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## DEPARTMENT OF BIOLOGY

## GRADUATION DISSERTATION

Presented by

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A Dissertation in The Field of Biological Sciences

for a Master Degree in Applied Biochemistry

**Speciality**: Applied Biochemistry

THEME

# Association Between 25(OH)Vitamin D and HbA1c Control in Type 2 Diabetes Mellitus in Mostaganem City, Algeria

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# DEDICATION

I would like to express the deepest appreciation to my family, particularly my father, Saleh Moqbel, and mother, Aysha Mohsen, who constantly made significant sacrifices, supported, fought relentlessly, often against apparently insurmountable hurdles, to guarantee that I could receive an education that would allow me to pursue professional and academic prospects. I dedicate my work to Um Rakan Al-majeed, for her encouragement, support, and patience over the previous few years as I sought the Master of Applied Biochemistry. Let the completion of this work inspire and serve as an example to my hero, Rakan Almajeed, of what he, too, may achieve through persistence and hard efforts.

This is also for my colleagues in the Applied Biochemistry. We study for various reasons, but for this, the main reason was for raising awareness of the importance of vit D to T2D patients in particular and society as a whole.

✍ Abdulrahman Saleh Moqbel Hagar

03-07-2022

# ACKNOWLEDGEMENTS

I offer all praise and honor to the Allah Almighty for providing me with the strength and wisdom I needed to accomplish this masters degree. I would like to send my great gratitude and thanks to my supervisors Dr. Said Bahmouni and Dr. Bengharbi Zineb for their patience, excellent academic supervision, support, enthusiasm and dedication inspired me to achieve the level of work I desired. Given the difficult task ahead of me, they kept me on my toes at all times. I'd like to extend my deepest gratitude and thanks to my other amazing prof. Dr. Chibani A (President) and Dr. Rebiche S (Examiners) for their guidance, support, and words of encouragement. Thank you from the bottom of my heart.

I am thankful to Dr. Ettalhi laboratory for supporting my study for two months. I am really grateful to Dr. Ettalhi Mehdi as well as all labs workers Soumia, Belgasm, Amina, Yasmina, Mohamed and Hamza). They put in a lot of time and effort to help me get the information I needed to answer my research inquiries. Also, my sincere love and appreciation go to my brothers (Ramzy, Abdelaziz) and sisters, as well as numerous other people who contributed to the successful completion of this research in various ways. Finally, I'd like to thank my immediate family, my queen, and hero Rakan Alsmajeeh, for their love, patience, and support. They accepted my frequent absences and travel to Algeria, to attend classes and do research without hesitation, despite the fact that it often interrupted their own life.

Needless to say, any failings of this work are entirely my responsibility.

Abdulrahman Saleh Moqbel Hagar

03-07-2022

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ADA** | American Diabetes Association |
| **AGE** | Advanced Glycation End products |
| **ATP** | Adenosine Triphosphate |
| **BFR** | Bone Formation Rate |
| **[Ca]i** | Intracellular Calcium |
| **Calbindin** | Calcium-Binding Protein |
| **COVID-19** | Coronavirus Disease-2019 |
| **CVD** | Cardiovascular diseases |
| **CYP27A1** | Cytochrome P450 Family 27 Subfamily A polypeptide 1 |
| **CYP24A1** | Cytochrome P450 Family 24 Subfamily A polypeptide 1 |
| **CYP27B1** | Cytochrome P450 Family 27 Subfamily B polypeptide 1 |
| **CYP2R1** | Cytochrome P450 Family 2 Subfamily R Member 1 |
| **DBP** | Vitamin D-binding protein |
| **DG** | Diacyl glycerol |
| **DM** | Diabetes Mellitus |
| **FGF23** | Fibroblast growth factor |
| **FFA** | Free Fatty Acid |
| **FPG** | Fasting Plasma Glucose |
| **GADA** | Glutamic Acid Decarboxylase Antibody |
| **GDM** | Gestational Diabetes Mellitus |
| **GDP** | Guanosine Diphosphate |
| **GTP** | Guanosine triphosphate |
| **GLUT-4** | Glucose Transporter-4 |
| **HbA1c** | Glycated hemoglobin |
| **HIP** | Hyperglycaemia in Pregnancy |
| **HLA** | Human Leucocyte Antibodies |
| **IA** | Insulin autoantibodies |
| **IAA** | Insulin Auto-Antibody |
| **IA-2A** | Insulinoma-Associated Antibody 2 |
| **ICA** | Islet cell autoantibodies |
| **IDF** | International Diabetes Federation |
| **IFG** | Impaired fasting glucose |
| **INS-R.** | Insulin Receptor |
| **IGT** | Impaired glucose tolerance |
| **PIP3** | phosphatidyl inositol tris phosphate |
| **IR** | Insulin resistance |
| **LADA** | Latent Auto-Immune Diabetes in Adults |
| **LDL** | Low-density lipoprotein |
| **MED** | Minimum Erythematic Dose |
| **MODY** | Maturity-Onset Diabetes of Youth |
| **NCX1** | Sodium/calcium exchanger 1 |

