more Skin

pigmentation decreases the effective light dose and greatly decreases vitamin D production with less than maximal sun exposure **(Kreiter et al., 2000).**

I.1.1.9.2. Dietary sources :

Most natural vitamin D is consumed in the form of vitamin D3 (D3, cholecalciferol). The only natural foods that contain the structurally related vitamin D2 (D2, ergocalciferol) are mushrooms. Ocean fish is the main dietary source of D3. Particularly rich sources are the fatty types of fish, such as salmon (0.1-0.3 j.Lg/g), sardines (0.4 j.Lg/g), and mackerel (0.1 j.Lg/g). Lean ocean fish, such as cod (0.01 j.Lglg), and freshwater fish, contain only little vitamin D egg yolk and milk **;** fortified cereals. and fortified margarines contribute smaller amounts **(Holick and al., 2021).**

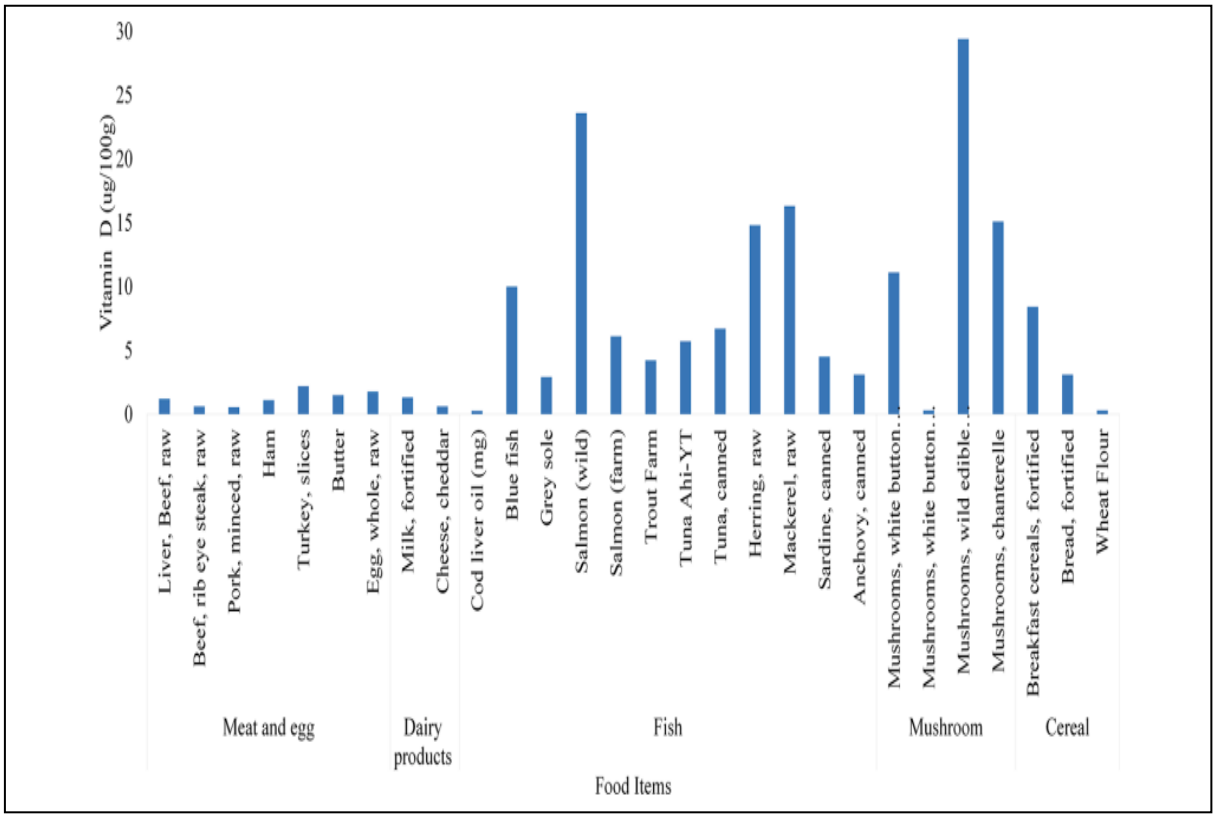

Figure 9 : Bar graph representation of different fortified and non-fortified food materials with Vitamin D and representation of its contenent in Ug/100mg **(Barnkob and al., 2020).**

Fortified foods (margarine, breakfast cereals, dairy products, orange juice), and vitamin supplements (both vitamins D2 and D3 are available**).** Dietary vitamin D provides only 10–20% of circulating levels of vitamin **(Dalan and al., 2014).**

I.1.1.9.3. Vitamin D roles:

Vitaminin D plays a major role in the growth and development **(Wimlavansa and al., 2018).**It is known to regulate the metabolism of calcium

and phosphate, playing an important role in the maintenance of musculoskeletal system **(Norman, 2012).**

It regulates the expression of neurotrophic factors. She can also act on neuronal plasticity processes. **(Martinaityte, 2018)**

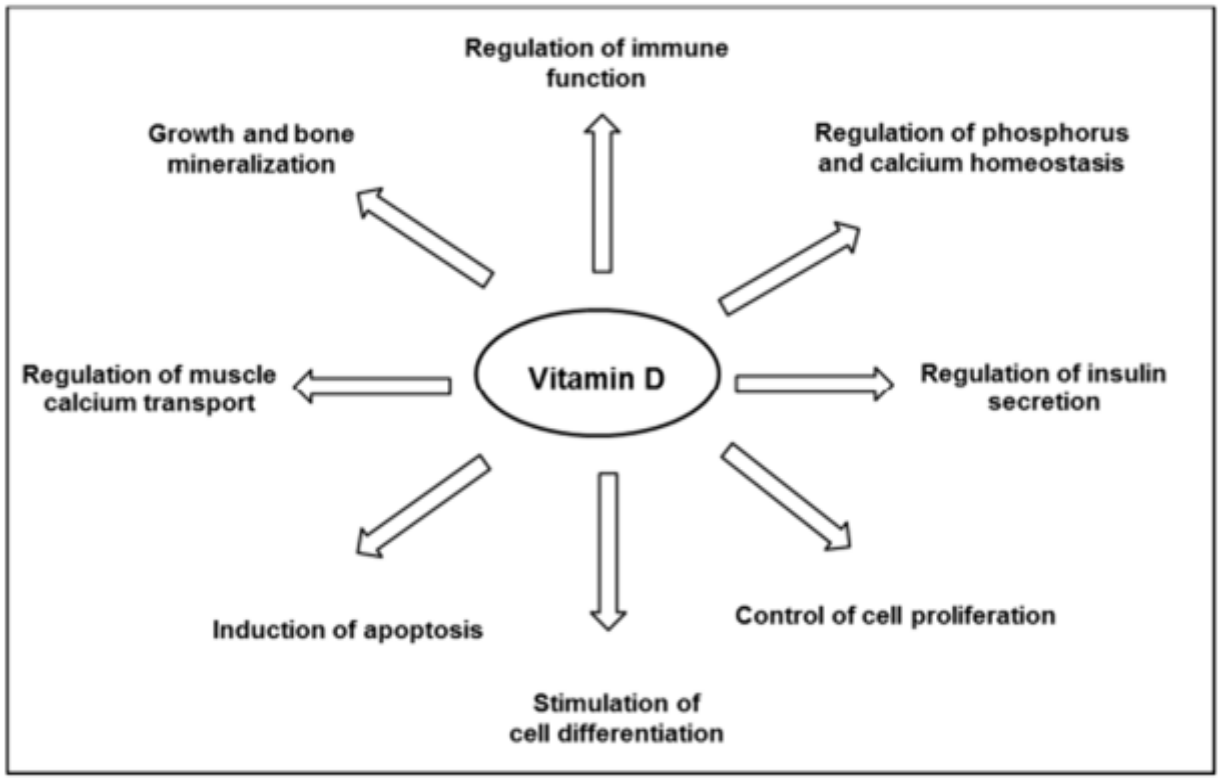

Figure 10 : Major biological function of Vitamin D **(Martinaityte, I. 2018).**

Vitamin D was reported to be involved in many different biological functions, such as cell differentiation, apoptosis with antiproliferative effects, and immunosuppression with anti-inflammatory actions, and promotes the necessary remodeling of bones and teeth **(Norman, 2012).**

Moreover, it is thought to be associated with various extra-skeletal diseases, including cancer, autoimmune, neurological and cardiovascular disorders (**Bokhari and Albaik, 2019**) .

These regulatory roles are mediated by vitamin D receptor (VDR), a nuclear receptor which controls both genomic and non-genomic effects **(Fashera and al., 2020).**

I.1.1.10. Vitamin D toxcity:

Normal cutaneous vitamin D synthesis is self-regulating with increased melanin synthesis being one regulatory mechanism. However, excessive oral intake can in rare circumstances lead to toxicity. Serious consequences are mediated via hypercalcaemia leading to cardiac, renal and soft-tissue damage. Symptoms might include varying symptoms of malaise, abdomen(al pain, confusion, constipation, itching, weakness, thirst, fever and chills. Other complications occur via increased urinary excretion of calcium **(Ribbans and al., 2021).**

Table 3: Sympthom of Vitamin D Toxicity **(Bouillon and al ., 2001).**

Symptoms of Vitamin D Toxicité
Hypercalciuria
Kidney stones
Hypercalcemia
Hyperphosphatemia
Polyuria
Polydipsia
Decalcification of bone
Ectopic calcification of soft tissues (kidney and lung)
Nausea and vomiting
Anorexia
Constipation
Headache
Hypertension

Hypercalcaemia does not usually occur until vitamin D levels approach 250nmol/L.135. However, some authorities caution on serum levels greater than 180nmol/L.18 A daily dose of ≤10000IU/day in the short term is not associated with toxicity.

Above these dosages, the duration of supplementation is important. In general, dosages of >50000IU for longer than 1month risks hypercalcaemia.18 Long-term intake of 4000IU/day has been advised as safe for most adults and children over 11 years of age, includingwith comorbidities predisposing to hypercalcaemia should be more restricted. The body attempts to control high doses of vitamin D by converting more substrate to 24,25(OH)D, which adversely effects 1,25(OH)D signalling and reduces 25(OH)D conversion to 1,25(OH)D **(Ribbans and al., 2021).**

Furthermore, treatments with 1-hydroxylated vitamin derivativeD, escaping physiological regulation, may have in the event of an overdose of consequences, in particular, renal **(Souberbielle and al ., 2006., Hmami and al ., 2014).**

I.1.1.11. vitamin D deficiency:

Vitamin D deficiency (or hypovitaminosis D) is now recognized as a global health issue that afflicts more than half of the world's population. The definition of vitamin D deficiency in the past by the clinical diagnosis of nutritional rickets has expanded to a definition based on the serum concentration of 25(OH)D. Although 25(OH)D has no physiologic function, it is widely used as an indicator to determine a person's vitamin D status because it is the major circulating form of vitamin Dwith a long half-life in the circulation of 2–3 weeks and it can reflect vitamin D supply from dietary exposure and endogenous synthesis. In contrast, 1a,25(OH)2D as the active form is not a suitable indicator because it is homeostatically regulated and has a short half-life (< 4h) **(Holick, 2006).**

The most common definitions include: deficiency <20ng/ml(< 50 nmol /l) , insufficiency 20-30 ng/ ml (50- 75 nmol/l) , and sufficiency >30 ng/mL (>75 nmol/L) or deficiency <12 ng/ ml (<30nmol/l) , insufficiency 12- 20 ng/mL (30-50 nmol/L) and sifficiency<20ng/ml (>50 nmol/L) **(Ross and al, 2011).**

I.1.1.12. Vitamin D supplementation

In people at risk of deficiency, it is interesting to take a blood test to measure the level of 25-OH-vitamin D. The blood concentration of 25-OH-vitamin D should be between 30 and 45 ng/ml of blood. If the blood level of vitamin D is below 30 ng/ml, the doctor prescribes vitamin D supplementation

to bring it back to normal values. When this goal is achieved, maintenance treatment is prescribed to maintain it throughout life.

This supplementation can take the form of a daily oral treatment (to be taken during meals) or monthly or quarterly injections (vitamin D accumulates in body fat). (**Martinaityte, I. 2018**)

I.1.2. Magnesium:

I.1.2.1. Brief history :

Magnesium was known to the ancient Romans as magnesia alba in acknowledgment of the white color of its carbonate salts found in Magnesia, Greece. Sir Humphry Davy in England first described the element in 1808, when he mixed magnesia, water, and mercuric oxide, heated them, and found that a previously unknown element, magnesium, was left after evaporation. Antoine Bussy further purified and isolated the metal in 1828. Magnesium was first detected in the serum of healthy adults by Denis in 1915. In 1926, Le Roy demonstrated that it was essential for the health and well-being of animals **(Grrifen, 2003) .**

In 1932, it was reported that severely magnesium-deficient rats had early violent deaths that were preceded by vasodilatation, hyperirritability of the nervous system, cardiac arrhythmia, spasticity, and tonic-clonic convulsions (Kruse et al., 1932). Although some early limited studies found that people with very low plasma magnesium exhibited twitching and convulsions, the incontrovertible signs of human magnesium deficiency were not described until the 1960s **(Nielsen, 2017).**

In recent years, much has been learned about its 0005 biological importance, but many areas of uncertainty remain, especially the effects of marginal magnesium status on human health **(Grrifen, 2003).**

I.1.2.2. Description of Magnesium:

Magnesium (Mg^{2+}) is an essential mineral **(Martens and al., 2018)**, chief of physiological importance in the body **(Barbagallo and al., 2015)**, and vital to many cellular processes **(Romani, 2011) ,** being the most abundant divalent intracellular

cation in the cells, the second most abundant cellular ion next to potassium and the fourth cation in general in the human body **(Barbagallo and Dominguez, 2015).**

I.1.2.3. Chemical and physiological characteristics of Magnesium:

Magnesium is the eighth most common element in the earth core**(Grrifen, 2003),** and it is a Group 2 (alkaline earth) element within the periodic table **(Jahnen-Dechent and Ketteler, 2012).**

Mg atomic weight is 24.305 g/mol, and its atomic number is **12 (Barbagallo and al., 2021)** has a specific gravity at 20°C of 1.738 , a melting point of 648.8°C and a boiling point of 1090°**C .** In the dissolved state, magnesium binds hydration water tighter than calcium, potassium and sodium. Thus, the hydrated magnesium cation is hard to dehydrate. Its radius is ~400 times larger than its dehydrated radius. This difference between the hydrated and the dehydrated state is much more prominent than in sodium (~25-fold), calcium (~25- fold) or potassium (4-fold). Consequently, the ionic radius of dehydrated magnesium is small but biologically relevant**. (Jahnen-Dechent and Ketteler, 2012).**

It exists as three natural isotopes, the most common being magnesium-24 (79%), with magnesium-25 and magnesium-26 each comprising 10–11% of naturally occurring magnesium **(Grrifen, 2003).**

Tableau 4: Chemical and physiological aspects of Magnesium (**Jahnen-Dechent and Ketteler, 2012**).

Magnesium
Chemical aspects **Name (symbol):**Magnesium (Mg) **Element category :** Alkaline earth metal **Abundance :** Eighth most abundant element in the crustof the Earth **Atomic number :** 12 **Valence :** 2 **Crystal structure :**Hexagonal **Atomic radius :**0.65 A **Atomic weight :**24.305 g/mol **Specific gravity :**1.738 (20°C) **Number of hydration shells :**Two layers **Radius after hydration :**-400 x larger than its dehydrated form **Isotopes:** Magnesium naturally exists in three stable isotopes: ^{24}Mg (most abundant isotope) ^{25}Mg ^{26}Mg ^{26}Mg (radioactive, ß-decay)
Physiological aspects **Availability in the human body:** Normal serum concentration range: 0.65-1.05 mmol/L, divided into three fractions: • Free, ionized (ultrafilterable fraction): 55-70% • Protein-bound (non-ultrafilterable): 20-30% • Complexed (citrate, bicarbonate, phosphate): 5-15% **Total body content in adults :** 24g **Function with respectto cell death:**Anti-apoptotic **Information attained by serum level:** Serum level does not represent total body content

I.1.2.4. Magnesium consumption:

Humans need to consume magnesium regularly to prevent magnesium deficiency, but as the recommended daily allowance for magnesium varies, it is difficult to define accurately what the exact optimal intake should be. Values of "300 mg are usually reported with adjusted dosages for age, sex and nutritional status. The Institute of Medicine recommends 310–360 mg and 400–420 mg for adult women and men, respectively. Other recommendations in the literature suggest a lower daily

minimum intake of 350 mg for men and 280–300 mg magnesium for women (355 mg during pregnancy and lactation) **(Jahnen-Dechent and Ketteler, 2012).**

I.1.2.5. Magnesium metabolism:

I.1.2.5.1. Magnesium absorption:

The majority of magnesium is absorbed by a passive paracellular mechanism in the ileum and distal parts of the jejunum, while a smaller amount is actively transported in the large intestine. Around 24–76% of ingested magnesium is absorbed in the gut and the remaining is eliminated in the feces **(Al Alawi, and al., 2018).**

Magnesium absorption takes place mainly in the distal small intestine and in the colon. Therefore shortening of ileum, results in a substantial decrease of Mg2+ absorption .Two Mg2+-absorbing pathways have been identified in the mammalian intestine **(Ayu, 2021).**

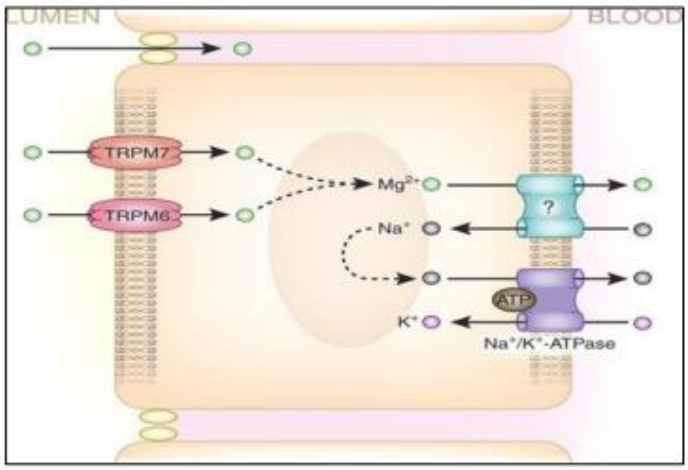

Figure 11: Pathways for Mg2+ absorption across the intestinal epithelium **(Ayu, 2021).**

Paracellular transport involves the absorption of Mg^{2+} through the small spaces between the intestinal epithelial cells and is a passive mechanism. Secondly, the transcellular pathway involves the active transport of Mg^{2+} to the blood through the interior of the epithelial cell mediated by apical channels; transient receptor potential channel melastatin members 6 and 7 (TRPM6/TRPM7). The transcellular pathway of Mg^{2+} transport is subject to tight regulation since the ions have to pass through two cell membranes. Paracellular Mg^{2+} absorption is responsible for 80–90% of intestinal Mg^{2+} uptake. The paracellular pathway is regulated by proteins comprising the tight junction, including claudins, occludin, and zona-occludens-1 .The driving force behind this passive Mg^{2+} transport is supplied by the high luminal Mg^{2+} concentration, which ranges between 1.0 and 5.0 mmol/L, and the lumen-positive transepithelial voltage of ~15 mV**(Ayu, 2021).**

Paracellular Mg^{2+} absorption relies on tight junction permeability. Tight junction assembly and function can be modulated by a number of signaling molecules that alter the phosphorylation state of the tight junctional proteins and the ionic permeability of the paracellular pathway. The ileum and distal parts of the jejunum are known to be the most permeable for ions because of the relatively low expression of tightening claudins 1, 3, 4, 5 and 8 **(Amasheh and al., 2011)** . As such, paracellular Mg^{2+} transport seems mainly restricted to these areas that lack the 'tightening' claudins. Claudins 16 and 19, known to be involved in Mg^{2+} permeability, are not expressed in the intestine. The exact mechanism facilitating paracellular Mg^{2+} absorption, therefore, remains unknown **(Ayu, 2021).**

The proportion of absorbed magnesium from the gut depends on the amount of ingested magnesium and the status of magnesium in the body**.** The magnesium homeostasis is primarily regulated by the kidneys**.** The glomeruli filter around 2400 mg of magnesium per day **(Al Alawi and al., 2018).**

I.1.2.5.2. Magnesium distribution :

The adult human body contains approximately 21–28 g of magnesium **(Saris and al., 2000)** ,which around 60–65% is mineralised in bones, whereas 33–34% is found in muscles and soft tissue (**Vormann, 2003**).