| | |
|---|---|
| **NF-KB** | Nuclear Factor-Kappa B |
| **OGTT** | Oral Glucose Tolerance Test |
| **OPG** | Osteoprotegerin |
| **PPG** | Post-prandial Glucose |
| **PTH** | parathyroid hormone |
| **PKCA** | Protein kinase C activation |
| **PKC** | protein kinase C |
| **PIP2** | Phosphatidyl Inositol Bisphosphate |
| **PLC** | Phospholipase C |
| **RXR** | Retinoid X Receptor. |
| **RPG** | Random Plasma Glucose |
| **RANKL** | Receptor Activator of Nucleus Factor-kapa B Ligand |
| **PPAR-$\delta$** | Peroxisome Proliferator-Activated Delta Receptor |
| **PMCA1b** | Plasma Membrane Calcium Pump1b |
| **SARS-COV-2** | Severe Acute Respiratory Syndrome Coronavirus-2 |
| **T1DM** | Type 1 Diabetes Mellitus |
| **T2DM** | Type 2 Diabetes Mellitus |
| **TSH** | Thyroid Stimulating Hormone |
| **TRH** | Thyrotropin-Releasing Hormone |
| **TRPV6** | Transient Receptor Potential Vanilloid 6 |
| **TRPV5** | Transient Receptor Potential Vanilloid 5 |
| **UI** | Unite International |
| **UV** | Ultraviolet Rays |
| **VSMC** | Vascular Smooth Muscle Cells |
| **VDR** | Vitamin D Receptor |
| **VDRE** | Vitamin D Response Elements. |
| **VLDL** | Very Low-Density Lipoprotein |
| **WHO** | World Health Organization |
| **ZnT8A** | Zinc Transporter 8 Antibody |
| **1,25(OH)2D$_3$** | 1,25-Dehydrox-Vitamin D$_3$ |
| **25(OH)D** | 25-hydroxy-Vitamin D |
| **7-DHC** | 7-dehydro-cholesterol |
| **7-DHCR** | 7-dehyro-cholesterol reductase |

# ABSTRACT

Type 2 diabetes (T2D) is amongst the most highly prevalent diseases in the world, and it is caused mostly by a combination of two fundamental factors: Insulin insufficiency is caused by a lack of pancreatic β-cell secretion and insulin-responsive tissue sensitivity. Vitamin D is a prehormone that is mostly produced at the epidermal level by ultraviolet B radiation action from its precursor, 7-dehydrocholestérol. It has lately attracted extensive attention in diabetes causation and prevention. Through its active form, calcitriol, vitamin D may play an important role in maintaining pancreatic β-cell function. There may be different explanations for this effect. It can be induced by activating VDR located on the phospholipid layer of the plasma membrane of pancreatic β-cells, muscle cell and adipocyte. Significant evidence exists to suggest the relationship between vitamin D insufficiency and T2D. This relationship is mediated by vitamin D's direct and indirect effects on insulin production, insulin sensitivity, and systemic inflammation.

The goal of this study is to determine if there is a link between vitamin D and T2D. A total of 55 people with T2D, aged 40 to 84 years, who attended analysis medical laboratory of Dr. Ettalhi for a diabetic examination between March and June 2022. We primarily attempted to link vitamin D levels with glycated hemoglobin (HbA1c) levels. The results showed that 72.7% of the 55 patients had a deficient vitamin D level and 27.3% had a vitamin D level in the insufficiency range, while HbA1C levels were found to be greater than normal in all T2D patients. We observed an inverse relationship between HbA1c and vitamin D. whereas, we concluded that Vitamin D was found to be strongly associated to glycemic control. Thus, when deficient or insufficient levels of vitamin D are identified in T2D patients, supplementation should be investigated since it may assist to improve glycemic control.