Only 1% of magnesium is present in blood plasma and erythrocytes , where the latter contain three times more than the former. About 10% of magnesium is free and the rest (90%) being bound, mainly to nucleic acids, ATP, negatively charged phospholipids and proteins. The highest magnesium concentrations were found in microsomes containing ribosomes, endoplasmic reticulum, mitochondria and nuclei **(Vormann, 2003).**

I.1.2.5.3. Magnesium Transcellular Transportation :

Due to the very important role of magnesium in the human body, the levels of cellular magnesium need to be strictly regulated. Several specific transporters controlling the cellular movements of magnesium have been identified **(Sontia and Touyz, 2007).** Using the electrochemical gradient of Na+ and through cations channels, magnesium enters cells via Mg2+/anion cotransport. Eight cation channels have been identified including transient receptor potential melastatin cation channels 6 and 7 (TRPM6, TRPM7), members 1 and 2 (SLC41A1, SLC41A2) channels, ancient conserved domain protein 2 (ACDP2), the mitochondrial RNA splicing 2 protein (Mrs2p), magnesium transporter 1 (MagT1), the human solute carrier family 41, and paracellin-1**.** TRPM7 is the most selective channel for magnesium, and it has been identified in the heart, blood vessels, lungs, liver, brain, intestine, and spleen. It is essential for regulating intracellular magnesium level, cell survival, and function**.** On the other hand, TRPM6 is mainly responsible for regulating the total body magnesium level via the kidney and intestines**.** Mrs2p, SLC41A1, and SLC41A2 are implicated in magnesium

transportation in the mitochondria and hence have a regulatory role related to metabolic, cardiovascular, and neurological functions. Magnesium efflux involves several exchanges including Na+ /Mg2+, Ca2+/Mg2+, Mn2+/Mg2+ antiporter, and Cl– /Mg2+ cotransporter**.** The most important exchanger is Na+ /Mg2+ exchanger which has been identified in many cells including cardiac and vascular smooth cells. Several factors have been found to effect the function of this exchanger such as vasopressin, angiotensin II, and insulin **(Al Alawi and al., 2018).**

I.1.2.5.4. Magnesium storage :

Magnesium can be stored in muscle fibres, where it plays an important role in the regulation of muscle contraction by antagonizing the action of Ca2+. At least 50% of the total body magnesium content resides in bone as hydroxyapatite crystals, where it also contributes to the density and strength of the skeleton **(Ayu, 2021).**

Dietary magnesium restriction causes decreased bone magnesium content**.** Depletion of magnesium is, therefore, a risk factor for osteoporosis. A model of Mg2+-induced bone loss has been proposed in which low blood plasma magnesium concentrations lead to activation of bone resorption by osteoclasts and decreased osteoblast bone formation**.** Moreover, bone surface magnesium concentrations (of ~30%) are closely related to serum magnesium concentrations, indicating a continuous exchange of magnesium between bone and blood **(De Baaij and al ., 2012)**. Although the bone magnesium stores are dynamic, the transporters that mediate magnesium flux in and out of bone have not yet been determined. PTH stimulates the release of magnesium from bone **(Ayu, 2021).**

I.1.2.5.5. Magnesium Excretion :

Magnesium is also excreted into the urine. Approximately 70% is filtered into the glomerulus, and most is subsequently reabsorbed. Between 30 and 40% is reabsorbed in the proximal convoluted tubule, and most of what

remains is reabsorbed in the ascending loop of Henle. This is a major source of magnesium homeostasis. Reabsorption in the proximal convoluted tubule appears to be passive, but reabsorption in the loop of Henle is active, although the hormone or hormones regulating this are not clear.

Under normal circumstances, at least 90% of filtered magnesium is reabsorbed in the kidney. During periods of severe magnesium restriction, however, urinary magnesium excretion can fall to very low levels within 7 days. High levels of magnesium supplementation, in contrast, increase the filtered magnesium load at the kidney, and urinary magnesium excretion increases significantly. Because of the high percentage of magnesium that is reabsorbed in the kidney, medications that interfere with this (such as some diuretics) may lead to magnesium deficiency and hypomagnesemia **(Grrifen, 2003).**

I.1.2.6. Magnesium sources:

Approximately 10% of the daily magnesium requirement is derived from wate **(Gröber and al., 2018)** .Dietary Sources of Magnesium Mg is present in all foods, but the Mg content varies substantially. Cereals and nuts have high Mg content (**Allen, 2005**), cocoa, nuts, almonds, whole seeds, unground grains, legumes and green leafy vegetables are rich in magnesium **(Saris, 2000)** and meat, eggs, and milk are poor in Mg (**Allen, 2005**).

A substantial amount of Mg may be lost during food processing, and refined foods generally have a low Mg content. In addition to Mg content, it is important to consider the Mg density of food (i.e., the quantity of Mg per unit of energy). Vegetables, legumes, and cereals thus contribute efficiently to daily Mg intake, whereas fat- and sugarrich products have a minor contribution **(Allen, 2005)** .

Table 5: Magnesium contenent in selected foods**(Mazur and Jam, 2016)**

Food	Magnesium (mg/100g)
Rye bread	110
Bread (with cereals and grains)	181
Bread (standard baguette)	20
Rice (complete, cooked)	50
Rice (white cooked)	11
Almonds (with skin)	231
Hazelnuts	88
Fish (cooked)	27
Chicken	25
Banana	32
Potatoes	17
Spinach (cooked)	53
Orange	12
Apple	6
Broccoli	11
Cauliflower	12
Lettuce	12
Milk	11
Yogurt	12
Cheese (white)	11
Bean (white, cooked)	60
Lentils (cooked)	35
Split peas (cooked)	20

I.1.2.7. Role of Magnesium in human body:

Magnesium is an essential cofactor for a diverse metabolic reactions involving more than 300 enzymes within the human body **(Viering and al., 2018).**

Table 6: Enzymes requiring Magnesium **(Fiorentini,2021).**

LOCALIZATION	ENZYME	Mg-ATP^{2-}	FREE Mg^{2+}
Cytosol: glycolytic pathway	Hexokinase		-
	Phosphofructokinase		-
	Phosphoglycerate kinase		-
	Pyruvate kinase		-
	Aldolase	-	
	Enolase	-	
Mitochondrion	Pyruvate dehydrogenase phosphatase	-	
	Isocitrate dehydrogenase	-	
	α-Ketoglutarate dehydrogenase	-	
	F_o/F_1-ATPase	-	
Muscle cytosol/Heart mitochondrion	Creatine kinase		-
Liver, cytosol	Phosphoenolpyruvate carboxykinase	-	
	Glucose-6-phosphatase	-	
β-subunit of the insulin receptor	Receptor tyrosine kinase activity		-

It may be required for substrate formation (Mg-ATP) and enzyme activation. It is critical for a great number of cellular functions, including oxidative phosphorylation, glycolysis, DNA transcription, and protein synthesis **(Allen ,2005)** .

Furthermore, Mg2+ acts as a modulator of synaptic transmission in the central nervous system (CNS), at the motoric endplate, in immunological pathways and in timekeeping. Importantly, Mg2+ is involved in the gating of ion channels. Many transient receptor potential (TRP) channels are regulated by Mg2+ in a voltage-dependent manner and are involved in the transport of cations across the ruminal epithelium. The modulation of channel function in the CNS by Mg2+ **(Martens and al., 2018).**

Recently, it has been shown that Mg++ plays an important role in the regulation of membrane channels as well as excitation contraction coupling in skeletal muscle **(Schweigel and Martens, 2000).**

Mg ions are necessary for the heart to function properly, playing an important role in the second and third stages of cardiac action potential by affecting potassium channels and calcium channels. As a natural calcium antagonist, Mg participates in cardiac activation–contraction coupling by competing with calcium to bind proteins and calcium transporters **(Michailova and al., 2004).**

Mg plays a role in the mitochondrial synthesis of adenosine triphosphate (ATP) to form MgATP **(Barbagallo and Dominguez, 2007).** Cell signaling needs MgATP for protein phosphorylation and activation of cyclic adenosine monophosphate (cAMP), which is involved in a number of biochemical processes (**Reinhart, 1988**).

Mg ions participate in the transport of other ions through cell membranes, in muscle contraction, and in controlling neuron excitability. Cellular Mg homeostasis is linked to the cellular metabolism of other ions, i.e., K, sodium (Na), calcium (Ca), via Na+/K+/ATPase, Ca++ activated K channels, and other mechanisms **(Resnick and al., 2001).**

Mg2+ is complexed with ATP, ADP and GTP, necessary for the activity of enzymes involved in phosphate group transfer such as glucokinase, phosphofructokinase, phosphoglycerate kinase and pyruvate kinase **(Castiglioni, 2011).**

Role in immunological functions Involved in macrophage activation, adherence, and bactericidal activity of granulocyte oxidative burst,

lymphocyte proliferation, and endotoxin binding to monocytes **(Al AlawiM, 2018).**

Magnesium is an important mineral for bone mineralization, muscular relaxation(**Martin and al., 2009**).

Magnesium involvement in ion fluxes regulation is mandatory for extra/ intracellular ion balance. Indeed, magnesium is central for the control of sodium and potassium transport across cell membranes. In addition, because of its antagonistic action on calcium fluxes, magnesium is considered as 'nature's calcium channel blocker'. Therefore, alterations of magnesium concentration lead to a variety of disturbances at the cellular level that may translate to severe dysfunctions and diseases (**Mazur and al., 2007**).

And is vital for maintaining genomic stability, through ensuring the fidelity of DNA replication and repair processes. More than half of the magnesium found in the cell nucleus is closely associated with nucleic acids and free nucleotides**,** and it is observed that DNA molecules adopt a more packed structure in the presence of Mg^{2+}. In addition, magnesium has a critical role in modulating cell cycle progression, cell proliferation, differentiation and apoptosis(**Blaszczyk and Duda-Chodak, 2013**).

magnesium is a controlling factor in nerve transmission, skeletal and smooth muscle contraction, cardiac excitability, vasomotor tone, blood pressure, and bone turnover glycemic control (**Al Alawi., 2018**).

I.1.2.8. Magnesium Deficiency :

The normal reference range for Mg^{2+} in the serum is 0.76–1.15 mmol/L. Magnesium deficiency (MgD) is a condition where the serum concentration of Mg^{2+} in the body is ≤0.75 mmol/L (1.8 mg/dL). Mg^{2+} concentrations ≤0.75 mmol/L may be considered as preclinical hypomagnesemia. Patients are

considered frankly hypomagnesemic with serum Mg^{2+} concentrations ≤0.61 mmol/L (1.5 mg/dL). MgD can be present without hypomagnesemia. However, hypomagnesemia, when present, is usually indicative of an important systemic Mg^{2+} deficit (**De Baaij and al., 2015**). Signs and symptoms of hypomagnesemia usually occur when serum Mg^{2+} is decreased below 0.5 mmol/L (1.2 mg/dL) **(De Baaij and al., 2015)**. A number of factors can negatively affect Mg^{2+} balance in the body and, in the long-term, may result in MgD. Such factors may be a decreased intake of Mg^{2+} from the food or drinking water **(Kostov and Halacheva, 2018)**, an increased Mg^{2+} loss through the kidneys, an impaired intestinal absorption of Mg^{2+} (**Swaminathan, 2003**), and prolonged use of some medications causing hypomagnesemia **(Kostov, 2019).**

I.1.2.9. Interactions Between Magnesium And Vitamin D Metabolism:

Magnesium homeostasis is maintained by the delicate interactions of the gut, bones and kidneys. Magnesium is an essential cofactor for the synthesis and activation of vitamin D and, in turn, can increase intestinal magnesium absorption and establish a feedback loop to maintain its homeostasis. Vitamin D, in turn, plays a key role in the metabolism of Mg both by stimulating intestinal Mg absorption and by preventing renal Mg excretion (figure 12) **(Kebir, 2024).**

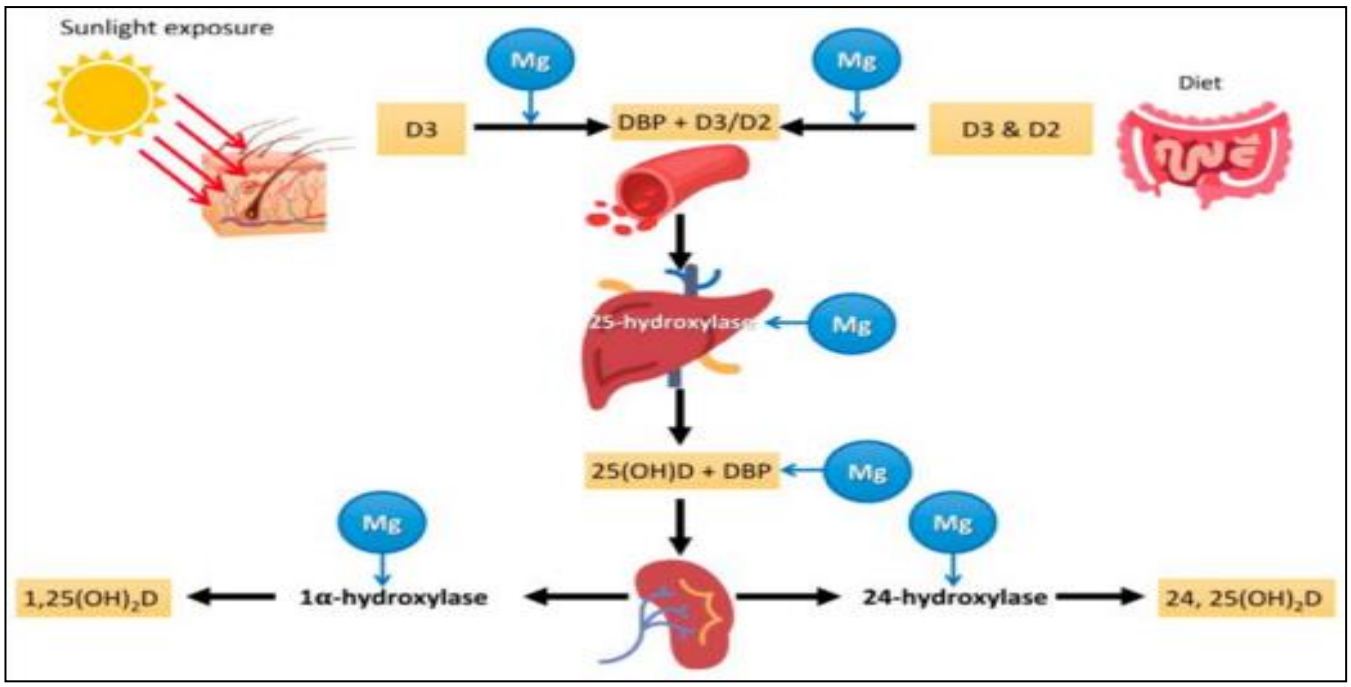

Figure 12: Mg and vitamin D metabolism **(Kebir, 2024).**

Mg is a cofactor that is required for the binding of vitamin D to its transport protein, for the conversion of vitamin D by hepatic 25-hydroxlation, for the transport of 25(OH)D, and for renal Iα-hydroxylation into the active hormonal form. Therefore, all these steps are Mg-dependent.

In vitro and in vivo studies have shown that magnesium deficiency affects the activities of the main enzymes that determine 25(OH)D concentrations, 1α-hydroxylase (CYP27B1) and 24- hydroxylase (CYP24A1), which synthesize and metabolize 25 (OH)D and 1,25(OH)2D,respectively, as well as vitamin D-binding protein (VDBP), is magnesium dependent. Magnesium deficiency results in reduced 1,25(OH)2D and impaired parathyroid hormone response. Magnesium status and supplementation influence vitamin D status and metabolism. Additionally, magnesium is crucial for normal function of the parathyroid glands, metabolism of vitamin D, and to ensure adequate responsiveness of target tissues to parathyroid hormone (PTH) and

active vitamin D metabolites. Hypomagnesemia is generally correlated with hypoparathyroidism, reduced production of active vitamin D metabolites, particularly 1,25(OH)2 of vitamin D3(calcitriol), and resistance to PTH and vitamin D. On the contrary, excess magnesium, similar to calcium, inhibits PTH secretion **(Kebir, 2024).**

I.1.2.10. Magnesium Supplements types :

Laboratories mix magnesium with other ingredients (such as calcium or lactic acid) to treat specific health conditions such as constipation, sleep disorders, or depression, or to support heart health or correct deficiencies. Magnesium supplements also come in different delivery forms and dosages.

Some types of magnesium are better for reaching certain health goals, or for managing specific symptoms **(Adam, 2023).**

The table below shows the different types of magnesium, including the different compounds, delivery methods, and what each one is best for **:**

Table 2 : Magnesium Supplements types**(Adam, 2023).**

TYPE OF MAGNESIUM	DELIVERY METHOD	RECOMMENDED USES
Magnesium chloride	Soluble powder , topical creams ,Tablets	DepressionFibromyalgia pain
Magnesium citrate	Liquid	Constipation, Depression
Magnesium glycinate	Soluble powder, pill	Managing deficiency
Magnesium lactate	Fruit-flavored supplementary beverages	Managing deficiency
Magnesium malate	Soluble powder, pill	Managing deficiency
Magnesium oxide	Tablet /capsule	Constipation
Magnesium sulfate	Injection	Constipation
Magnesium taurate	Capsule / powder	Heart health

I.1.3. Insulin :

I.1.3.1. Breif history:

The history of the discovery of insulin and its therapeutic utility defined a paradigm for the integration of physiologic and biochemical approaches in experimental medicine . At the end of the 19 th century Von Mering and Minkowski noted that removal of the pancreas led to the development of DM in dogs . In 1916 Schafer first speculated that an antidiabetic hormone, which he named "insuline," was secreted from pancreatic islets . Barron noted in 1920 that ligation of the pancreatic duct, with destruction of the exocrine pancreas, only resulted in DM if the islets, so named by Langerhans in 1869 , were also destroyed . Subsequently, the work of Banting, Best, Collip and MacCleod in the early 1920's resulted in the identification of a substance in extracts of pancreas that had the remarkable ability to reduce blood glucose levels in diabetic animals . By 1923 these pancreatic extracts were employed to successfully treat patients with DM. The dramatic clinical utility of insulin encouraged broad public support for medical research **(Weiss and al., 2015).**

I.1.3.2. Insulin definition:

Insulin is a peptide hormone secreted by islet B cells Langerhans of the pancreas. It is the only hypoglycemic hormone: during a increase in blood sugar, it promotes a return to normal by activation of glycogenogenesis, inhibition of glycolysis, inhibition of gluconeogenesis, activation of lipogenesis, inhibition of lipolysis **(Magnan and al., 2005).**

I.1.3.3. Insulin structure :

Insulin is a peptide hormone comprised of 51 amino acids distributed among two peptide chains, the A and B chains of 21 and 30 amino acid

residues, respectively. Disulfide bonds of cysteine residues connect the 2 chains (figure 13) (**Vargas, E and al., 2018**).

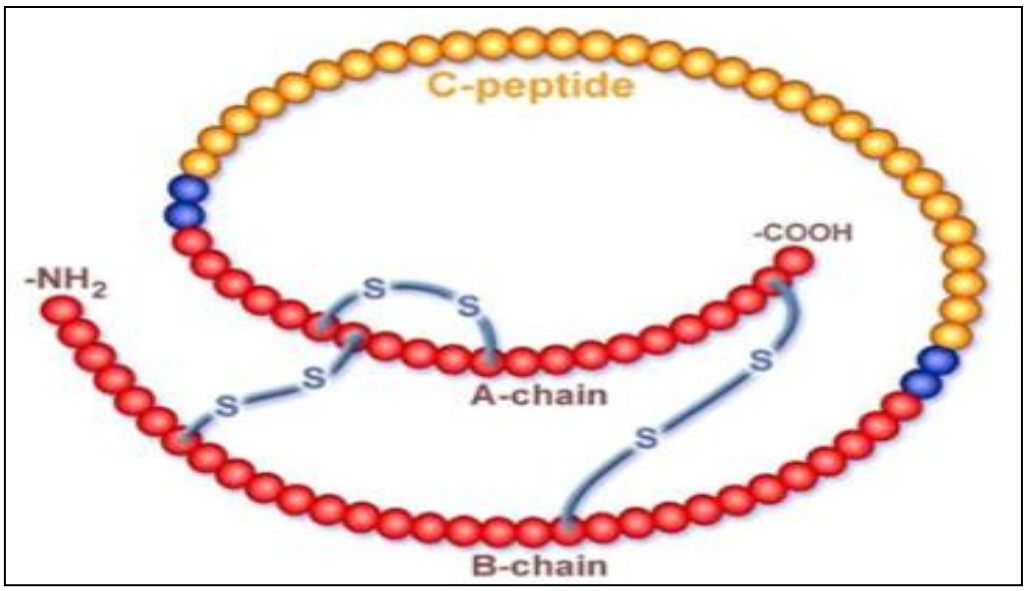

Figure 13: Insulin structure **(Akinlade and al., 2014).**

Preproinsulin is the original precursor protein of insulin. It is a single-chain polypeptide consisting of proinsulin and signal peptide sequences. Upon its translocation into the endoplasmic reticulum, preproinsulin is cleaved at its signal peptide, releasing proinsulin. Proinsulin is a single-chain containing insulin's A, and B chains in a continuous fashion joined through a segment known as the C domain. Dibasic residues flank the C domain at each end. At the site of each dibasic residue, a trypsin-like enzyme cleaves proinsulin. This cleavage finally releases insulin along with a C-peptide. Until it is metabolically needed, insulin is stored within glucose-regulated secretory vesicles as zinc insulin hexamers (**Cano and al., 2007**).

I.1.3.4. Insulin synthesis:

Insulin synthesis begins in the nucleus of pancreatic β cells by transcription of a gene carried by the short arm of chromosome 11, its intracellular course continues in the rough endoplasmic reticulum after transcription of the gene into RNA coding for a high molecular weight precursor molecule of 11.5 kDa which are pre pro-insulin which has a short lifespan (figure 14) **(Read and al., 1993) .**

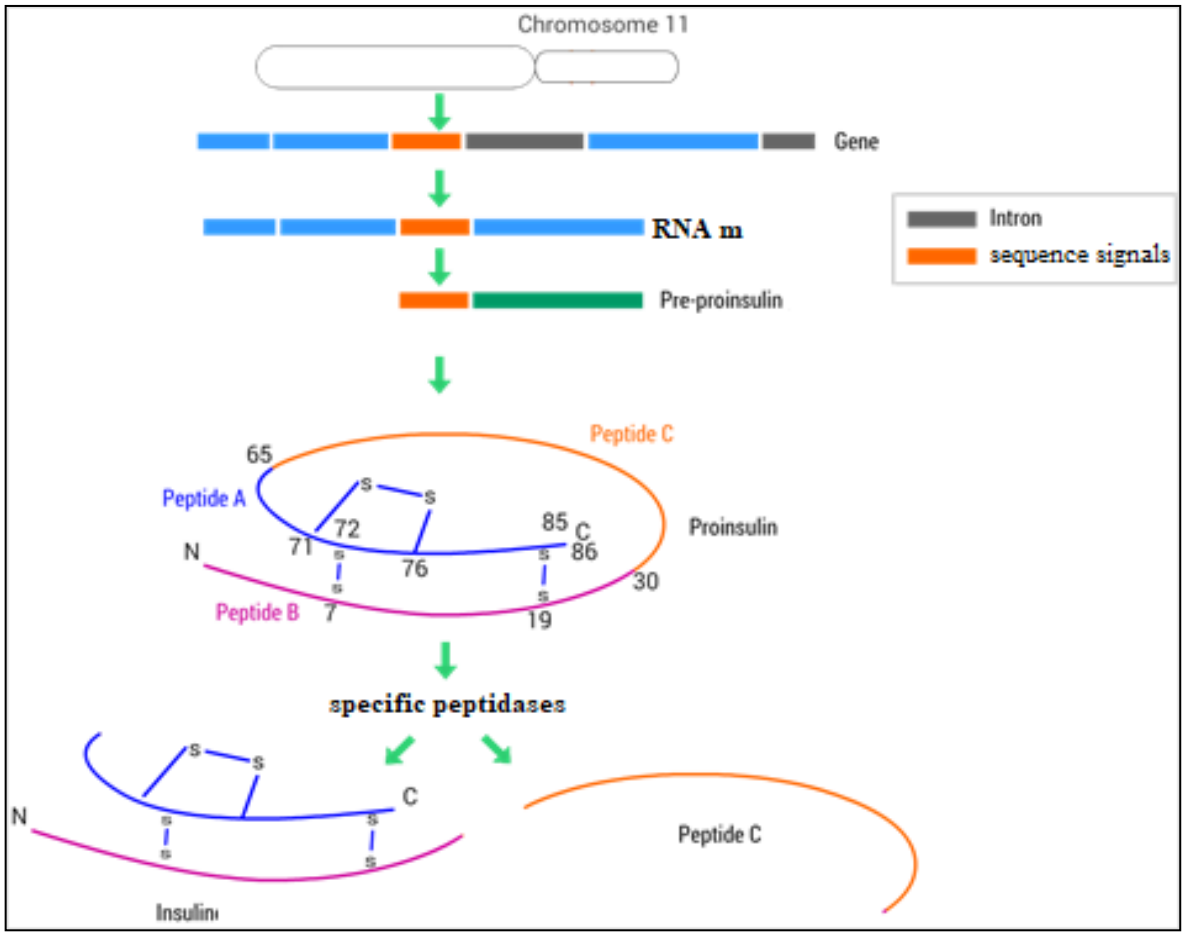

Figure 14: Insulin synthesis (**Magazine science, 2018).**

Under the action of numerous specific endopeptidases, the central part of proinsulin,C peptide, is cleaved to generate mature insulin: 51 amino acids divided into twoα and β chains linked by disulfide bridges **(Capeau, 2003).**

Mature insulin is incorporated into secretory vesicles with C peptide at thelevel of the Golgi apparatus. These vesicles accumulate in the cytoplasm until itthere is a signal to secrete insulin **(Girard, 1999).**

I.1.3.5. Insulin secretion mechanism:

The levels of glucose in the blood need to be tightly maintained into a well-defined range in order to guarantee the availability of this sugar as source of energy and contex- tually prevent organ damage due to its excessive concentration.

Insulin is secreted from beta pancreatic cells, respectively **(Aronoff and al, 2004)** . Insulin decreases glucose concentration in the blood stream by promoting its uptake and utilization through the glycolytic pathway. Insulin secretion depends on the open or closed status of the ATP-dependent potassium channels (K+/ ATP channels); when blood glucose levels are high, pancreatic beta cells promote glycolysis, a process leading to increased intracellular ATP concentration, and consequently K+ /ATP channel inhibition and cellular membrane depolarization. This event opens the voltage-dependent calcium channels and the entering of Ca2+ ions enables insulin release into the bloodstream .

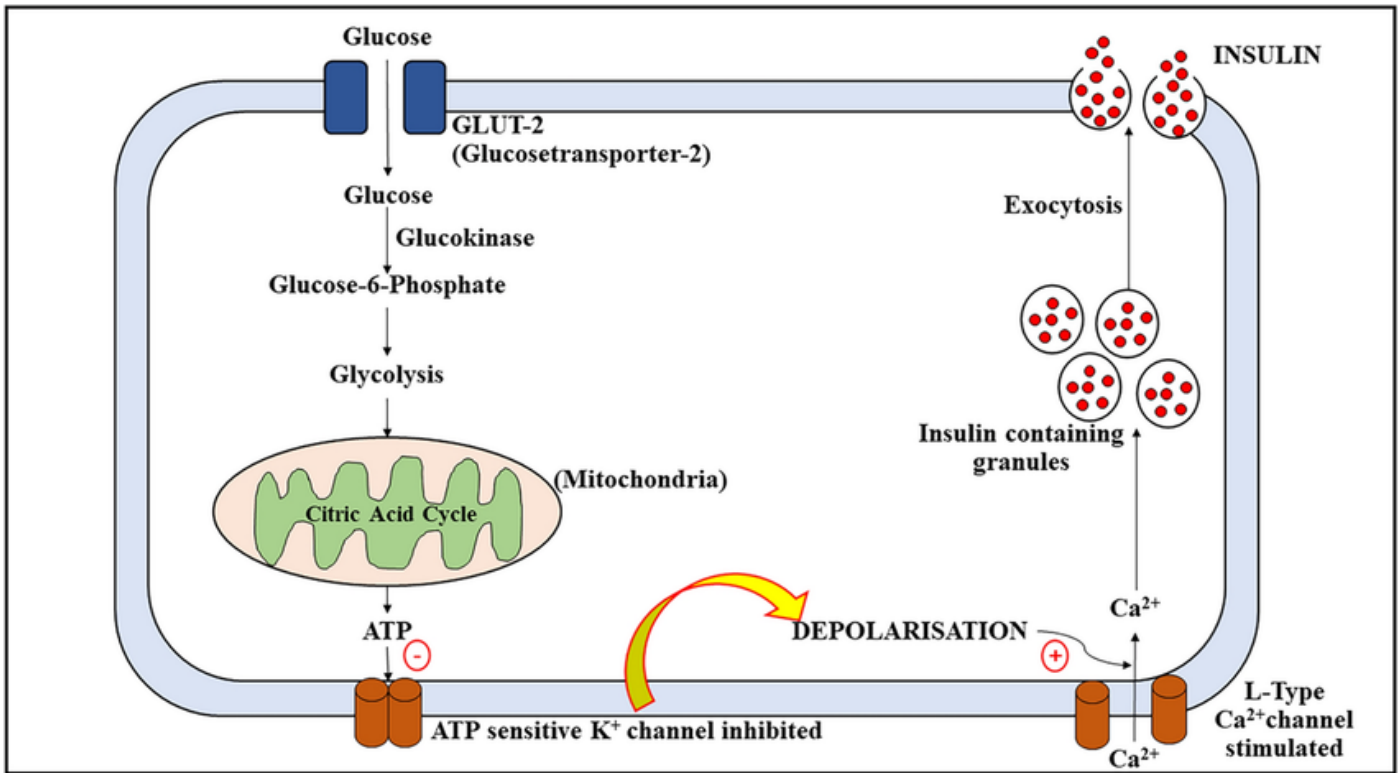

Figure 15: insulin secretion mechanism in beta cells **(Arora and al., 2021).**

Glucose is transported into pancreatic beta cells through facilitated diffusion by GLUT2 glucose transporters. Intracellular glucose is metabolized to ATP through glycolysis and oxidative phosphorylation. A high ATP/ADP ratio induces closure of K+ channels, leading to cell membrane depolarization. This event promotes the opening of Ca2+ chan- nels, facilitates extracellular Ca2+ influx into the beta cell, and in turn triggers insulin exocytosis. **(Liguori, F and al., 2021).**

I.1.3.6. insulin signaling pathway:

Once produced, insulin is delivered to target tissues (liver, adipose cells, muscles, brain) where it binds insulin receptor (IR) and triggers a cascade of phosphorylation events, named insulin/insulin-like growth factor signaling

(IIS), ultimately lead- ing to glucose uptake and storage in the form of glycogen, thus decreasing glucose blood levels. The name IIS clearly indicates that the same pathway may be activated not only by insulin but also by insulin-like growth factors (IGFs) through binding to their specific re- ceptors. After its activation, insulin receptor (IR) phosphorylates several substrates in- cluding insulin receptor substrate (IRS) protein which provides specific docking sites for phosphatidylinositol 3-kinase (PI3K) activation. This enzyme generates phosphatidylino- sitol (3,4,5)-triphosphate (PIP3), which in turn recruits phosphoinositide dependent pro- tein kinase 1 (PDK1) and AKT to the plasma membrane, where PDK1 activates AKT. PI3K activity is counteracted by the activity of PTEN (phosphatase and tensin homolog). Full AKT activation also requires the activity of the mammalian target of rapamycin com- plex (mTORC2). Once activated, AKT phosphorylates several downstream targets such as GSK-3 and TBC1D4, responsible, respectively, for glycogen synthesis and glucose uptake through GLUT4 glucose transporter translocation. Other AKT targets are the ra- pamycin complex (mTORC1), involved in cellular growth, and FOXO proteins, whose ex- pression impacts on gluconeogenesis and apoptosis. IIS is finely regulated by negative feedback signals. The mTORC1 and S6-kinase (S6K) complex—downstream components of the pathway—phosphorylates mTORC2, attenuates its activity and hence reduces AKT action. More recently, Kearney and collaborators showed that AKT-mediated post-translational modifications of the IRS represent another feedback signal that controls PIP3 abundance(**Liguori and al., 2021**).

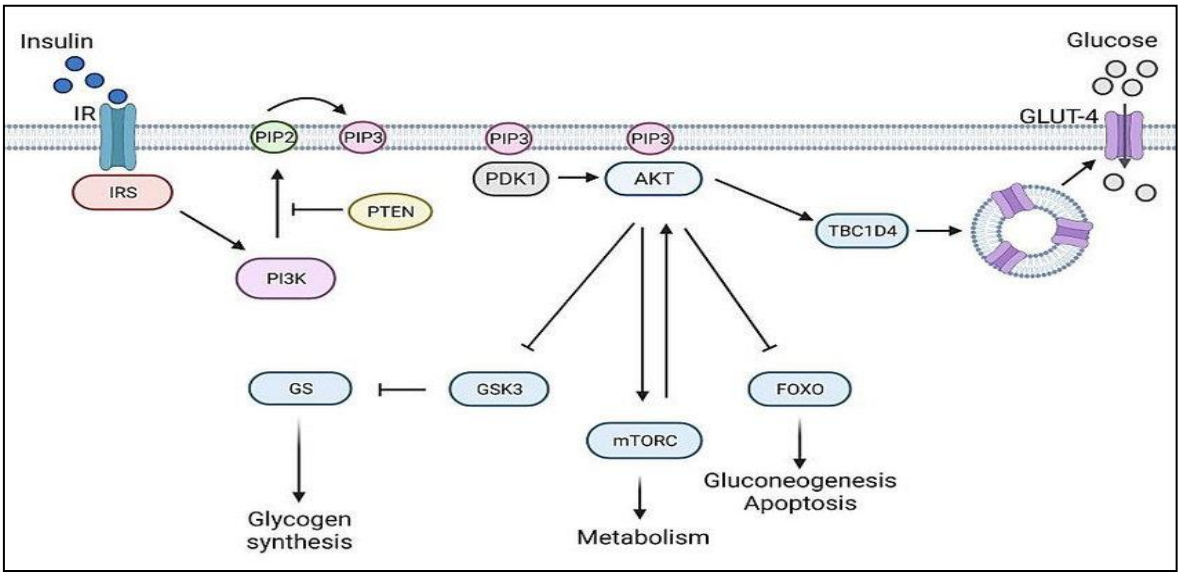

Figure 16 : The mammalian insulin signaling pathway(**Liguori and al., 2021**).

I.1.3.7. Insulin functions :

I.1.3.7.1. Role in Glucose Metabolism:

The homeostasis of glucose metabolism is carried out by 2 signaling cascades: insulin-mediated glucose uptake (IMGU) and glucose-stimulated insulin secretion (GSIS). The IMGU cascade allows insulin to increase the uptake of glucose from skeletal muscle and adipose tissue and suppresses glucose generation by hepatic cells. Activation of the insulin cascade's downstream signaling begins when insulin extracellularly interacts with the insulin receptor's alpha subunit. This interaction leads to conformational changes in the insulin-receptor complex, eventually leading to tyrosine kinase phosphorylation of insulin receptor substrates and subsequent activation of phosphatidylinositol-3-kinase. These downstream events cause the desired translocation of the GLUT-4 transporter from intracellular to extracellular onto skeletal muscle cell's plasma membrane. Intracellularly, GLUT4 is present

within vesicles. The rate at which these GLUT4-vesicles are exocytosed increases due to insulin's actions or exercise. Thus, by increasing GLUT-4's presence on the plasma membrane, insulin allows for glucose entry into skeletal muscle cells for metabolism into glycogen **(Naaji, 2019)**.

I.1.3.7.2. Role in Glycogen Metabolism:

In the liver, insulin affects glycogen metabolism by stimulation of glycogen synthesis. Protein phosphatase I (PPI) is the key molecule in the regulation of glycogen metabolism. Via dephosphorylation, PPI slows the rate of glycogenolysis by inactivating phosphorylase kinase and phosphorylase A. In contrast, PPI accelerates glycogenesis by activating glycogen synthase B. Insulin serves to increase PPI substrate-specific activity on glycogen particles, in turn stimulating the synthesis of glycogen from glucose in the liver.

There are a variety of hepatic metabolic enzymes under the direct control of insulin through gene transcription. This affects gene expression in metabolic pathways. In gluconeogenesis, insulin inhibits gene expression of the rate-limiting step, phosphoenolpyruvate carboxylase, as well as fructose-1,6-bisphosphatase and glucose-6-phosphatase. In glycolysis, gene expression of glucokinase and pyruvate kinase increases. In lipogenesis, the expression is increased of fatty acid synthase, pyruvate dehydrogenase, and acetyl-CoA carboxylase **(Daghlas, 2019)**.

I.1.3.7.3. Role in Lipid Metabolism:

As previously mentioned, insulin increases the expression of some lipogenic enzymes. This is due to glucose stored as a lipid within adipocytes. Thus, an increase in fatty acid generation will increase glucose uptake by the cells. Insulin further regulates this process by dephosphorylating and

subsequently inhibiting hormone-sensitive lipase, leading to inhibition of lipolysis. Ultimately, insulin decreases serum free fatty acid levels.(**Vargas and al., 2018**).

I.1.3.7.4. Role in Protein Metabolism:

Protein turnover rate is regulated in part by insulin. Protein synthesis is stimulated by insulin's increase in intracellular uptake of alanine, arginine, and glutamine (short-chain amino acids) and gene expression of albumin and muscle myosin heavy chain alpha. Regulation of protein breakdown is affected by insulin's downregulation of hepatic and muscle cell enzymes responsible for protein degradation. The impacted enzymes include ATP-ubiquitin-dependent proteases, and ATP-independent lysosomal proteases, and hydrolases.(**Vargas and al., 2018**).

I.1.3.7.5. Role in Inflammation and Vasodilation:

Insulin's actions within endothelial cells and macrophages have an anti-inflammatory effect on the body. Within endothelial cells, insulin stimulates the expression of endothelial nitric oxide synthase (eNOS). eNOS functions to release nitric oxide (NO), which leads to vasodilation. Insulin suppresses nuclear factor-kappa-B (NF-kB) found intracellularly in endothelial cells. Endothelial NF-KB activates the expression of adhesion molecules, E-selectin, and ICAM-1, which release soluble cell adhesion molecules into the circulation. Studies have linked the presence of cell adhesion molecules on vascular endothelium to the development of atherosclerotic arterial plaques.

Insulin suppresses the generation of O_2 radicals and reactive oxygen species (ROS). Within the macrophage, insulin inhibits NADPH oxidase expression by suppressing one of its key components, p47phox. NADPH oxidase aids in generating oxygen radicals, which activate the inhibitor of NF-

kB kinase beta (IKKB). IKKB phosphorylates IkB, leading to its degradation. This degradation releases NF-kB, allowing for its translocation in the macrophage's nucleus. Once in the nucleus, NF-kB stimulates gene transcription of pro-inflammatory proteins that are released into the circulation, such as inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein (MCP-1), and matrix metalloproteinase (MMP). **(Slater and al., 2019).**

I.1.3.8. Insulin sensitivity:

I.1.3.8.1. Definition:

insulin sensitivity is defined as the effectiveness of insulin in reducing blood glucose by directly promoting glucose uptake into muscle and fat cells as well as by increasing hepatic glycogen storage and reducing hepatic glucose production. A significantly reduced levelof insulin sensitivity is called insulin resistance **(Cefalu ,2000).**

I.1.3.8.2. The importance of Determining insulin sensitivity:

Determining insulin sensitivity is of critical importance. First, it can enable identification of diabetic individuals before irreparable damage appear**.** Second, for patients whose insulin sensitivity changes over time, such as critical care patients, it enables personalised adaptation of insulin dosing for glycemic control. Insulin sensitivity computation can be conducted using mathematical models (**Pretty, and al., 2012**)

I.1.3.9. Insulin resistance (IR):

I.1.3.9.1. Definition :

is a metabolic disorder characterized by impairment of insulin-mediated glucose transport to peripheral cells, which leads to increased concentrations of this hormone in the circulatory system as a compensatory mechanism. IR is associated with the development of various diseases, such as cardiovascular disease, metabolic syndrome, obesity, cancer, and type 2 diabetes mellitus (T2DM) **(Morais and al., 2017).**

I.1.3.9.2. Pathophysiology:

The 3 primary sites of insulin resistance are the skeletal muscle, liver, and adipose tissue. In a state of chronic caloric surplus, the tissues in the body become resistant to insulin signaling. Skeletal muscle is a large reservoir for circulating glucose, accounting for up to 70% of glucose disposal as measured by the hyperinsulinemic-euglycemic clamp. The direct result of muscle insulin resistance is decreased glucose uptake by muscle tissue. Glucose is shunted from muscle to the liver, where de novo lipogenesis (DNL) occurs. With increased glucose substrate, the liver develops insulin resistance as well. Higher rates of DNL increase plasma triglyceride content and create an environment of excess energy substrate, which increases insulin resistance throughout the body, contributing to ectopic lipid deposition in and around visceral organs. (**Samuel and Shulman, 2016**).

I.1.3.9.2.1. Skeletal Muscle Tissue :

After intake of a caloric load and conversion to glucose, muscle is the primary site for glucose disposal, accounting for up to 70% of tissue glucose uptake. In chronic caloric excess, muscle tissue accumulates intramyocellular fatty acids. Diacylglycerol is an intramyocellular fatty acid that signals energy excess within the cell. Diacylglycerol activates protein kinase C theta (PKC-theta), decreasing proximal insulin signaling. The direct result is decreased

glucose transporter type 4 (GLUT4) translocation to the cell membrane and reduced glucose uptake by the muscle tissue. The excess glucose in the blood is shunted to the liver to be metabolized or stored.(**Freemanand Pennings, 2018**).