**Keywords**: 25-Hydroxyvitamin D; Type 2 Diabetes; Glycated Hemoglobin.

# RÉSUMÉ

Le diabète de type 2 (DT2) est l'une des maladies les plus répandues dans le monde, et il est causé principalement par une combinaison de deux facteurs fondamentaux : L'insuffisance d'insuline est causée par un manque de sécrétion pancréatique des cellules β et une sensibilité des tissus sensibles à l'insuline. La vitamine D est une préhormone majoritairement produite au niveau épidermique par l'action des rayonnements ultraviolets B à partir de son précurseur, le 7-déhydrocholestérol. Il a récemment attiré une grande attention dans la cause et la prévention du diabète. Par sa forme active, le calcitriol, la vitamine D pourrait jouer un rôle important dans le maintien de la fonction des cellules β pancréatiques. Il peut y avoir différentes explications à cet effet. Il peut être induit en activant le VDR situé sur la couche phospholipidique de la membrane plasmique des cellules β pancréatiques, des cellules musculaires et des adipocytes. Des preuves significatives existent pour suggérer la relation entre l'insuffisance en vitamine D et le DT2. Cette relation est médiée par les effets directs et indirects de la vitamine D sur la production d'insuline, la sensibilité à l'insuline et l'inflammation systémique.

Le but de cette étude est de déterminer s'il existe un lien entre la vitamine D et le DT2. Un total de 55 personnes atteintes de DT2, âgées de 40 à 84 ans, qui se sont présentées au laboratoire médical d'analyse du Dr. Ettalhi pour un examen diabétique entre mars et juin 2022. Nous avons principalement tenté de relier les niveaux de vitamine D aux niveaux d'hémoglobine glyquée (HbA1c). Les résultats ont montré que 72,7 % des 55 patients avaient un taux de vitamine D déficient et 27,3 % avaient un taux de vitamine D dans la plage d'insuffisance, tandis que les taux d'HbA1C étaient supérieurs à la normale chez tous les patients DT2. Nous avons observé une relation inverse entre l'HbA1c et la vitamine D. alors que nous avons conclu que la vitamine D était fortement associée au contrôle glycémique. Ainsi, lorsque des niveaux déficients ou insuffisants de vitamine D sont identifiés chez les patients atteints de DT2, une supplémentation doit être étudiée car elle peut aider à améliorer le contrôle glycémique.

**Mots clés** : 25-Hydroxyvitamine D ; Diabète de Type 2 ; Hémoglobine Glyquée.

# نـــبذة مخـــتصرة

يـــعد مـرض السكر نـوع 2 من بين أكـثر الأمـراض انتشـاراً في العـالم، ويكون في الغـالب ناتج عن مزيج من عـاملين أساسيين: العامـل الأول حدوث قصور في الأنسولين بسبب نقص إفرازه عن طريق خلايا بيتا البنكرياسية والعـامل الثاني حدوث خلل في حساسية الأنسجة (العضلية، الشحمية والكبدية) المستجيبة للأنسولين.

فيتامـــين دال هـو هـرمون يتم إنتاجه في الغـالب على مستوى البشرة (تحت الجلد) عن طريق أشعة الشمس فوق البنفسجية التي تعمل على تحول 7- ديهيدروكوليسترول الى كوليكالسيفيرول. في الاواني الاخيرة وجد فيتامين دال اهتماماً واسعاً في أسباب مرض السكري والوقاية منه. فمن خلال شكله النشط، كالسيتريول، يلعب دوراً مهماً في الحفاظ على وظيفة خلايا البنكرياس المسؤولة على تصنيع الأنسولين وخلايا الانسجة المستجيبة للأنسولين. توجد أدلة مهمة تشير إلى العلاقة بين نقص فيتامـــين دال ومرض السكر نوع 2، فهناك تفسيرات مختلفة لهذا التأثير. فيمكن أن يحدث عن طريق تنشيط مستقبلات فيتامين دال الموجودة على طبقة الفسفوليبيد في غشاء البلازما لخلايا بيتا البنكرياسية لتخليق الأنسولين وإفرازه الى الدم وقمع السيتوكينات التي تعمل ع تحفيز موت خلايا بيتا عن طريق الجهاز المناعي الذي تسرع بالانتقال الى مرض السكر نوع 1، كذلك في الخلايا العضلية والخلايا الشحمية والكبدية فيتامين دال يحفز على تخليق مستقبلات الأنسولين ومن ثم زيادة حساسية استجابة هذه الانسجة للأنسولين. وتتوسط هذه العلاقة التأثيرات المباشرة وغير المباشرة لفيتامين دال على إنتاج الأنسولين واستجابة الانسجة للأنسولين وقمع الالتهاب الناتجة عن مقاومة الأنسولين.