I.1.3.9.2.2. Hepatic Tissue :

The liver is responsible for processing energy substrates. It packages, recirculates, and creates fatty acids and processes, stores, and creates glucose. If the liver becomes insulin-resistant, these processes are severely affected, resulting in significant metabolic consequences. When skeletal muscle develops insulin resistance, excess glucose in the blood is shunted to the liver. When the liver tissue senses an excess of energy substrate, particularly in the form of diacylglycerol, a process similar to that in skeletal muscle occurs. In the liver, the diacylglycerol content activates protein kinase C epsilon (PKC-epsilon), which decreases proximal insulin signaling. Excess glucose enters hepatocytes via insulin-independent pathways stimulating DNL via substrate push, creating more fatty acids from the glucose surplus. The excess fatty acid is deposited in the liver or as ectopic lipid throughout the viscera. Additionally, immune-mediated inflammatory changes contribute to excess lipolysis from adipose tissue, which is re-esterified by the liver and further adds to circulating fatty acid and ectopic lipid deposition. Finally, normal insulin-mediated suppression of gluconeogenesis is defective, and the liver continues to create more glucose, adding to the circulating glucose surplus. (**Freemanand Pennings, 2018**).

I.1.3.9.2.3. Adipose Tissue:

Using the hyperinsulinemic-euglycemic clamp technique, researchers determined that lipolysis is sensitive to insulin. The failure of

insulin to suppress lipolysis in insulin-resistant adipose tissue, especially visceral adipose tissue, increases circulating free fatty acids (FFAs). Higher levels of circulating FFAs directly affect both liver and muscle metabolism, further exacerbating insulin resistance in these tissues and contributing to lipotoxicity-induced beta-cell dysfunction**(Freeman and Pennings, 2018).**

I.1.4. The glycemic response:

I.1.4.1. Definition :

After eating a meal, the digestible or glycaemic carbohydrates are absorbed from the intestine into the bloodstream, producing an increase in blood glucose concentration. In time and in response to its tissue disposal, facilitated by the hormone insulin, the blood glucose concentration falls back to or below fasting levels. The magnitude of the rise and fall in blood glucose and the duration over which it occurs has been termed the blood glucose (or glycaemic) response**(Sadler. 2011).**

I.1.4.2. Glycaemic index (GI):

The GI is a ranking of carbohydrate foods from 0 to 100 based on how quickly and how much they raise blood sugar levels after being eaten. This is related to how quickly a carbohydrate containing food is broken down into glucose **(Nemo, 2016).**

Considering the GI of carbohydrate foods can help with good diabetes management as:

- Lower GI foods produce lower, more stable blood sugar levels and therefore can help improve control of diabetes.

- Higher GI foods produce higher, faster rising blood sugar levels.

- Lower GI foods also help you to feel fuller for longer, which can help to control appetite and assist with weight management **(Nemo, 2016)** .

I.1.5. Diabetes :

I.1.5.1. Definition :

Diabetes is a group of metabolic diseases in which there are high levels of sugar in the blood. characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of differentorgans, especially the eyes, kidneys, nerves, heart, and blood vessels. **(American Diabetes Association. 2014).**

I.1.5.2. Diabetes classification:

The classification of diabetes has been extensively reviewed and revised since its firstclassification in 1979. Finally, the American Diabetes Association (ADA) proposed tonew diagnostic criteria as well as a new classification according to which diabetesis primary or secondary (figure 17) (**Peter Riesch and al., 2002**).

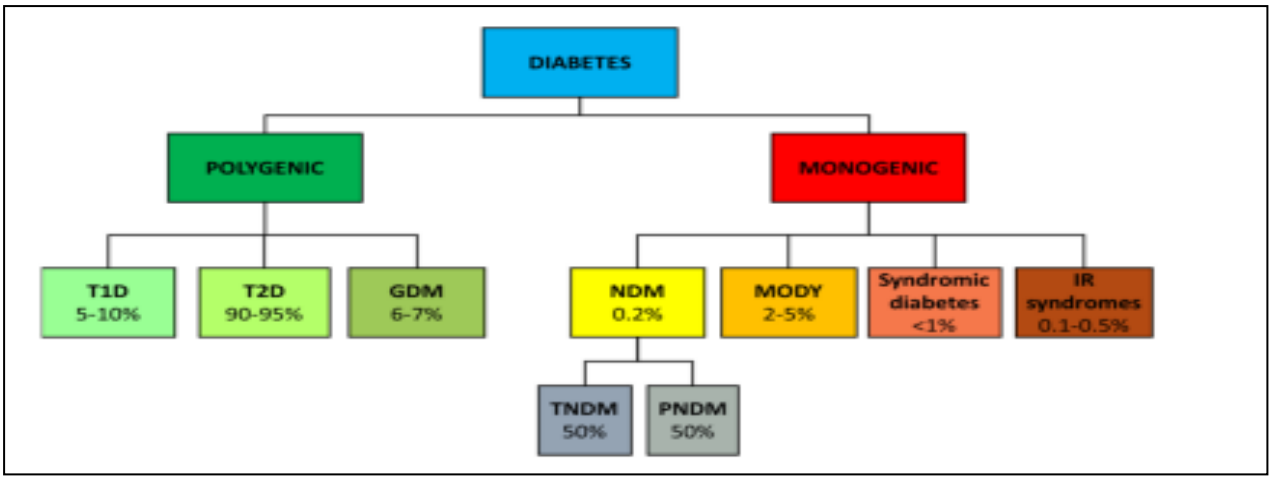

Figure 17: Classification of diabetes .Diffrent diabetes and their rough frequencies **(Liguori, F and al., 2021).**

I.1.5.2.1. Primary diabetes:

Primary diabetes is classified into 3 types: type 1 diabetes (approximately 5 to 10% ofthe diabetic population), type 2 diabetes (90 to 95%) and gestational diabetes.

I.1.5.2.1.1. Type 1 diabetes(TD1) or insulin-dependent diabetes(IDD):

Represents approximately 10% of global diabetes cases(**Peter Riesch and al., 2002**). It appears most often in children and young adults, which is why it is also called "juvenile diabetes" (**Grimaldi and al., 2001**).It is an autoimmune disease leading to destructionselective and progressive of pancreatic β cells, producing insulin (**Boitard. 2002 ;Thivolet. 2002**).This destruction results from the production of autoantibodies directed against the antigens of β cells, it seems to appear in genetically predisposed subjects(**Peter Riesch and al., 2002**).

This process of destruction leads to an absolute and definitive insulin deficiency responsiblethe appearance of permanent chronic hyperglycemia (**Grimaldi and al., 2001**).

I.1.5.2.1.2. Type 2 diabetes (TD2) or non-insulin-independent diabetes (NIDDM):

Is by far the form of the most common disease since it presents 90% of global cases (**King et al., 1998**). Type 2 diabetes is also called "mature diabetes" because it occurs most frequently often in adults(**Peter Riesch and al., 2002**). Its main characteristic is insulin resistance of target tissues (decrease inthe inhibitory action of endogenous production and the stimulatory action of useperipheral glucose insulin) which leads to reactive hyperinsulinemia (**Rigalleau and al., 2007**).

I.1.5.2.1.3. Gestational diabetes:

Gestational diabetes is defined as glucose intolerance that occurs or occurs is first identified during pregnancy. This type of diabetes occurs during pregnancy, especially during the 2nd or 3rd trimester when insulin needs are high more important than normal. In addition, certain factors such as hormones growth and placentals decrease the action of insulin (**Landon and al., 2009**).

I.1.5.2.2. Secondary diabetes:

Other types of diabetes are often called specific diabetes, Rare forms of diabetes, including neonatal (NDM) and maturity onset diabetes of the young (MODY) are, instead, due to single gene mutations.

since they are linked to a well-defined cause. These causes can be genetic in nature, such as diabetesMODY (Maturity Onset Diabetes Of the Young), and affect β-cell function. THE Secondary diabetes may also result

from the development of another disease, such asendocrine diseases (Cushing's syndrome, hyperthyroidism), pancreatic diseases(pancreatitis, pancreatic cancer) and liver diseases (cirrhosis, hepatitis C). Somemedications such as corticosteroids can also induce this type of diabetes **(Stumvoll and al., 2005).**

I.1.5.3. Methods to detect diabetes

Diabetes is defined by an increase in blood sugar fasting above 7 mmol/L (1.26 g/L) . The diagnosis clinical hyperglycemia is carried out by measuring plasma glucose, measured either fasting and/or randomly at any time of the day and/or during an oral glucose load (**Tenenbaum, M and al., 2018**).

Hemoglobin A1C (HbA1C), Fasting glucose (FPG) and Insulin Resistance (HOMA-IR) are the main methods to detect diabetes.Diabetics will have HbA1C higher than 6.5% (48 mmol/l), HOMA-IR higher than 1.9 and FPG higher than 126 mg/dl (**Goyal, R and al., 2023**).

I.1.5.4. Organestrageted by type 2 diabetes:

Type 2 diabetes mainly affects 4 main organs:

the pancreas, adipose tissue, liver and muscle. Dysfunction at the β cell levelcauses insufficient insulin secretion (INS). An alteration in the liver to an overproduction of hepatic glucose. At the level of adipose tissues, we notice a activation of lipolysis leading to abnormal levels of fatty acids (FA). An anomaly in level of the muscle leads to a reduction in glucose absorption and its use. All of these factors lead to hyperglycemia **. (Vargas, E and al., 2018)..**

I.1.5.5. Type 2 diabetes Causes :

I.1.5.5.1. Heredity:

The majority of patients have a parent with type 2 diabetes: 20% of their first-degree relatives will have over the course of their life a glycoregulation disorder, the risk increases with the number of affected parents, and concordance among monozygotic twins approach 100%. Genetic studiesmade it possible to discover the cause of monogenic formsparticular types of diabetes (maturity onset diabetes of the young [MODY]), and the involvement of the PPARc genes, IRS1, KIR6.2, calpain and more recently TCF7L2 in formscommon types of type 2 diabetes, but they are complex becauseseveral genes are probably involved. It should be noted that in addition to heredity, the nutritional environment in utero plays a rolea very early role: maternal hyperglycemia duringpregnancy, a cause of neonatal macrosomia, as well asconversely, low birth weight promotes diabetes of type 2 in adulthood.

I.1.5.5.2. Obesity:

The majority of patients are, or have been, obese, and the current epidemic of obesity and diabetes highlights the importance of environmental factors: food abundance and sedentary lifestyle. Excess or weight gain promote the occurrence of a "minor" disorder of glycoregulation, its evolution towards type 2 diabetes, and increased blood sugar levels once this is established, controlling weight the first therapeutic objective throughout the disease.

I.1.5.5.3. Age:

The majority of patients are between 55 and 75 years old: beyond prevalence falls due to excess mortality associated with the disease.

The increase in life expectancy therefore plays a role in the diabetes epidemic, but its recent appearance in children harshly recalls the importance of the other factors already cited **. (Vargas, E and al., 2018)..**

in the case of obesity, hyperglycemia caused orally studies reveal glucose intolerance in 25% of children, and type 2 diabetes in 4% of adolescents. Age is physiologically accompanied by a reduction progressive insulin secretion, a reduction in lean mass using glucose and perhaps a decreased sensitivity to insulin which promotes all the expression of the disease. The late start also reflects the delay diagnostic linked to its insidiousness, and its progressive nature, long preceded by a phase of "prediabetic" state.

I.1.5.5.4. Scalability:

These minor disorders of glycoregulation, such as moderate fasting hyperglycemia and glucose intolerance more frequent, expose to a risk of type 2 diabetes assessed at approximately 7%/year. In the case of women, they can be expressed decades ago, in the form of a gestational diabetes: even if the diagnosis has not been made at the time, the patients concerned generally remember having given birth to overweight children.

Non-alcoholic steatohepatitis probably also exposes people to this risk, a level of transaminases higher by a few units is associated with a marked increase in the risk of diabetes later type 2.

But above all the worsening does not stop once the diagnosis of type 2 diabetes is made. The United Kingdom study Prospective Diabetes Study (UKPDS) showed that regardless of the type and intensity of treatment, HbA1C then tends to rise gradually over the years, leading clinicians to a therapeutic escalation which ultimately makes each year 5 to 10% of patients

on insulin. Even if the therapeutic failure of patients tired of their diet and taking multiple oral antidiabetics exist, we should no longer consider this explanation sufficient, particularly when weight control is achieved.

The high rates of glucose and free fatty acids play roles through their effects deleterious on cells sensitive to insulin and especially on b cells: there is "glucotoxicity", and "lipotoxicity", responsible for functional disorders, but also accelerated apoptosis of these cells. Pancreatic deposits amyloids formed from the amylin that they co-secrete with insulin can also contribute to this worsening . (**Vargas and al., 2018**)..

I.1.5.5.5. Involvement of hormones counter-regulation:

It is legitimate to consider the role of hormones (glucagon, catecholamines, cortisol, growth hormone) which are involved in normal glycemic homeostasis, especially as their hyperglycemic influence accounts for rare diabetes secondary to endocrinopathies: glucagonoma, pheochromocytoma, adrenal hypercortisolism, acromegaly. They are involved in the response to the aggression, accounting for the usual increase in hyperglycemia when patients present with an intercurrent pathology. Their involvement in common type 2 diabetes is less important, but it exist.

Adrenal hypercortisolism shares many aspects clinical conditions encountered in type 2 diabetes: overload abdominal fat, high blood pressure, dyslipemia. A systematic research in type 2 diabetics done detect proven autonomic hypersecretion of glucocorticoids (Cushing's disease or cortisol adenoma) in 2%cases, and up to 3.5% more could be linked tocortisol adenomas (**Vargas and al., 2018**).

I.1.5.5.6. Insulin resistance :

Insulin resistance concerns virtually all type 2 diabetics. Detectable 10 to 20 years before diagnosis, even in the absence of obesity.

clinically marked by excessive waist circumference, which leads to insulin resistance. The secretion products of Excess adipose tissue are therefore widely studied. Even if the body cannot directly generate glucose from free fatty acids, these have deleterious effects on the insulin sensitivity, well established and detectable with in a few hours in humans (**Vargas and al., 2018**).

CHAPITRE II :

EFFECT OF VITAMIN D AND MAGNESIUM IN TYPE 2 DIABETES

I.2. Effect of Vitamin D on glycemic response and insulin sensitivity:

I.2.1. Role of Vitamin D in insulin secretation :

Beta cells in the pancreas express both vitamin D receptor VDR and 1α-hydroxylase, which suggests that vitamin D can have a direct effect on beta cell function by binding to its VDR ; or an autocrine effect via 1α-hydroxylase within the beta cell.

Vitamin D also has an indirect effect by regulating calcium concentrations. Calcitriol mediates calcium influx from intracellular stores and the extracellular space (L-type calcium channels on islet β-cells are stimulated by 1, 25(OH) 2D which then controls calcium levels., influencing insulin release from beta cells. (**Ahmad and Haque, M**).

Beta cells also express calbindin, a calcium-binding protein in the cytosol, which is a regulator of intracellular calcium, and thereby can modulate depolarization-stimulated insulin release. **(Sooy, 1999).**

Expression of the insulin gene is promoted through the vitamin D response element **(Liao, 2018).**

Suppressing the production of pro-infammatory mediators and cytokines(**Musazadeh, 2023).**

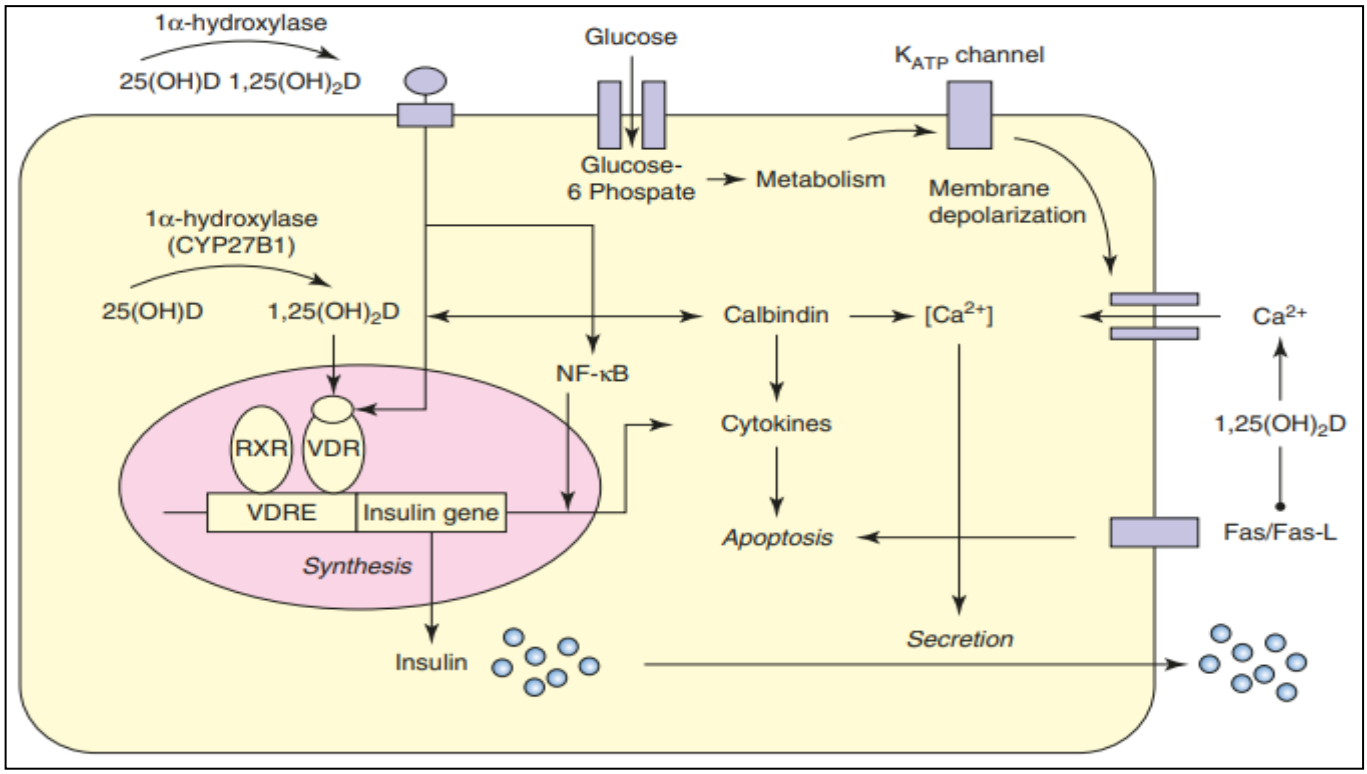

Figure 18: Role of Vitamin D in insulin secretation in beta cell**(Liao, 2018).**

I.2.2. Vitamin D and insulin sensitivity :

There are several ways in which vitamin D could affect insulin sensitivity **(JOANNA and ANASTASSIOS, 2014):**

Vitamin D increases expression of insulin receptor number (but not affinity), via vitamin D response element (VDRE) in the human insulin receptor gene, thus increasing insulin sensitivity (**Mitri and Pittas, 2014**)

Vitamin D also activates peroxisome proliferator activator receptor (reported with PPARδ and PPARγ), which is involved in the regulation of fatty acid metabolism in skeletal muscle and adipose tissue, there by increasing insulin sensitivity**(Liao, 2018)** .

Vitamin D has also been found to improve muscle oxidative phosphorylation after exercise.

Another potential effect of 1,25(OH)2D on insulin sensitivity might be exerted via its regulatory role in extracellular calcium concentration and flux through cell membranes. Calcium is essential for insulin-mediated intracellular

processes in insulin-responsive tissues such as muscle and fat, with a narrow range of intracellular calcium needed for optimal insulin-mediated functions. Changes in intracellular calcium in insulin target tissues may contribute to peripheral insulin resistance via an impaired insulin signal transduction leading to a decreased glucose transporter activity. **(JOANNA and ANASTASSIOS, 2014).**

I.2.3. Vitamin D and systemic inflammation:

Vitamin D could directly and/or indirectly lessen the effects of systemic inflammation in patients with type 2 diabetes in several ways. For example, 1,25(OH)2D may protect against beta cell cytokine-induced apoptosis by directly modulating the expression and activity of cytokines, hence improving insulin sensitivity **(JOANNA and ANASTASSIOS, 2014)** . One such pathway may be through the down-regulation of NF-kB, a major transcription factor for TNF-alpha and other proinflammatory molecules **(Cohan and al, 2007).** Another pathway that may mediate the effect of 1,25(OH)2D on beta cell function is through counteracting cytokine-induced Fas expression, which in turn will have anti-apoptotic effects.(180)Several other immune-modulating effects of 1,25(OH)2D such as blockade of dendritic cell differentiation, inhibition of lymphocyte proliferation, inhibition of foam cell formation and cholesterol uptake by macrophages and enhanced regulatory T-lymphocyte developmentmay provide additional protective pathways against beta cell destruction mediated by the systemic inflammation caused by type 2 diabetes**(JOANNA and ANASTASSIOS, 2014)**.