الهدف من هذه الدراسة هو تحديد إذا كان هناك ارتباط بين فيتامين دال ومرض السكر نوع 2. إجمـــالي 55 شخصاً يعانون من مرض السكر نوع 2، تتراوح أعمارهم بين 40 و84 عاماً، حضروا مختبر التحاليل الطبية للدكتور إطلحي مهدي لفحص السكر بين مارس ويونيو 2022. لقد حاولنا في المقام الأول ربط مستويات دال بمستويات الهيموجلوبين السكري (السكر التراكمي). وأظهرت النتائج أن 72.7٪ من 55 مريضاً يعانون من نقص فيتامـــين دال و27.3٪ لديهم مستوى فيتامـــين دال في نطاق القصور، بينما وجد أن مستويات السكر التراكمي أعلى من الطبيعي في جميع مرضى السكر نوع 2. ولاحظنا وجود علاقة عكسية بين السكر التراكمي وفيتامين دال. حيث خلصنا إلى أن فيتامين دال يرتبط ارتباطًا وثيقًا بالتحكم في نسبة السكر في الدم. وبالتالي، عندما يتم تحديد مستويات ناقصة أو غير كافية من فيتامين دال في مرضى السكر نوع 2، ينبغي وصف مكملات فيتامين دال لمرضى السكري نوع 2 لأنها قد تساعد في تحسين السيطرة على نسبة السكر في الدم.

**الكلمات المفتاحـــيه:** 25-هيدروكسي فيتامين دال؛ داء السكري من النوع 2؛ الهيموجلوبين السكري.

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# GENERAL INTRODUCTION

Type 2 diabetes (T2D) is the most common form of diabetes, contributing for more than 90% of all diabetes globally. The International Diabetes Federation (IDF) predicts that 537 million people will have diabetes by 2021, increasing to 643 million by 2030 and 783 million by 2045. Changes in lifestyle, less physical activity, and high calorie snacks have increased the incidence of obesity, which is linked to T2D.

Vitamin D deficiency has been linked to T2D and metabolic syndrome in studies. It has led to the idea that vitamin D insufficiency is linked to insulin resistance and that 25-(OH) D supplementation lowers insulin resistance. Vitamin D deficiency was thought to be rare in areas of the world that received sufficient of sunlight all year. However, according to the WHO vitamin D deficiency currently affects more than half of the population of all ages. thus, there is evidence that vitamin D supplementation is beneficial improves insulin resistance and related parameters, many studies have implicated vitamin D in cardiovascular disease (CVD) prevention, cancer prevention, inhibiting parathyroid hormone secretion, promoting insulin secretion, inhibiting adaptive immunity while promoting innate immunity, and inhibiting proliferation and inducing cell differentiation.

The study's goal is to evaluate the degree of vitamin D deficiency in T2D patients and observe if there is a link between vitamin D and glycemic control. This research, named " the association between vitamin D and glycated hemoglobin (HbA1C) control in T2D" was divided into six chapters. The first chapter explains T2D (background, classifications, risk factors, diagnosis and complications); the second chapter discusses vitamin D (its structural formula, consu-mables, metabolic activity, biological roles, regulation, and so on); the third chapter talks about the relationship between vitamin D and T2D; and the fourth chapter focuses on the methods and materials used to perform various dosages (glycemia, HbA1c levels, and vitamin D levels), as well as the fifth chapter explains the results and discussions that lead to a general conclusion.

## 1.1 Foreword

T2D is one of the utmost prevalent metabolic diseases in the world, and the development of which is mainly caused by a collection of two major factors: pancreatic β-cell deficiency in insulin secretion and insulin insufficiency insulin-responsive tissue responsiveness. Insulin secretion and action must be in full compliance with metabolic demands; therefore, the molecular mechanisms participatory in insulin synthesis and release and insulin response in tissues must be tightly regulated. Thus, deficiencies in any of the mechanisms involved may lead to metabolic imbalances that contribute to the pathogenesis of T2D (Unai *et al.,* 2020).

## 1.2 Background

According to (IDF) diabetes mellitus (DM), more simply called diabetes, it is a chronic, long-term, or "severe" disease that occurs when blood sugar levels rise due to the body's inability to produce or adequately produce the hormone insulin, or to effectively use the insulin produced. Insulin is the pancreatic production of essential hormones. It allows glucose in the blood to enter body cells, where it is converted or stored as energy. Insulin is also essential for the metabolism of protein and fat. A lack of insulin or the inability of cells to respond to it can lead to high blood sugar levels (hyperg-lycemia), a clinical indicator of diabetes. threshold at (**Fig. 3).** Chronic uncontrolled insulin deficiency can damage many organs in the body, which can lead to disabling and life-threatening health complications such as CVD, nerve damage (neuropathy), kidney damage (nephropathy) amputations and eye disease (mainly affects the retina), resulting in decreased vision and even blindness. However, if diabetes is adequately managed, these serious complications can be delayed or completely prevented (IDF, 2021).

## 1.3. Classification of Diabetes Mellitus

According to American Diabetes Association (ADA) in the document of the committee report on diabetes mellitus diagnosis and classification in the US, diabetes can be divided into four major types **(Fig. 1)**: type 1 diabetes, type 2 diabetes, gestational diabetes, and secondary or other particular types of diabetes **(**Lee *et al.,* 2021).

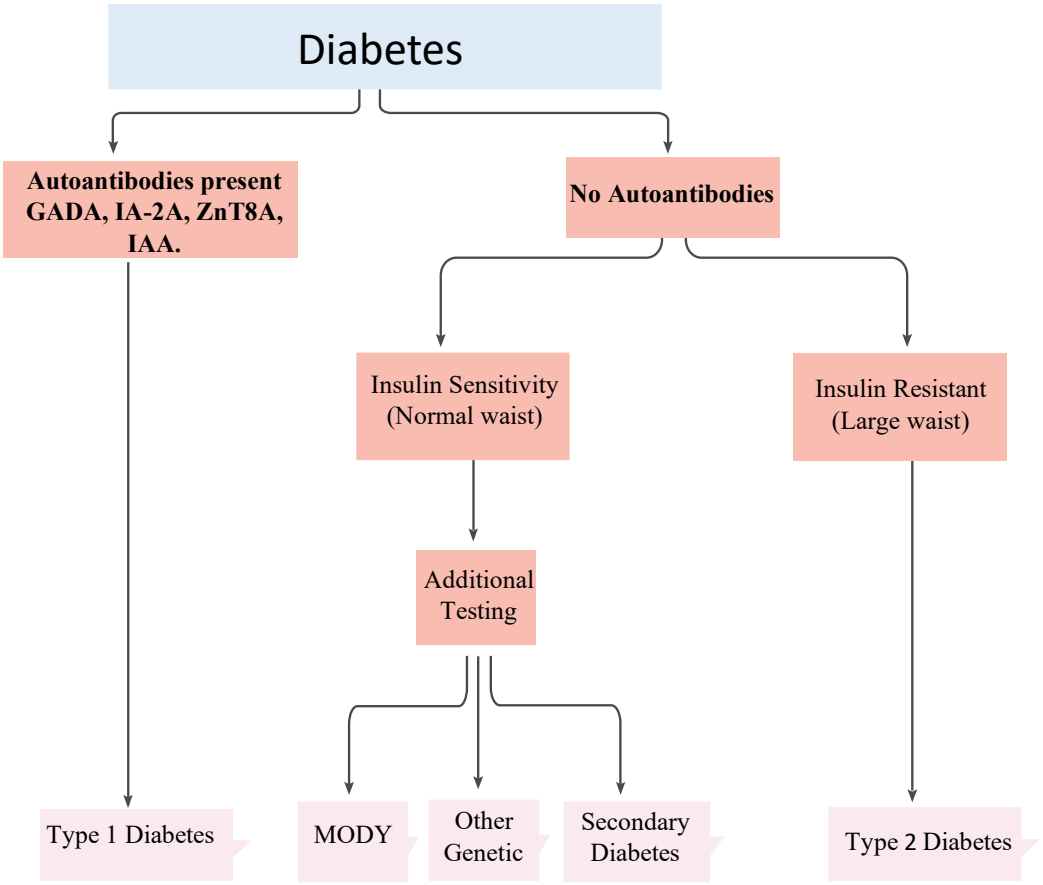



**Figure 1 | Algorithm for classification of diabetes.** *(GADA) Glutamic Acid Decarboxylase Antibody; (IA-2A) Insulinoma-Associated Antibody 2; (IAA) Insulin Autoantibody; MODY; (ZnT8A) Zinc Transporter 8 Antibody (Zheng et al., 2018).*