I.3. Vitamin D and Type 2 Diabetes :

Vitamin D plays an important role in modifying the risk of type 2 diabetes, an effect which is likely mediated by an effect of vitamin D on beta cell function, insulin sensitivity and systemic inflammation **(JOANNA and ANASTASSIOS, 2014)**.

Vitamin D protects against type 2 diabetes. **(Wu and al., 2023)** and it is related complications through its antioxidant, anti-infammatory, and

immunemodulatingefects which plays an important role in insulin resistance (**Mazur and Maier, 2016**).

I.3.1. The genetic component of vitamin D and diabetes:

In addition to low serum vitamin D levels, VDR polymorphisms are also associated with genetic sensitivity in type 2 diabetic patients. This would exert its effect through this receptor, and genetic variations of the VDR gene lead to dysfunctional vitamin D signaling(**Ma, L and al., 2020**).

Studies have demonstrated a nonfunctional VDR in mice, which resulted in consequence an impairment of peripheral glucose tolerance and a reduction in secretion of insulin at the pancreatic level(**Zeitz and al., 2003**). In humans, the analysis of organization genomics of the VDR locus showed that the gene is quite large, which suggests that more than 100 polymorphisms may exist, among which 25 have been mapped, four of which have been identified and described: FokI, BsmI, ApaI and TaqI **(Uitterlinden and al., 2004).**

These polymorphisms could play a role in the pathogenesis of multi-diabetes levels:

- Reduction of insulin secretion (ApaI, BsmI and TaqI)(**Ogunkolade and al., 2002**)

- Glucose intolerance (ApaI)

- Increased insulin sensitivity (FokI) (**Oh and Barrett-Connor , 2002**).

- Alteration of calcium metabolism and a modification of calcium flow in the pancreatic β cells (BsmI) (**Palomer and al., 2008**).

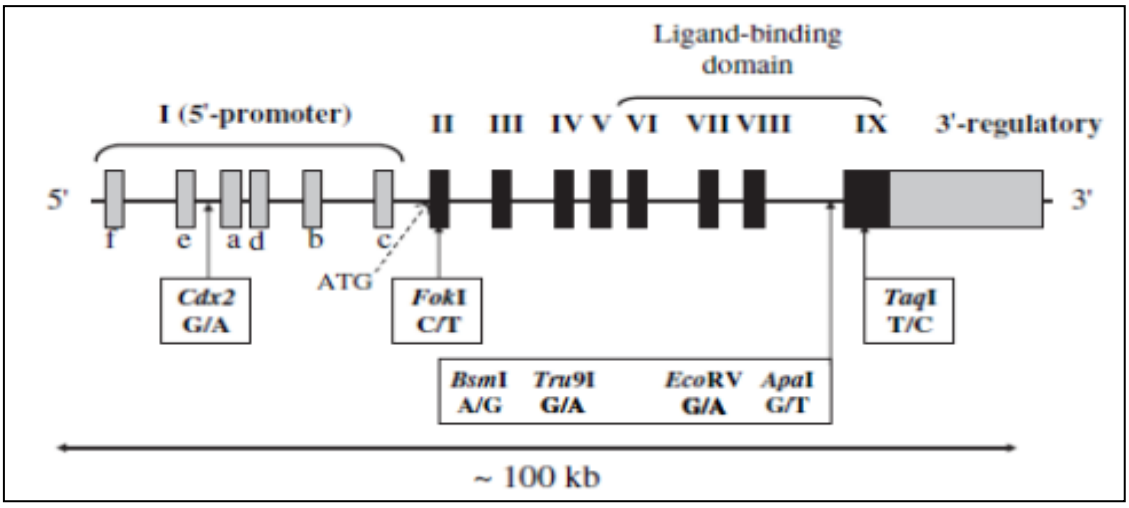

Figure 19: Polymorphisms of the vitamin D receptor gene in humans **(Uitterlinden and al., 2004).**

In addition, polymorphisms of the BPD genes where certain variants would affect the insulin secretion by affecting the availability of active forms of vitamin D in the β cells have been demonstrated(**Kebir and Zahzeh, 2022**).

Finally, the 1-α-hydroxylase gene would not be major in the occurrence of T2DM. However,

it could be associated with T2DM in obese subjects. However, additional studies are necessary to confirm these data (**Piuri and al., 2021., Hodson and al., 2014).**

I.3.2. Vitamin D mechanism action in Type 2 Diabetes :

1,25 (OH)2 D3 , the active form of vitamin D3, is produced from cholesterol through successive hydroxylation of UVB generated 7-dehydrocholesterol (DHC).

1,25 (OH)2 D3 activates the vitamin D receptor (VDR) retinoid X receptor (RXR) heterodimer in the major metabolic tissues. The active VDR/RXR heterodimer binds to vitamin D response elements (VDREs) to induce changes in gene expression that in combination, improve islet function and decrease insulin resistance.

1,25(OH)2D, as a ligand, binds to vitamin D receptor (VDR), a ligand-dependent nuclear receptor that functions as a transcription factor by generating a heterodimer with the retinoid X receptor (RXR) upon ligand binding. The VDR/RXR complex recognizes vitamin D-responsive elements (VDRE)) to induce changes in gene expression that in combination, improve islet function and decrease insulin resistance **(Wu and al., 2023).**

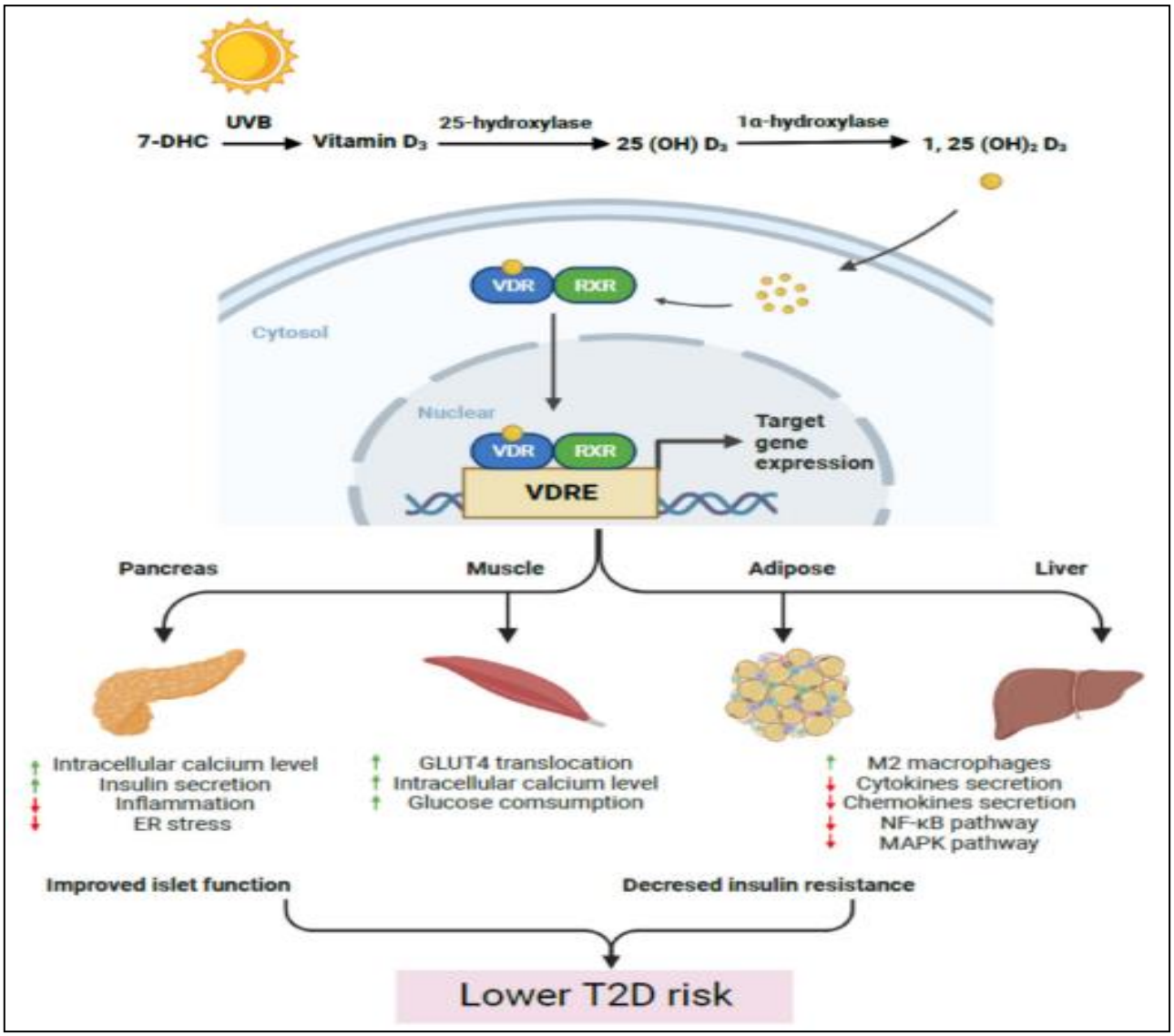

Figure 20 : Vitamin D mechanism action in Type 2 Diabetes **(Wu and al., 2023).**

I.3.3. Deficiency of Vitamin D and Type 2 Diabetes :

I.3.3.1. Vitamin D deficiency and Insulin Secretion:

In type 2 diabetes (T2D) patients Vitamin D deficiency reduce insulin secretion and the expression of proteins involved in the glucose response, including insulin ; and lead to insulin resistance (**von Hurst and al., 2010., Pittas and al., 2006**).

I.3.3.2. Vitamin D deficiency and metabolic Pathways:

Vitamin D deficiency affects the glycolytic pathway after the d-glyceraldehyde step and alters oxidative events within the tricarboxylic acid cycle, potentially leading to beta cell dysfunction and death (**Takiishi and al., 2012**).

Vitamin D deficiency increased insulin resistance through reduced Ppar-γ expression, a regulator of glucose metabolism and inflammation (**Liu and al., 2010**).

Vitamin D deficiency also leads to an increase in the levels of parathyroid hormone (PTH), which has been associated with insulin resistance. **(JOANNA and ANASTASSIOS, 2014)** Vitamin D may also affect insulin resistance indirectly through the renin-angiotensinaldosterone system (RAAS), as described below. Finally, vitamin D insufficiency has been associated with increased fat infiltration in skeletal muscle, which appears independent of body mass and is thought to contribute to a decreased insulin action. (**Gilsanz and al., 2010**)

I.3.3.3. Vitamin D deficiency Calcium Dependence:

insulin release is dependent on calcium, vitamin D deficiency, which causes hypocalcemia, reduces insulin secretion**(Liao, 2018)** .

I.3.3.4. Vitamin D deficiency Clinical Implication :

Vitamin D deficiency (defined as serum 25(OH)D levels of ≤15 ng/mL) is associated with higher risks for metabolic syndrome, lowered insulin secretion, and impaired glucose tolerance (**Vanherwegen and al.,2018**).

Observational studies consistently show an association between low vitamin D levels and the incidence of T2DM. Low vitamin D concentrations predict an increased future risk of T2DM (**Sergeev and Rhoten, 1995**).

Vitamin D deficiency is also associated with multiple risk factors for diabetes and its associated conditions, such as obesity and metabolic syndrome **(Liao, 2018).**

I.3.4. Vitamin D supplemetation and Type 2 Diabetes:

Vitamin D supplementation may help improve type 2 diabetes mellitus (T2DM) biomarkers by enhancing insulin sensitivity and glucose regulation (**Monapati and al.,2023**). Meta-analyses have shown that vitamin D significantly decreases HbA1c levels, suggesting its potential to delay or reduce the development of diabetic complications (**Musazadeh, 2023**). Additionally, vitamin D supplementation has been linked to increased insulin secretion by the pancreas (**Monapati and al.,2023**).

I.3.4.1. Vitamin D supplemetation and Inflammatory Cytokines:

Vitamin D3 supplementation reduces inflammatory cytokines such as IL-6 and TNF-a, which significantly contribute to insulin resistance (**Liu and al., 2023**).

I.3.4.2. Vitamin D supplemetation and Insulin Sensitivity and β-Cell Function:

Vitamin D3 improves insulin sensitivity in response to an oral glucose load and enhances peripheral insulin sensitivity and β-cell function (**Tracy and Mazen, 2010., Liu and al., 2023).**

I.3.4.3. Vitamin D supplemetation and Clinical Implications:

While vitamin D supplementation may not be therapeutic after the onset of diabetes, it has a high potential to delay the risk of diabetes in high-risk individuals (**Liu and al., 2023).**

I.4. Effect of Magnesium on glycemic response and insulin sensitivity:

I.4.1. Regulatory role of Mg+2 in the insulin secretion from pancreatic beta cells:

The normal intracellular Mg2+ concentrations are of utmost importance for the optimal insulin secretion. **(Kostov, 2019).**

Magnesium acts as a cofactor in the regulation of glucokinase, an enzyme that catalyzes the conversion of glucose into glucose-6-phosphate in pancreatic β-cells, particularly in situations of high concentrations of glucose in the blood **(Sousa Melo and al., 2022)**, which subsequently results in a rise in intracellular ATP (**Kostov, 2019**).

In addition, magnesium is a cofactor of the ATPase enzyme required in several intracellular reactions, such as the glycolytic pathway and phosphorylation of cellular receptors(**Gommers and al., 2016**). It is noteworthy that magnesium participates as a cofactor of the ATPase enzyme via the sulfonylurea receptor (SUR1), which is a subunit that regulates the opening of potassium channels. In parallel, due to the increased metabolism of glucose-6 phosphate, there is excessive production of ATP, which induces the closure of ATP-sensitive potassium channels (KATP), triggering plasma membrane depolarization and the opening of L-type calcium channels, thus increasing calcium infux. Subsequently, with the increase in intracellular calcium concentration, insulin granules are released via exocytosis **(Piuri and al., 2021., Hodson and al., 2014).**

Magnesium is necessary for the activation of protein kinase C, which in turn activates adenylate cyclase. The activation of adenylate cyclase results in ATP hydrolysis and an increase in the intracellular content of cAMP, a substrate with a high afnity for protein kinase A binding sites. Protein kinase A is activated by this binding, and contributes to the mobilization of insulin-secreting granules by pancreatic β-cells. In summary, insulin secretion directly depends on the increase in ATP and Ca2+ concentrations within pancreatic β-cells, which is regulated by magnesium **(Soriano and al., 2022).**

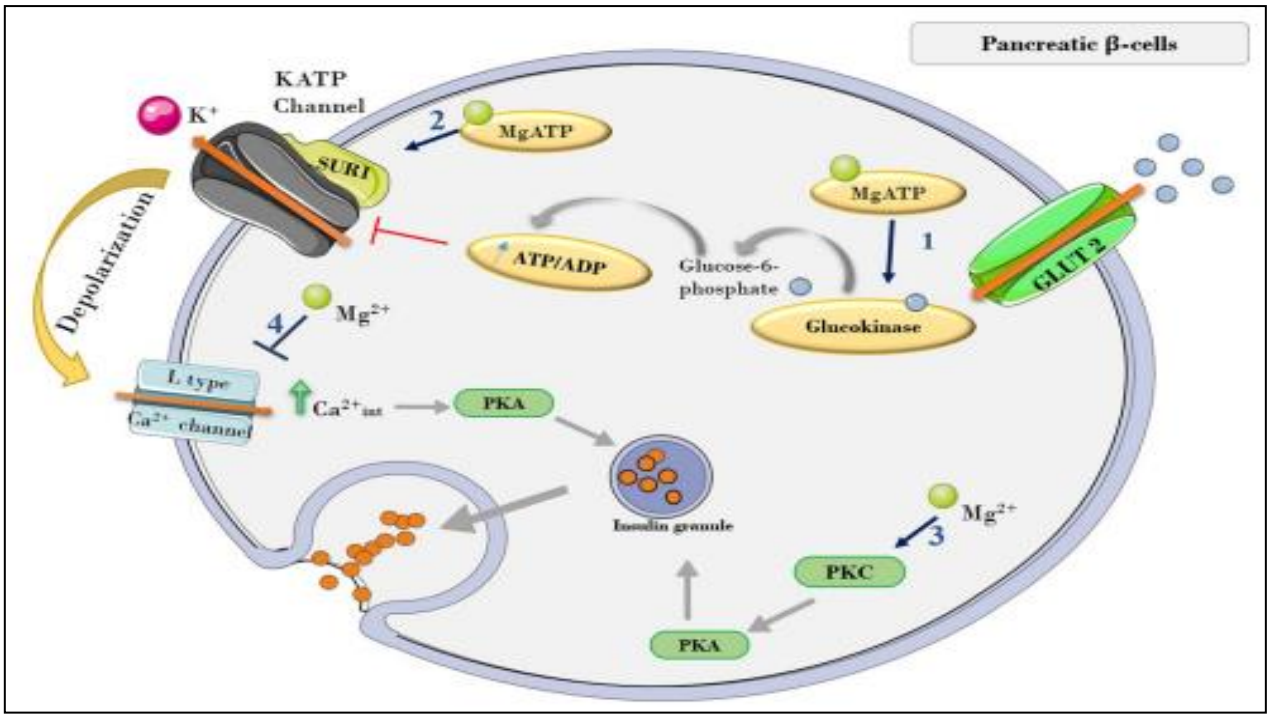

Figure 21: Regulatory role of Mg+2 in the insulin secretion from pancreatic beta cells **(Soriano and al., 2022).**

I.4.2. Regulatory role of Mg2+ on insulin sensitivity:

Magnesium has received considerable attention for its potential role in improving insulin sensitivity (**Kebir and Zahzeh, 2022**).

Intracellular Mg2+ concentrations are critical for the phosphorylation of INSR and the activity of other signal kinases, where Mg2+ operates together with ATP as a kinase substrate Mg2+ may exert regulatory influence on TK of INSR and other enzymes (**Kebir and Zahzeh, 2022**).

I.4.2.1. Mechanism of action :

In particular, Mg2+ and Mg-ATP complex are key regulators of the PI3K/Akt kinase pathway downstream to the Insulin receptor INSR. This pathway starts with INSR auto-phosphorylation, which triggers the downstream kinase cascade. Insulin receptor substrate (IRS) mainly activates phosphatidylinositol-4,5-bisphosphate-3-kinase (PI3K), which generates the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3

activates 3-phosphoinositide dependent protein kinase-1 (PDK1), which activates Akt. Akt regulates the metabolic actions of insulin, including glucose uptake by GLUT4 mobilization in skeletal muscle and adipose tissue, glycogen and protein synthesis and lipogenesis **(Piuri and al., 2021).**

The Ras/MAPK pathway regulates gene expression and insulin-associated mitogenic effects. The PI3K/Akt kinase pathway regulates the metabolic actions of insulin, which include glucose uptake by GLUT4 mobilization, glycogen and protein synthesis, and lipogenesis. Intracellular Mg^{2+} concentrations are critical for the phosphorylation of INSR and the activity of other signal kinases, where Mg^{2+} operates together with ATP as a kinase substrate. Mg^{2+} may exert regulatory influence on TK of INSR and other enzymes, mediating the metabolic effects of insulin (**Kostov, K. 2019**).

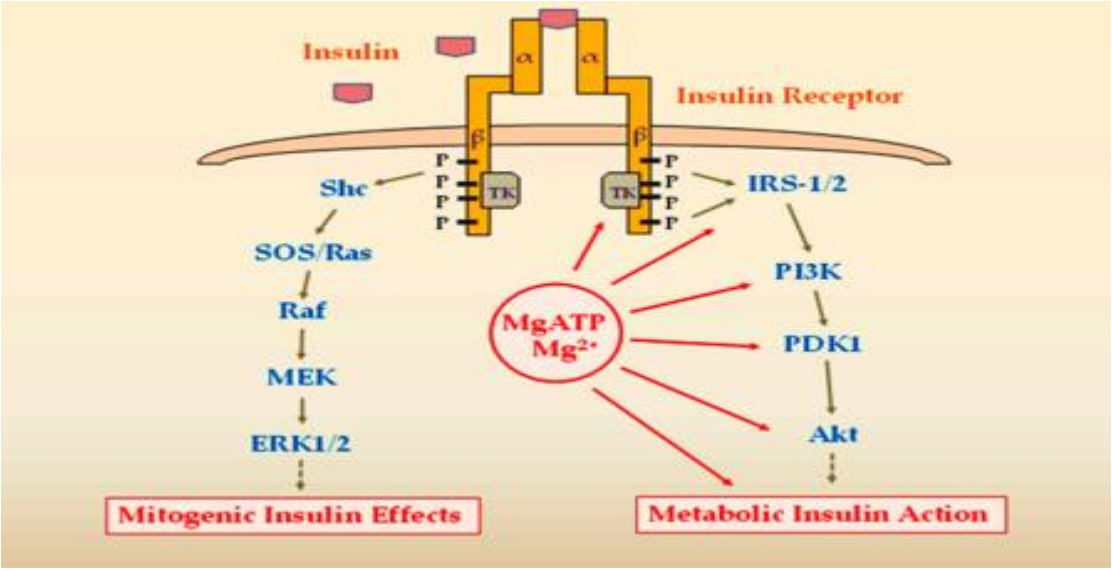

Figure 22: Schematic diagram showing the two major insulin signaling pathways and the role of Mg^{2+} in metabolic signaling (**Kostov, 2019**).