### 1.3.1. Type 1 Diabetes

β-cell destruction that leads to an absolute insulin, deficiency mostly transmitted immunologically, LADA (latent autoimmune diabetes in adults): classified as T1D **(Tab. 1).**

### 1.3.2 Type 2 Diabetes

Can range from primary insulin resistance with accompanying insulin insufficiency to a mostly secretory dysfunction with insulin resistance **(Tab. 1).**

### 1.3.3 Gestational Diabetes

Glucose tolerance disease that manifests itself or is diagnosed for the first-time during pregnancy (Petersmann *et al.,* 2019).

### 1.3.4 Secondary Specific Types of Diabetes

Secondary diabetes is a diabetic condition that arises as a result of the death of beta cells in the pancreatic islets and/or the development of insulin resistance by an acquired illness (e.g., endocrinopathies, cystic fibrosis) or others (Petersmann *et al.,* 2019).

**Table 1** | Differential diagnostic criteria for common DM (Petersmann *et al.,* 2019**)**.

| | **Type 1 diabetes** | **Type 2 Diabetes** | **MODYs** |
|---|---|---|---|
| **Aetiology** | Autoimmune disease, genetic predisposition | Genetic predisposition Multi-factorial | Monogenic |
| **Heredity** | Variable | Variable | Autosomal dominant; diabetes in ≥ 3 generations |
| **Frequency among all diabetes types** | 5–10 % | 90–95 % | Approx. 2 % |
| **Pathogenesis** | Autoantibodies, absolute insulin deficiency | Insulin resistance and secretion disorder up to insulin deficiency | mutation of genes of transcription factors or glucokinase of β-cells |
| **Typical age of manifestation** | Childhood to adulthood | Adulthood | Youth to early adulthood |
| **Clinical manifestation** | Acute polyuria, polydipsia, severe hyperglycaemia, ketoacidosis | slow onset, often secondary diseases, moderate hyperglycaemia | Slow onset, variable hyperglycaemia |
| **Comorbidities** | Autoimmune thyroiditis, celiac disease | Visceral obesity, hypertension, Diabetes (also called Metabolic Syndrome) | Renal cysts depending on MODY type |
| **Tendency to ketosis** | Yes | No | No |
| **Weight** | Normal weight | Overweight | Normal weight |
| **Plasma insulin/ C-peptide HOMA-B2** | Reduced to lacking | Often high at beginning, then reduced | mostly diminished |
| **autoantibodies** | Yes | No | No |
| **Insulin resistance HOMA-R3** | No | Yes | No |
| **Therapy** | Insulin | Lifestyle modification measures, oral antidiabetics, insulin | possibly none, OADs, insulin (Depending on MODY type) |
| *LADA (latent insulin-dependent diabetes in adulthood) is associated with a slow loss of beta cell function. The LADA has a rapid failure of oral antidiabetics. In case of suspicion of LADA: recommend analysis of GAD antibodies.* | | | |

## 1.4 Epidemiology

Diabetes mellitus is a serious health problem that has reached alarming proportions. Today, more than 500 million people worldwide have diabetes. Diabetes is the 9th leading cause of death. About 1 in 10 adults worldwide now has diabetes. An estimated 537 million people will have diabetes in 2021, and this number is expected to increase to 643 million by 2030 and 783 million by 2045 **(Fig. 2)**. Additionally, an estimated 541 million people will have impaired glucose tolerance by 2021. It is also estimated that over 6.7 million people aged 20–79 will die from diabetes-related causes in 2021. The number of children and adolescents with diabetes (i.e., under the age of 19) is increasing every year. By 2021, more than 1.2 million children and adolescents will have type 1 diabetes. Hyperglycemia in pregnancy (HIP) is also estimated to affect approximately one in six pregnancies. Another problem is the consistently high proportion of people with undiagnosed diabetes (45%), most of them T2D. This highlights the urgent need to improve the ability to diagnose people with diabetes, many of whom do not know they have it, and to provide appropriate and timely care to all people with diabetes as early as possible (IDF, 2021).