I.5. Effect of Magnesium deficiency in Type 2 Diabetes:

I.5.1. Effect of Magnesium deficiency on insulin secretation:

In MgD, intracellular levels of ATP and MgATP decrease. This disturbs the coupling between the chemical signal (blood glucose) and the electrical stimulation of the beta cells, resulting in a disturbance of the normal phases of insulin release **(Kostov, 2019).**

Magnesium defciency can directly infuence the conversion of glucose into glucose-6-phosphate by reducing glucose binding to glucokinase, and influencing the rate of GK activity because the enzyme's action depends on MgATP and inhibit pancreatic β-cell depolarization, consequently compromising insulin release ; which then results in insulin deficiency **(NikN. F. and al., 2023).**

Magnesium deficiency conditions decrease Ca2+ transport, lowering insulin secretion (**Soriano-Pérez, 2022**) , as found in T2D patients, may cause insulin resistance (**EL Derawi and al., 2018).**

I.5.2. Effect of Magnesium deficiency on insulin sensitivity:

A large body of evidence shows a link between hypomagnesemia and reduced tyrosine kinase activity at the level of insulin receptors, which may lead to decreased insulin action and the development of insulin receptors, insulin resistance has gradually accumulated in previous years (**Kebir and Zahzeh, 2022**).

I.6. Effect of Mg supplementation inType 2 Diabetes

Oral magnesium supplementation and appropriate dietary habits improve insulin sensitivity and metabolic control in patients with type 2 diabetes mellitus (T2DM), suggesting that magnesium is crucial in the management of this rapidly growing global health issue (**Kebir and Zahzeh, 2022**). Higher magnesium levels correspond to increased insulin sensitivity, explaining the improvement in glycemic control indicators following magnesium supplementation (**EL DerawiA and al., 2018**)

I.6.1. Mg supplementationand Insulin Receptor and Intracellular Signaling:

Suppressed intracellular magnesium concentration may result in defective tyrosine kinase activity, altering insulin sensitivity by influencing receptor activity post-binding or by affecting intracellular signaling and processing (**Kebir, N. E., &Zahzeh, T, 2022**).

Magnesium supplementation increases insulin receptor binding constant and capacity. Insulin receptor tyrosine kinase activity can also vary with magnesium concentrations, as reported by several in vitro studies (**Soriano-Pérez, L and al.,2022**).

I.6.2. Mg supplementationand Gene Expression and Protein Synthesis:

Magnesium supplementation increases the mRNA and protein expression of IRS-1 in skeletal muscle and IRS-2 in the liver It also increases the mRNA expression of the glucagon receptor, which may improve insulin sensitivity.

I.6.3. Mg supplementation and Glucose Transport and Metabolism:

Magnesium supplementation improves insulin signaling, showing positive results by increasing insulin resistance and GLUT4 expression levels in T2DM rats.

Oral magnesium supplementation increased muscle GLUT4 expression in STZ-induced rats, thereby lowering serum glucose levels to normal ranges (**Oost and al., 2023**).

I.6.4. Mg supplementation and Metabolic Pathways:

Magnesium supplementation may improve glucose metabolism through increased expression of phosphofructokinase 1 (PFK-1), an enzyme involved in regulating the glycolytic pathway, and glucagon-like peptide 1 (GLP-1), an incretin that stimulates insulin production (**Oost and al., 2023**).

I.6.5. Mg supplementation Clinical Evidence:

Accumulated evidence suggests that magnesium intake is inversely associated with preclinical conditions such as insulin resistance and chronic diseases like T2DM. Cause higher circulating magnesium levels or higher magnesium intake are linked with a lower risk of developing T2DM (**Soriano and al., 2022**).

Magnesium supplementation has been hypothesized to benefit T2DM patients by improving circulatory glucose parameters (**Soriano-Pérez and al.,2022**).

I.7. Association of Vitamin D and Magnesium in Insulin and Glycemic control:

I.7.1. Interrelation of vitamin D and magnesium in the regulation of insulin synthesis and release from pancreatic β-cells:

Calcitriol, an activated form of vitamin D, enters pancreatic β-cells and binds to vitamin D receptors (VDR) to form a heterodimer complex with retinoid X receptor (RXR). The calcitriol-VDR-RXR complex later binds to the vitamin D response element which is located in the promoter region of the insulin gene in the nucleus. This is followed by increased insulin gene transcription to enhance its synthesis.

Magnesium in the form of magnesium adenosine triphosphate and magnesium adenosine diphosphate plays vital roles in regulating glucokinase, an enzyme that converts glucose to glucose-6-phosphate. Adequacy of intracellular magnesium level is paramount to allow optimal glucokinase activity. The subsequent process in glycolytic pathway, tricarboxylic acid cycle, and oxidative phosphorylation adenosine triphosphate (ATP). The rise in ATP results in the closure of potassium-ATP-channel which increases intracellular potassium level. The resulting membrane depolarization causes the opening of voltage-gated calcium channel that triggers insulin degranulation and secretion (figure 22) **(Nik and al., 2023).**

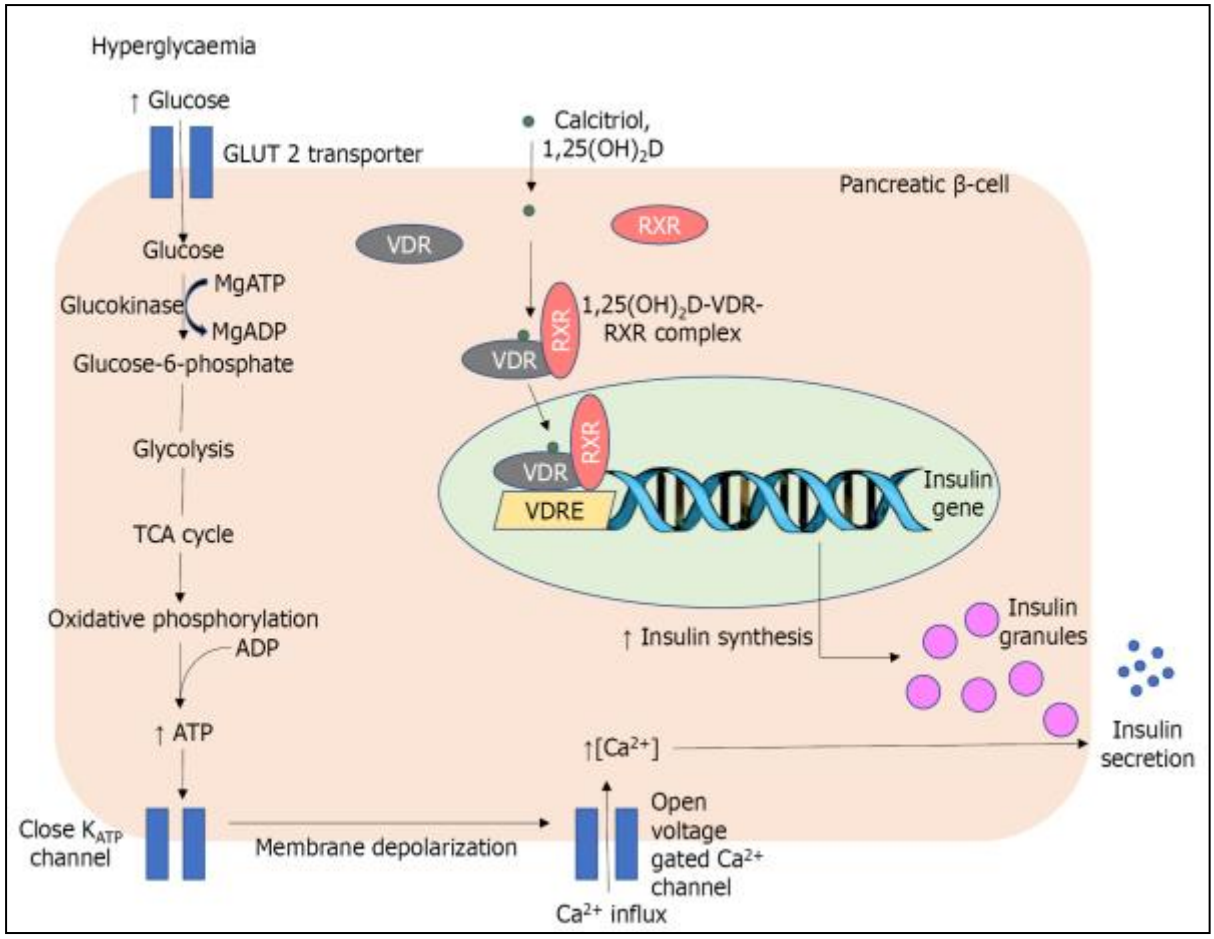

Figure 23: Interrelation of vitamin D and magnesium in the regulation of insulin synthesis and release from pancreatic β-cells**(Nik and al., 2023).**

I.7.2. Interrelation of vitamin D and magnesium in the action of insulin at target organs:

Magnesium adenosine triphosphate plays a role in the autophosphorylation process of B-subunits of insulin receptor tyrosine kinase, a crucial step in initiating an intracellular signaling pathway.

The calcitriol-vitamin D receptors-retinoid X receptor-vitamin D response element complex also modulates the synthesis of glucose transporter type 4 (GLUT4), a predominant glucose transporter present in muscle cells and adipocytes **(Nik and al., 2023).**

Upon stimulation by insulin, the activated tyrosine kinase receptor will stimulate the fusion of vesicles containing GLUT4 to the cellular membrane. This process increases cellular glucose uptake **(Nik and al., 2023).**

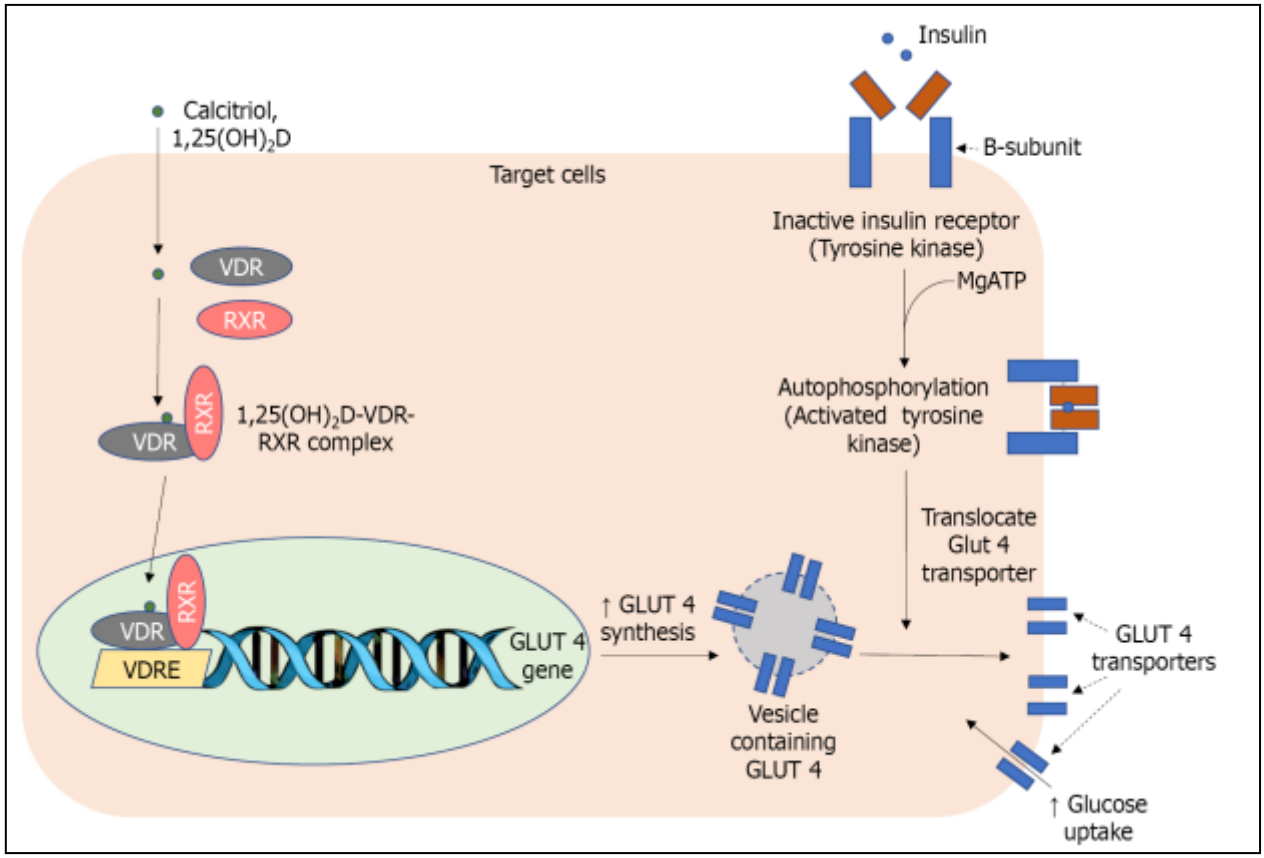

Figure 24: Interrelation of vitamin D and magnesium in the action of insulin at target organs**.(Nik and al., 2023).**

I.8. Association of Vitamin D and Magnesium in Type 2 Diabetes:

Most studies agree that both vitamin D and magnesium are important regulators of glucose homeostasis and, in turn, play a crucial role in the management of T2DM.

Different studies on diabetes patients which showed that those with poor glycemic control had significantly lower mean vitamin D and serum magnesium levels than those with good glycemic control (5); and other studies showed that the association of serum vitamin D with the incidence of T2DM appeared to differ between the low magnesium intake group (< 267 mg/d) and the high magnesium intake group (> 267 mg/d). It is reported that

the interaction between vitamin D levels and high magnesium intake decreases the incidence of T2DM(**Huang and al., 2021**).

PARTIE IV .CHAPITRE III :MATERIAL AND METHOD

I.9. Research methodology:

In this study we use the descriptive statistical method, because it depends on the accurate description of a phenomenon and expressing it as a whole through Ratio tables and circles and how to analyse and discuss them.

I.10. Study domain :

I.10.1. Temporal domain :

The data for this study lasted from March to june 2024.

I.10.2. Spatial domain:

The field study was conducted in the wilaya of Saïda.

I.10.3. Parts of study:

In this study, we focused on the results of measuring vitamin D, given that Magnesium constitutes only 1% of the human body, and that methods for measuring it accurately and easily are more expensive and still pose a challenge to many countries including Algeria .

The data for this study lasted was obtained from three parts :

- **The first part :**we carried out a questionnaire relating to the vitamin D table 1.
- **The second part :** was the collection of vitamin D analyzes from laboratories in the wilaya of Saïda table 2 .
- **the third part :** was the study of 78 patients suffering from Diabetic disease from the Wilaya of Saïda table 4 and who carried out the vitamin D analysis.

I.10.4. The objective of the study:

The first step : The main objective of this study is to estimate the prevalence of vitamin D deficiency in a group of 690 patients of different age groups in Saïda.

Secondary step: the secondary objective is to assess the influence of vitamin D levels on diabetic disease of 78 diabetic patients in Wilaya of Saïda.

I.10.5. Materials and Methods:

To know the materials used and methods followed to Calculate vitamin D dosage and the blood sugar level we relied on the assistance of the analysis laboratory .

I.10.5.1. Calculate vitamin D dosage:

Vitamin D analysis is one of the many tests that the VIDAS device can perform.

The VIDAS device uses the Enzyme-Linked Fluorescent Assay (ELFA) principle for analysis. The main difference in this technology is the use of enzyme-linked to antibodies instead of radiation to bind known substances in liquid samples.

Using this technology, the VIDAS device can analyze Vitamin D accurately and efficiently, providing important information about Vitamin D levels in the body.

I.10.5.1.1. Materials :

- Blood sample : patient serum in a 4ml dry or heparinized tube.
- Vitamin D assay reagent (plate).
- Centrifuge.
- 100 ul micropipette with a sterile tip.
- Instrument from the VIDAS family.
- Patient results software (softlam).

I.10.5.1.2. Methods :

- Collection of venous blood.
- Centrifugation of the samples at 4000 revolutions for 4 minutes to obtain the serum.

- Using a micropipette add 100ul of serum In the vitamin D reagent cartridge.
- Place the cartridges in the mini vidas machine carefully.
- Click on the start button of the automaton.

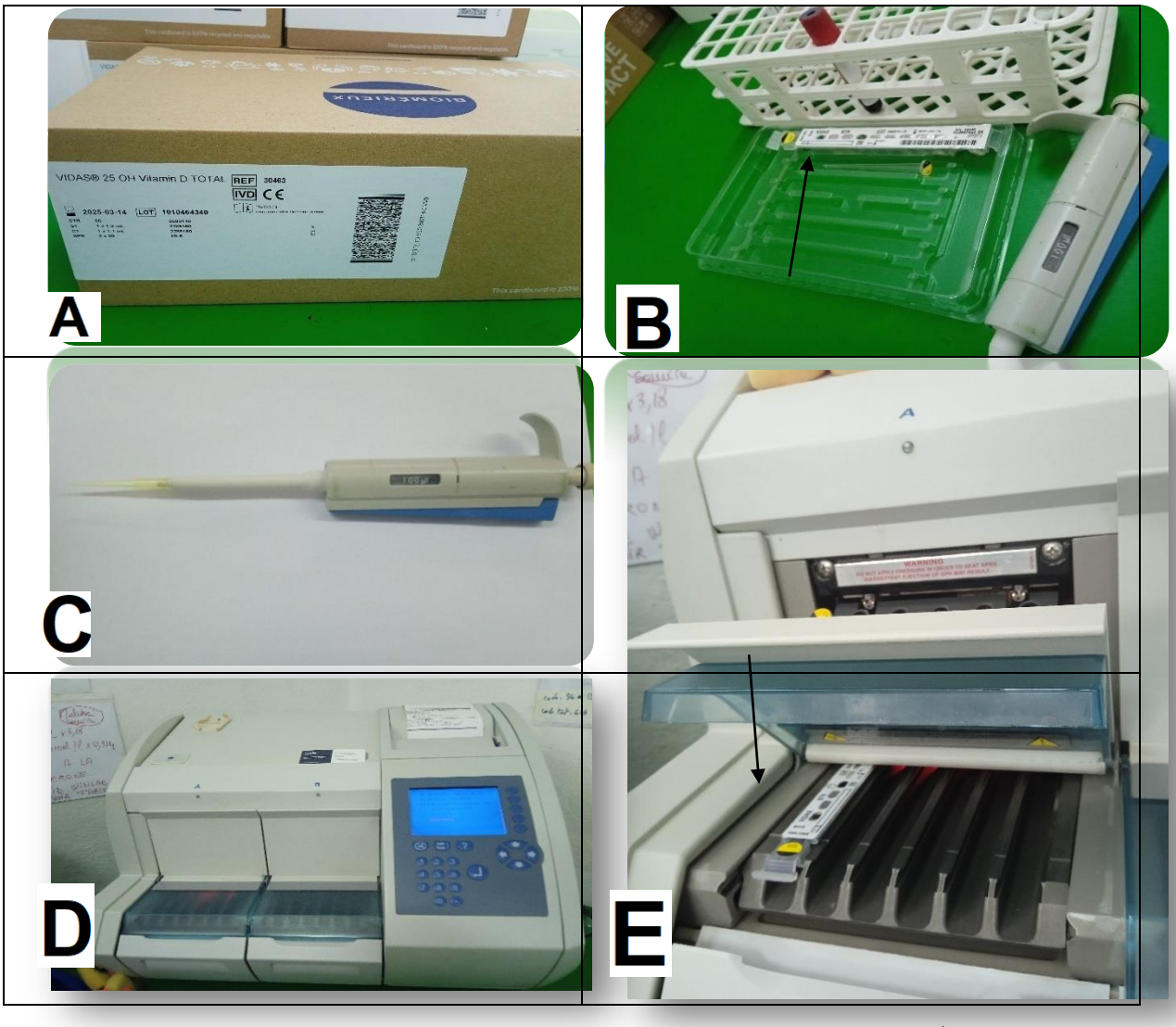

Figure 25: Materials used to Calculate vitamin D dosage (A. vitamin D box , B. vitamin D cartridge , C. micropipette with tip , D. the minividas automaton ,E equipment of the minividas automaton.

I.10.5.2. calculates the blood sugar level:

Blood glucose measurement is used for the diagnosis of carbohydrate metabolism disorders such as diabetes.

Glucose PAPSL is an in vitro diagnostic reagent. Intended for the quantitative determination of glucose in human serum and plasma samples.

I.10.5.2.1. Meteriales:

- Venous sampling sample .
- Centrifuge .
- Glucose reagent .
- The biolis automaton and their equipment .

I.10.5.2.2. Methods:

- Collect venous blood in a heparinized tube.
- Centrifuge the samples to obtain serum or plasma.
- Pipette a defined volume of serum or plasma into the reaction cuvette.
- Add the glucose PAPSL chemical reagents
- Place the cuvettes in the machine.
- Start the measurement cycle and wait for the results.

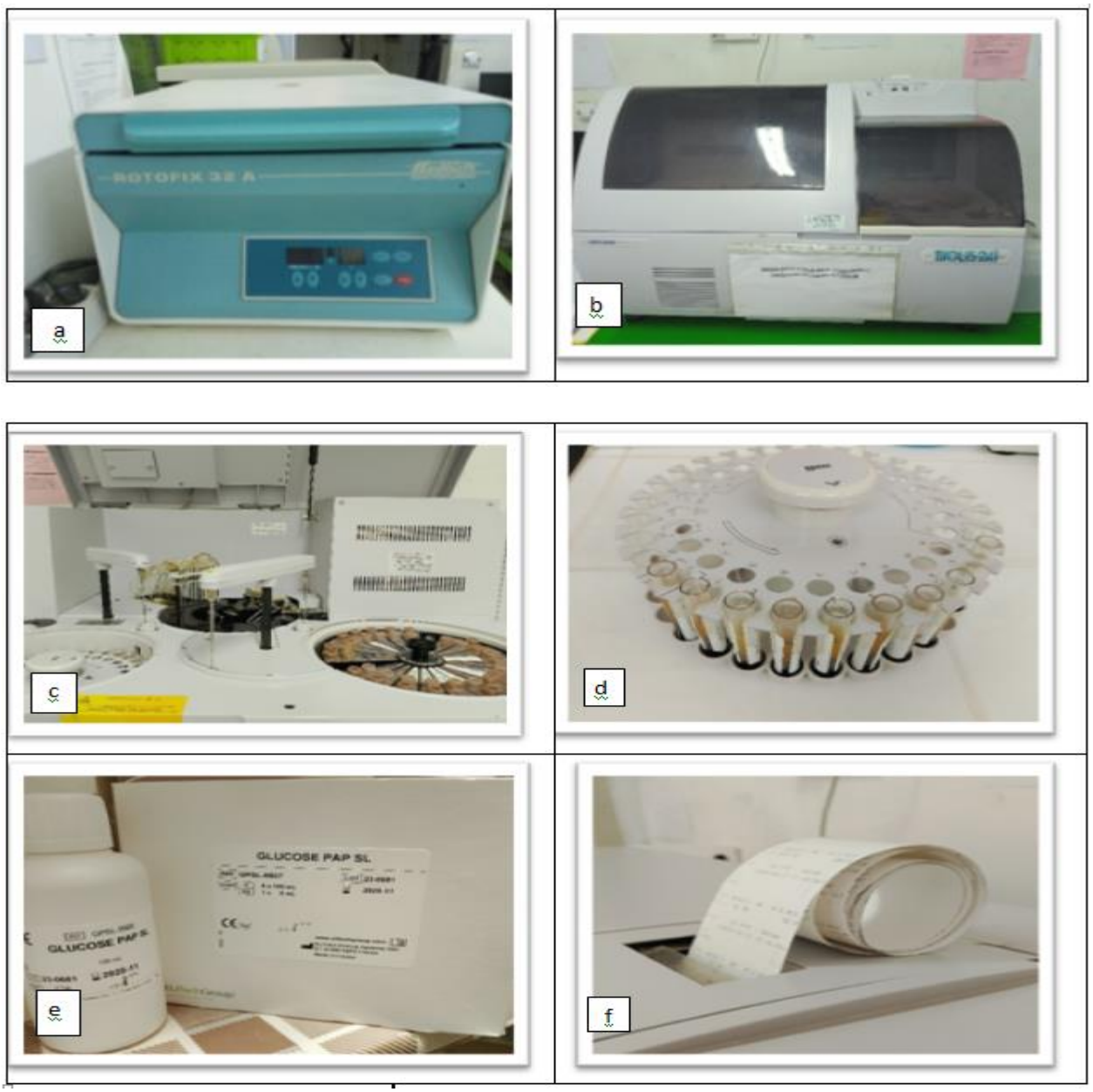

Figure 26: : Materials used to Calculate blood sugar level(a. Centrifuge , b. The Biolisautomaton, c. Samples in the automaton, d. Serum pipetting, e. The PAPSL glucose reagent, f. Exit result).

I.10.6. Target population and sampling :

I.10.6.1. Target population :

The target population of this study are subjects aged 1 to +50 years old, in Saïda, who do not present 100% of major health problems interfering with the metabolism of Vitamin D.

I.10.6.1.1. Inclusion criteria :

The inclusion criteria were all anonymous patients from three laboratories, where they were divided male/female and divided into 3 age groups.

I.10.6.1.2. Non-inclusion criteria :

The exclusion criteria for the study were essentially all chronic pathologies responsible for hypovitaminosis (with Crohn's disease, ulcerative colitis or celiac disease) or the lifestyle/diet of the subject (spending most of the time at indoors or outdoors) interfering with the development of vitamin D insufficiency.

I.10.6.2. Sampling method and sample size:

Our study is composed of three samples as follows:

The first sample questionnaire covering a population of 173 people categorized according to sex and age; 99 women, 70 men aged 18 to ≥50 years and a adolescents aged between 10 and 18 years. **Table 10.**

The second sample is composed of 685 people including 572 women and 113 men spread over 3 age groups, the first group from 1 to 15 years old, the second group from 15 to 50 years old and the third group over 50 years old **Table 11.**

The third sample is made up of 78 people including 49 women and 36 men spread over 3 age groups, the first group from 1 to 15 years old, the second group from 15 to 50 years old and the third group from over 50 years old **Table 13.**

I.10.7. Statistical processing of data :

Statistical tests were carried out using Microsoft Office Excel 2007 for the analysis of the quantitative data collected through the questionnaire.

CHAPITREIII : RESULTS AND DISCUSSION

Results and discussion:

The present study evaluated 25(OH)D levels in a cohort of patients with T2DM from the wilaya of Saïda. The assessment focused on vitamin D deficiency, on the one hand in the general population and on the other hand in diabetic people of both sexes and different age groups.

1. First evaluation based on questionnaire:

1.1.Vitamin D based on population's daily life :

From a sample questionnaire of a population of 173 person categorized on sex and age; 61women, 54 men between the ages 18 to ≥70 years, and teenage group were 4 all of them boys their age varied between 10 and 18 years.

Table 8: Percentage of men and women participing in the questionnaire.

Gender	Frequency	Perrcentage
Man	74	%43
Women	99	%57
Total	173	%100

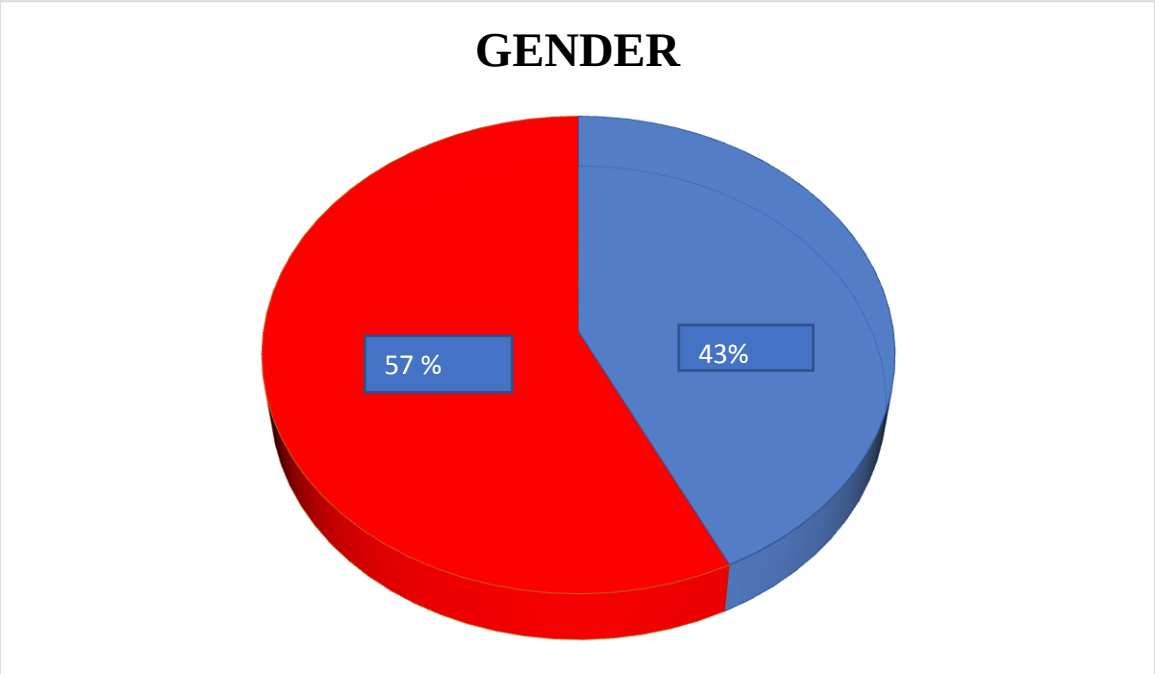

Figure 25 :A proportional circle representing Percentage of men and women participing in the questionnaire.

Table 9: percentage and age of men and women participing in the questionnaire.

Age	Frequency	Percentage
man: 18 -70	71	%41
woman: 18-70	98	%56,6
-18	4	%2,4
Total	173	%100

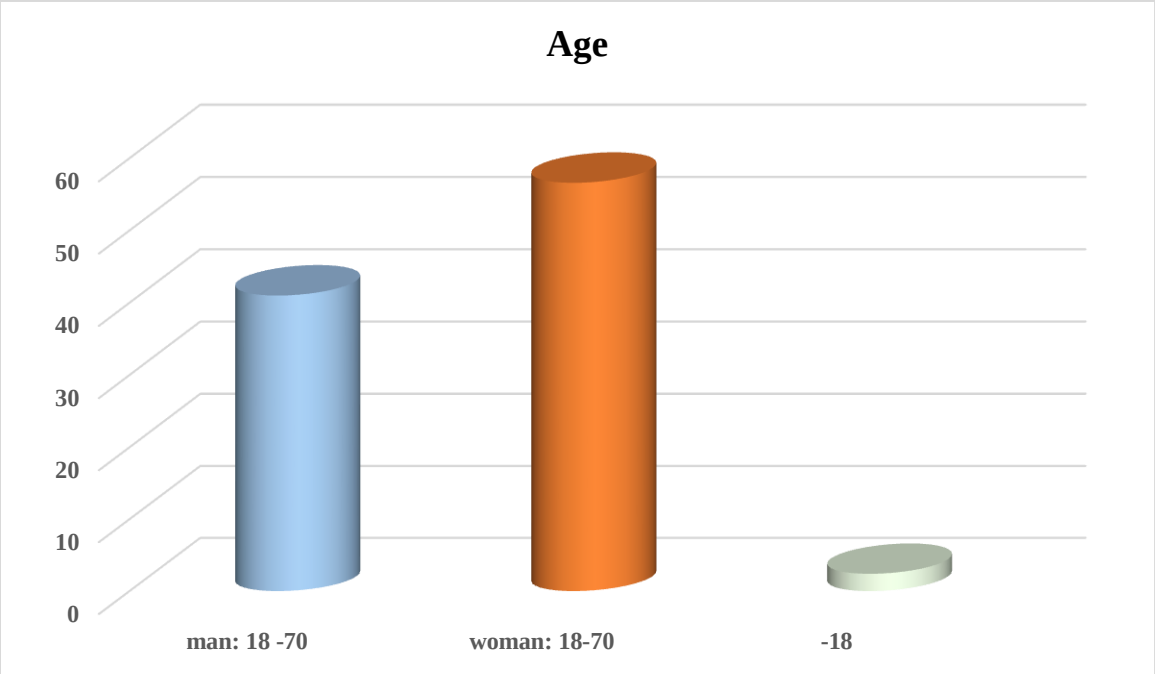

Figure 26 : Bar graf representing age of men and women participing in the questionnaire .

The questions were practically related to their knowledge about vitamin D (source, intake, sun exposure) and the possibility to have any chronic diseases or health problems, consumption, ability to eat well (diverse products from different origins) beside their daily habits and working environment (indoors/outdoors), also the questions were to their knowledge about Magnesium (source , symptoms deficiency), Insulin function and the possibility to have diabetes disease .

Table 10 : Vitamin D sample questionnaire

	Man		**Woman**		**Child**	
	18 - 70years		**18 - 70 years**		**10-18 years**	
	yes/in/house	no/out	yes/in	no/out	yes/in	no/out
Have you ever heard of vitamin D?	85%	15%	83%	17%	90%	10%
Do you work mainly indoors or outdoors?	33%	67%	79%	21%	100%	0%
Do you live in an apartment or a house?	51%	49%	33%	67%	50%	50%

Do you like being in contact with the sun?	57%	43%	72%	28%	25%	75%
Are you afraid of sun exposure	60%	40%	50%	50%	50%	50%
Did you know that sunlight can provide you with vitamin D?	70%	30%	87%	13%	75%	25%
Did you know that darker skin tones are more prone to vitamin D deficiency?	31%	69%	32%	68%	25%	75%
Do you use sunscreen? SPF factor?	5%	95%	63%	37%	25%	75%
What are the sources of vitamin D?	79%	21%	72%	28%	75%	25%
Do you take vitamin D supplements?	35%	65%	55%	45%	25%	75%
Do you consume a lot more products of animal or plant origin?	50%	50%	60%	40%	50%	50%
Do you have the ability to eat well? (Allow red meat, cow's milk, butter for example).	80%	20%	75%	25%	50%	50%
Do you take cow's milk, do you eat butter, meat (approximate quantity)	65%	35%	70%	30%	75%	25%
Have you been diagnosed with Crohn's disease, ulcerative colitis or celiac disease?	41%	59%	32%	68%	0%	100%
Have you suffered from vitamin D deficiency before?	44%	56%	51%	49%	0%	100%
Do you have an idea about the role of vitamin D in the body?	77%	23%	64%	63%	0%	100%
Do you have an idea about the symptoms of vitamin D deficiency in the body?	43%	57%	57%	43%	0%	100%
Do you have an idea about magnesium?	44%	56%	58%	42%	0%	100%
Do you have an idea about food sources rich in magnesium?	43%	57%	47%	53%	0%	100%
Did you know that magnesium deficiency can cause symptoms such as headaches and fatigue?	56%	44%	54%	46%	0%	100%
Do you take nutritional supplements rich in magnesium?	31%	69%	36%	64%	0%	100%

Do you have any idea about insulin?	69%	31%	74%	26%	100%	0%
Do you have an idea about the function of insulin in the body?	81%	19%	56%	44%	100%	0%
Do you suffer from diabetes?	11%	89%	49%	51%	100%	0%
If your answer is yes, has your doctor prescribed vitamin D supplements for you?	11%	89%	20%	80%	100%	0%
Has your doctor prescribed magnesium supplements?	10%	90%	12%	88%	100%	0%
Did he describe both to you?	7%	93%	8%	2%	100%	0%

1.2.Additional evaluation:

questionnair**e** table consists of 27 question that were asked to 173 person from different age groups, sex.

The answers depended on the individual's knowledge, occupation, place of living, their interaction with sun light and protection, dietary and their health state .

The answers varied from a subject to another depends on their life routine, occupation, age and sex.

Both of men (85%) and women(83%) , as well 90% of teenage group were having an idea about vitamin D.

The exposure to sunlight was basically depending on their occupation (in/out doors), activities and also the type of habitat they were living in, that men were most likely to be exposed to sunlight unlike women that were less exposed to it due to their occupation where 67% of men and 21% of women were working outdoors, therefore the majority of women 90% were afraid to be exposed to sunlight

63% of women were using a sunscreen with spf 50, and all of them didn't know that it affects the vitamin D absorption, because the women who didn't know about the fact that sunlight exposure is the main source of vitamin D were frightened to be exposed to sunlight due to preserve their skin from

getting darker (aesthetic prevention). 5% of men were not using a sunscreen with spf 50 because they think it is a beauty product when it is a skin care product .

Population's dietary was balanced between products of animal and vegetal sources with, in the other hand 79% of men and 72% of women had a good knowledge about vitamin D sources (fish,mushrooms,dairy products, egg yolk), and both of them have the ability to eat well (red meat, dairy product, butter) except the the subjects who were diagnosed with diseases (Crohn's disease, ulcerative colitis or celiac disease) 41% of men and 32% of women; these were on a diet to avoid stomach/intestinal inflammation.

Most of men and women have an idea about Magnesium and all of teenage have not idea about Magnesium. But few of them take nutritional supplements rich in magnesium.

Most of men and women have an idea about Insulin and his function and all of teenage have idea about that.

49 % of women and 11% of men suffer from diabetes.

11% of men and 20% women diabetic patients has their doctor prescribed vitamin D supplements for them.

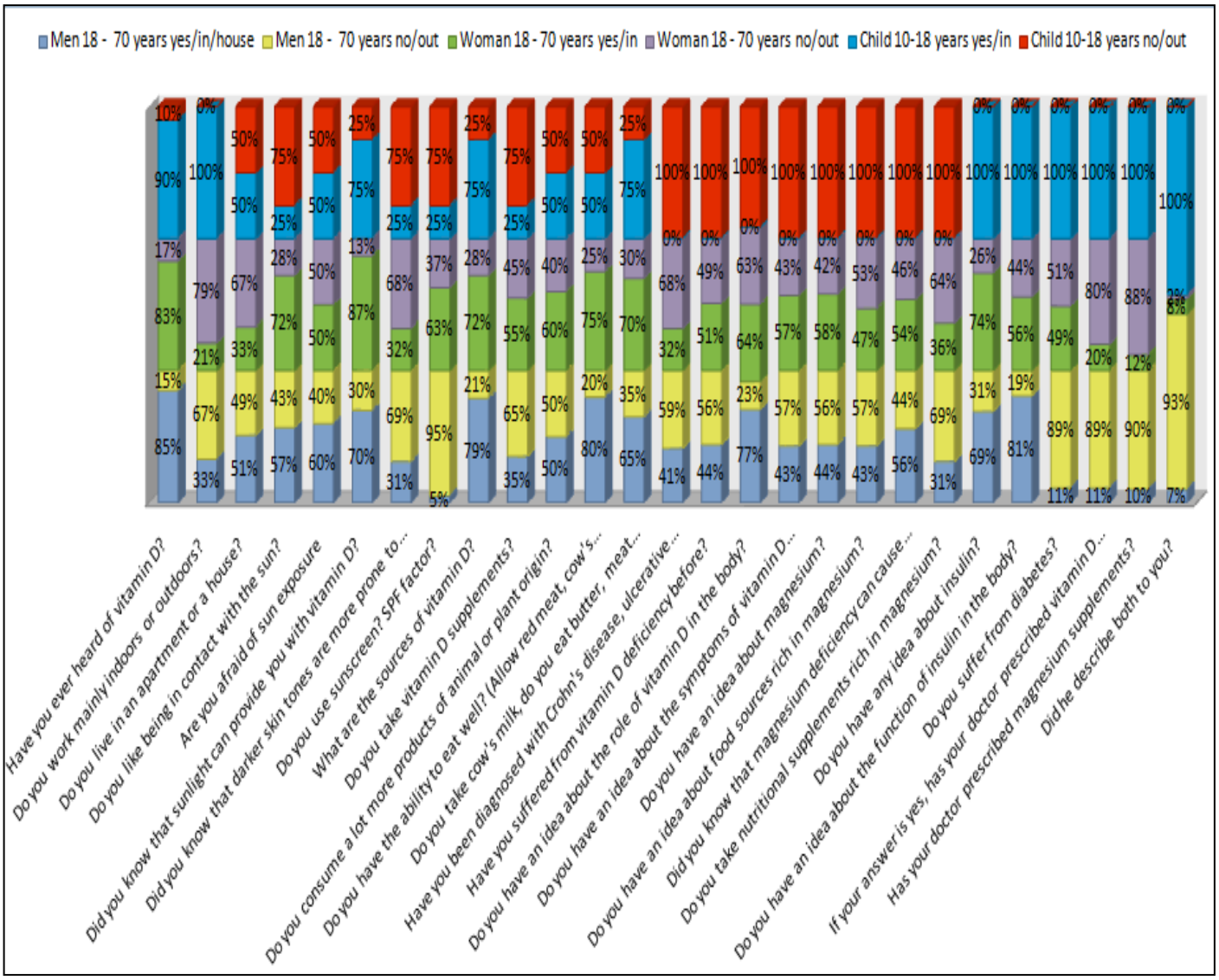

Figure 27: Vitamin D sample questionnaire to person from different age groups, sex.

1.3. Risk factors related to the questionnaire

A large percentage of the population isaware of the existence of vitamin D, but living in apartmentsratherthanhouseswithterraceswhereyou have the choice and surface area to expose yourself to the sunadds to thisfearexposure in the sun and the use of sunscreenrepresents an obstacle to the synthesis of vitamin D.

1. therefore the majority of women 90% were afraid to be exposed to sunlight
2. 40% of women were using a sunscreen with spf 50, and all of them didn't know that it affects the vitamin D absorption, because the

women who didn't know about the fact that sunlight exposure is the main source of vitamin D were frightened to be exposed to sunlight due to preserve their skin from getting darker (aesthetic prevention).

2. Evaluations of vitamin D levels:

2. 1. First evaluation: Vitamin D analysis survey :

Table 11 : Vitamin D level (ng/ml)

Vitamin D level (ng/ml)									
	Man	Woman	1-15 years		15 -50 years		≥50 years		Total
			Man	Woman	Man	Woman	Man	Woman	
Vitamin D <30 ng/mL	79	423	23	38	28	223	28	162	502
Vitamin D 30-50 ng/mL	18	111	4	6	3	56	11	49	129
Vitamin D 51-70 ng/mL	9	20	3	1	5	11	1	8	29
VitaminD 71-100 ng/mL	7	13			1	7	6	6	20
Vitamin D ≥100 ng/mL		5		2		2		1	5
Total	113	572	30	47	37	299	46	226	685

First evaluation: the subject group consists of 685 people whose minimum age was 1 year and maximum age was over 50 years, classified according to gender and age groups.

The general prevalence is 685 people; among them, 572 (84%) are women and 113 (16%) are men.

Group age 1-15 consists 77 people ,contained 61% girl and 39% boys.

Group age 15-50 consists 336 people contained 89% women and 11% men.

Group age +50 consists 272 people contained 83% women and 17% men .

Prevalence of vitamin D deficiency (<30ng/ml) is the highest: 502 person from a sum of 685 person (73%), where 84% of them are women and 16% men.

Prevalence of vitamin D sufficiency (30-50 ng/mL) medium: 129 person from a sum of 685 person(19%), where 86% of them are women and 14% men.

Prevalence of vitamin D adequacy (50-70 ng/mL) medium low: 29 person from a sum of 685 person(4%), where 69% of them are women and only 31% of them are men.

Prevalence of vitamin D therapeutic(≥70 ng/mL) low: 20 persons from a sum of 685 person (3%), where 65% are women and 35% of them are men.

Prevalence of vitamin D level (≥100 ng/mL) lowest : there's only 5

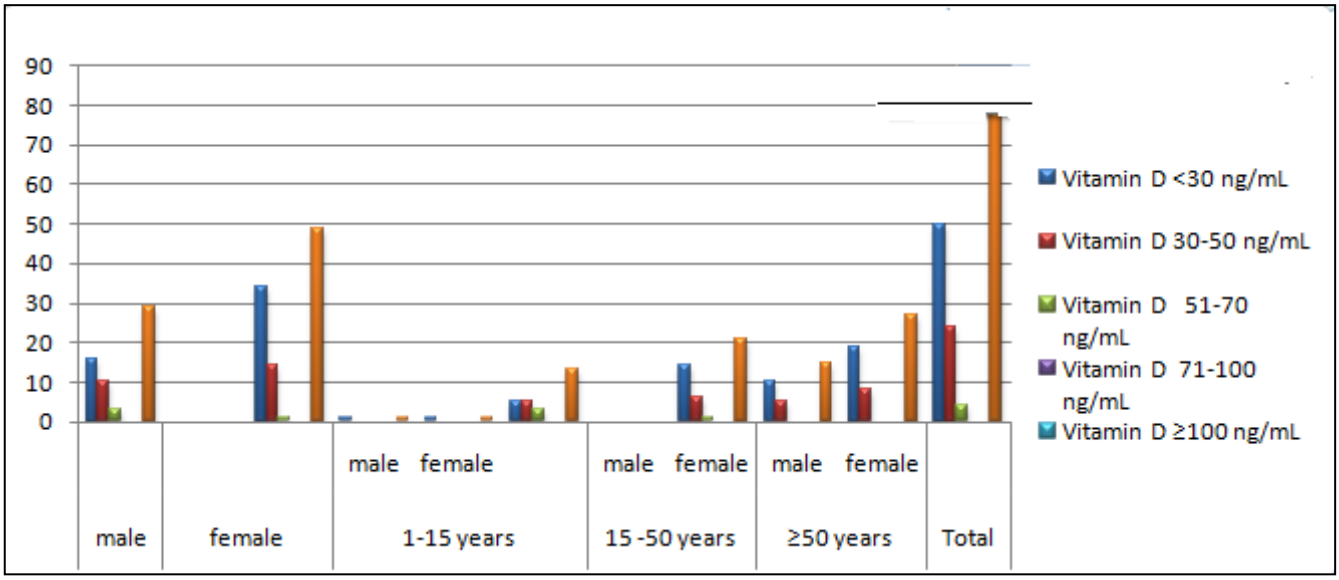

Figure 28: Bar graphs discripe Vitamin D level (ng/ml) person from different age groups, sex.

persons from a sum of 685 person (1%) it includes only women

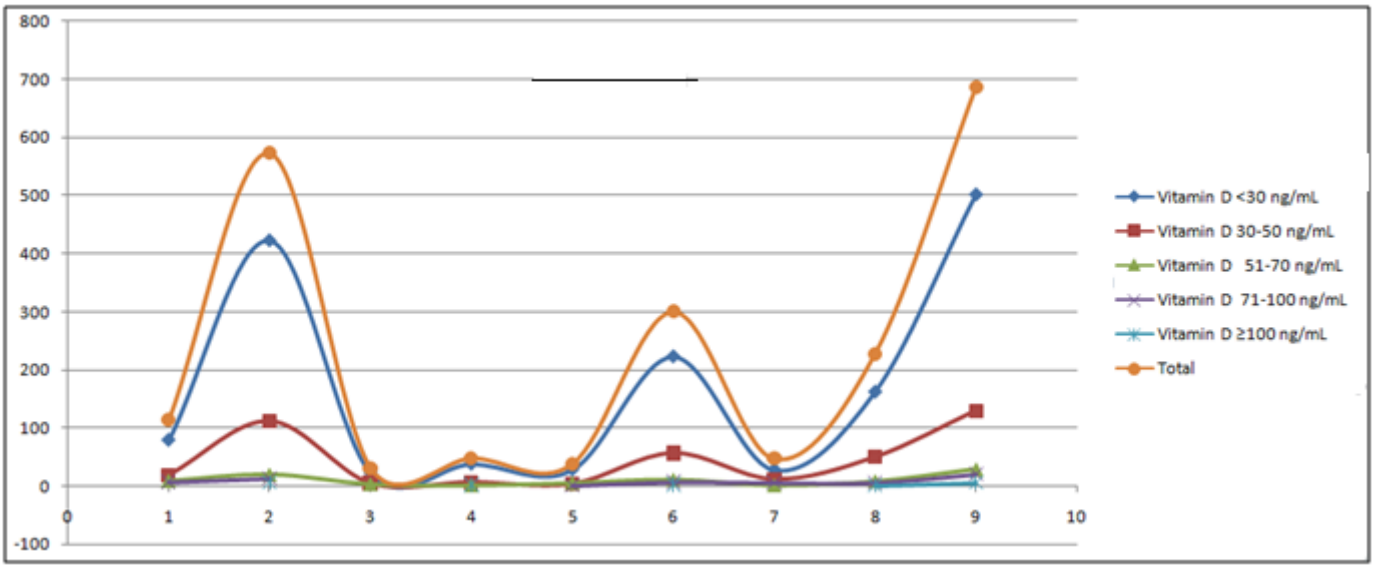

Figure 29: graphs discripe Vitamin D level (ng/ml)to person from different age groups, sex.

The first study found that vitamin D deficiency (<30ng/ml) was highly prevalent, affecting 502 out of 685 individuals (73%), with 84% of them being women and 16% men. In the general population, women are more prone to vitamin D deficiency than men, largely because 90% of women avoid sun exposure and tend to cover up more than men. Women also have higher obesity rates, and 40% use SPF 50 sunscreen, unlike men. Additionally, living in apartments reduces access to sunlight, contributing to vitamin D deficiency.

Table 12 : Vitamin D level(<30ng/mL)

Population	Women	Men	Total
1-15 years	38	23	61
15-50 years	223	28	251
≥50 years	162	28	190
Total	423	79	502

Prevalence of vitamin D deficiency (<30ng/ml) demonstrated that from 502 subject, women in all group ages are more prone to be deficient than men, where :

Group age 1-15 contained 8% girl and 4% boys.

Group age 15-50 contained 44% women and 6% men.

Group age +50 contained 32% women and 6% men.

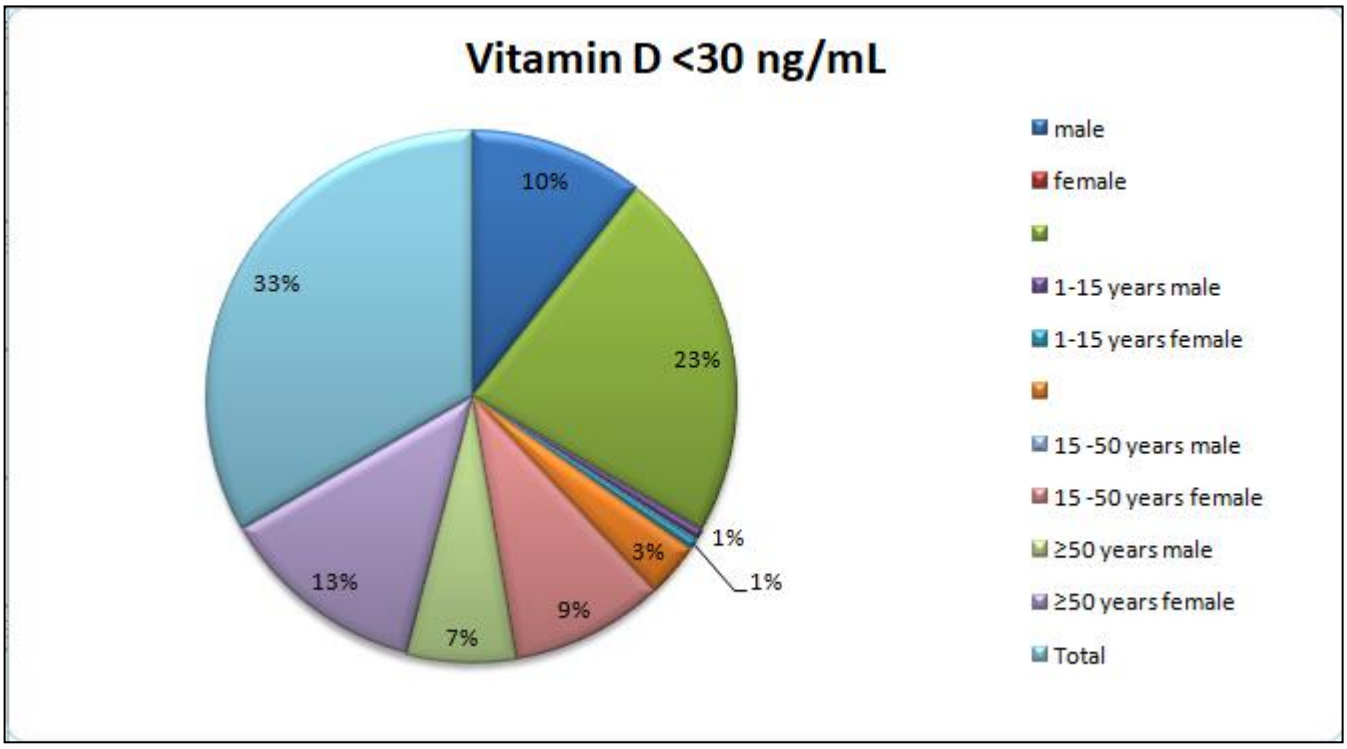

Figure 30: Propotional circle discripe Vitamin D level(<30ng/mL) to person from different age groups, sex.

Second evaluation: Vitamin D deficiency and diabetes.

From a 78 subject that were struggling from it was found that 50 of them were having vitamin D deficiency.

Table 13: Vitamin D level of patients with diabetes disease

Vitamin D level (ng/ml)									
	Man	Woman	1-15 years		15 -50 years		≥50 years		Total
			Man	Woman	Man	Woman	Man	Woman	
Vitamin D <30 ng/mL	16	34	1	1	5	14	10	19	50
Vitamin D 30-50 ng/mL	10	14			5	6	5	8	24
Vitamin D 51-70 ng/mL	3	1			3	1			4
VitaminD 71-100 ng/mL									
Vitamin D ≥100 ng/mL									
Total	29	49	1	1	13	21	15	27	78

Diabetics patients that were having VDD (64%) from 78 subject, where women represented 68% and men 32% varied on age groups that :

Group age 1-15 contained 1% girl and 1% boys .

Group age 15-50 contained 52% women and 29% men.

Group age +50 contained 14% women and 3% men.

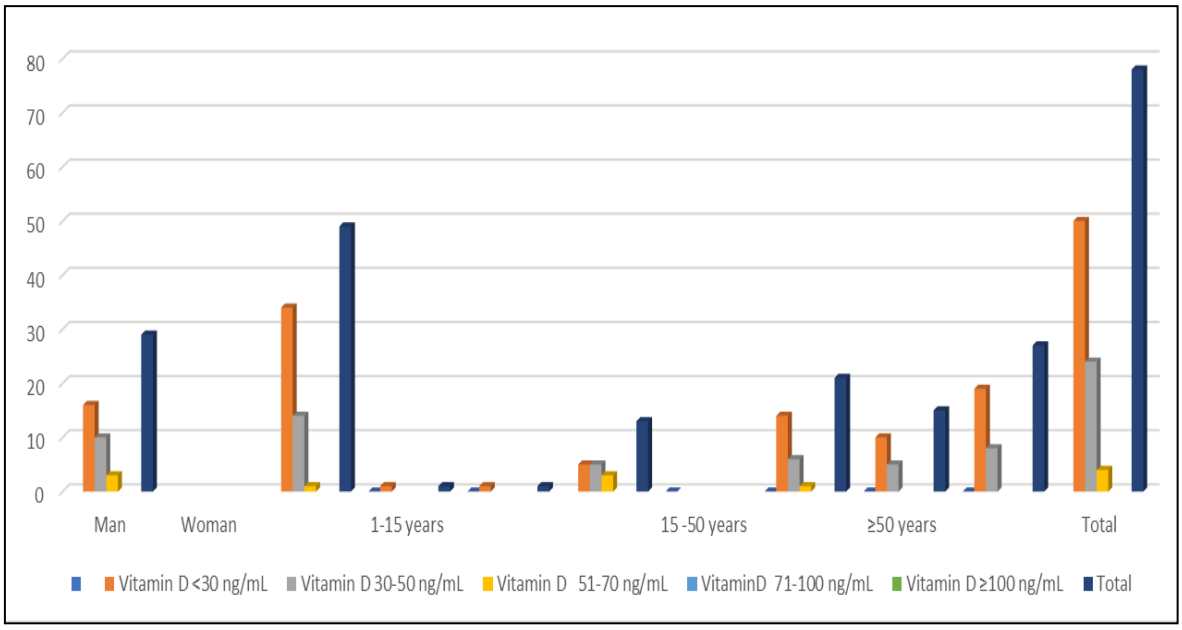

Figure 31: Bar graphs discripe Vitamin D level (ng/ml) in diabetic persons from different age groups, sex.

- The second study showed that 64% of diabetic patients had vitamin D levels below 30 ng/ml, with the deficiency associated with diabetes this agrees with the results of Several cross-sectional and epidemiological studies have indicated that vitamin D deficiency contributes to a higher incidence of diabetes and numerous studies have investigated the connection between vitamin D and diabetes, demonstrating a link between low vitamin D levels and a higher risk of developing type 2 diabetes and its associated complications(**Md Isa, Z and al., 2023 , Talaei, A and al., 2013 , Wang, F and al., 2022**) , and the results of **Salhi, H., & El Ouahabi, H. 2021)** which showed that the relationship between vitamin D insufficiency or deficiency in individuals with T2DM and an increased risk of insulin resistance can be explained by the reduced anti-inflammatory action of vitamin D. This reduction leads to increased secretion of pro-inflammatory cytokines, which subsequently decreases the sensitivity of insulin receptors to the hormone.
- Among these participants, 68% were women and 32% were men,this agrees with the results of Lee, J and al in 2021 also with the results of Taderegew, M. M and al., 2023 Which showed that Vitamin D deficiency is more closely associated with a high prevalence of prediabetes and diabetes in women than in men. And it matches also wthresultes of Vranić, L and al in 2019 which showed that the high prevalence of vitamin D deficiency among obese individuals, which is more pronounced in women than men, is well-documented. This is likely due to volumetric dilution in the larger volumes of fat, serum, liver, and muscles found in obese people.

Ather studies showed the relationship between Magnesium and T2D are following**:**

- Barbagallo, M and Dominguez, L. J in 2015**:**Magnesium (Mg) is a vital mineral essential for various physiological activities in the body. Low magnesium intake and serum magnesium concentrations are

associated with metabolic syndrome, insulin resistance, and Type 2 diabetes. An increased prevalence of magnesium deficits has been identified in T2DM patients, especially those with poorly controlled blood sugar levels, longer disease duration, and chronic microvascular and macrovascular complications.

- Dasgupta A and al in 2012 **:**Among the endocrine and metabolic disorders linked to magnesium deficiency, diabetes mellitus is the most prevalent. Numerous studies have demonstrated that average plasma magnesium levels are lower in patients with both type 1 and type 2 diabetes compared to nondiabetic control subjects. And Studies have reported incidence rates of hypomagnesemia between 13.5 and 47.7% in diabetic subjects.
- Lynette J Oost and al in 2023 **:**inypomagnesemia is reported approximately 10-fold more than in the general population and is associated with insulin resistance, hyperglycemia, and rapid diabetes disease progression.
- Ramara K and al in 2023 and Fiorentini D in 2021 **:**Magnesium deficiency has been correlated to type 2 diabetes mellitus, metabolic syndrome, and insulin resistance. As well as all other protein kinases, the tyrosine kinase activity of the β-subunit of the insulin receptor is dependent on magnesium concentration, therefore a magnesium deficiency may result in an impaired insulin signal.
- Magnesium is an essential cofactor for vitamin D synthesis and activation, and in turn, vitamin D can increase the intestinal absorption of magnesium, establishing a feed-forward loop to maintain homeostasis. Magnesium is crucial for the activity of hepatic 25-hydroxylase and renal 1α-hydroxylase, which convert 25(OH)D into its biologically active form, 1,25(OH)2D3. Additionally, magnesium facilitates the transfer of vitamin D to target tissues via the vitamin D binding protein. Conversely, magnesium is involved in the inactivation

of vitamin D by being required for the activity of renal 24-hydroxylase, which forms 24,25(OH)2D. Dysregulation in either of these nutrients can be associated with various disorders, including skeletal deformities, cardiovascular disorders, and metabolic syndrome.

The difficulties face in the study :

One of the difficulties we faced in the study was the refusal of some analysis laboratoiries to provide results of Vitamin D level , especially the results of Vitamin D level of patients with diabetes disease only laboratory that helped us.

CONCLUSION

Conclusion

This comprehensive analysis highlights the importance of vitamin D and magnesium in maintaining good health, particularly for individuals with diabetes. It underscores the crucial roles these elements play at the cellular level, emphasizing the need for strategies that promote behaviors and diets that meet recommended daily values. Additionally, it is essential to develop reliable and minimally invasive methods for quickly identifying deficiencies in vitamin D and magnesium across various body areas and accurately monitoring the effectiveness of supplements to prevent and combat related diseases. Magnesium should be regarded as a vital metabolite, not merely an electrolyte, given its significant impact on various physiological functions, particularly its role in activating vitamin D to enhance its biological effects.

Globally, studies indicate that approximately one billion people are vitamin D deficient. Despite magnesium's critical role in human health, 60% of people do not meet the recommended daily intake (RDI) of 320 mg/day for women and 420 mg/day for men, with 19% not even reaching half of the recommended amount. This deficiency can be attributed to common dietary practices, medications, and farming techniques, as well as a significant decline in the mineral content of vegetables by 80–90% over the last 100 years.

Data from numerous studies show that around 60% of adults have insufficient magnesium intake from their diets, and subclinical magnesium deficiency is widespread in Western populations. Therefore, greater attention should be given to the preventive role of magnesium in addressing social health issues, encouraging adequate dietary intake and supplementation. As previously described, magnesium is abundant in a variety of unrefined foods and is one of the more affordable supplements available. Moreover, clinical trials have demonstrated that magnesium supplements are well tolerated and generally improve multiple markers of disease status.

A high prevalence of vitamin D deficiency would increase the global burden of diseases associated with its deficiency. Therefore, adequate magnesium supplementation should be considered a crucial aspect of correcting the in vivo synthesis of vitamin D. Governments, policymakers, health workers, and individuals should recognize the significance of the widespread deficiencies in both vitamin D and magnesium and prioritize their prevention as a public health issue.

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