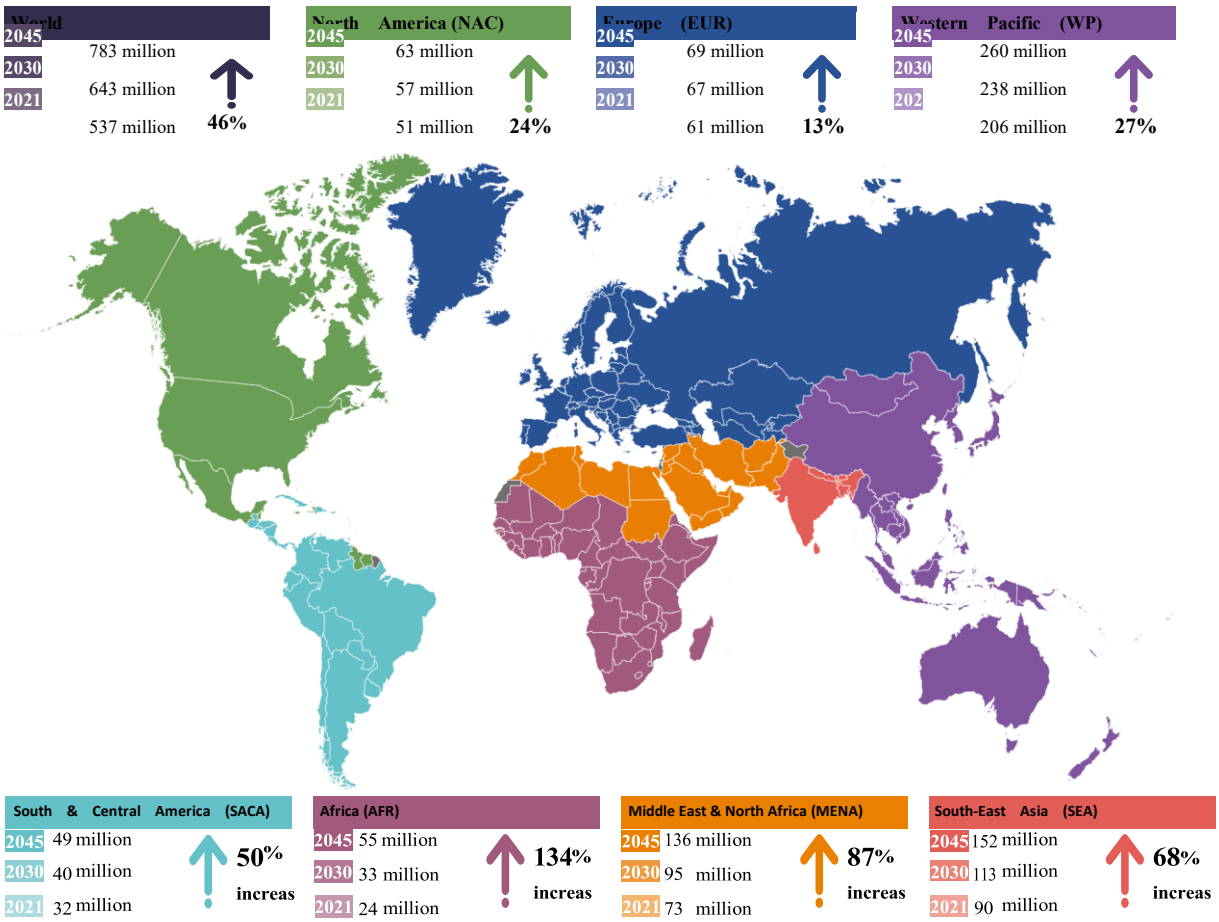



**Figure 2** | Number of people (20–79 years) with diabetes worldwide (IDF, 2021).

## 1.5 Risk Factors

A study by Tang et al. (2021) SARS-CoV-2 antigens have been detected in pancreatic beta cells collected from autopsy specimens of COVID-19 patients. Studies have shown that insulin expression is reduced in SARS-CoV-2-infected beta cells, which may be undergoing transdifferentiation. Another study by Wu et al. From 2021 it was also shown that infected B cells secreted less insulin, and researchers found evidence that SARS-CoV-2 can induce beta cell apoptosis (Wu *et al.,* 2021). In addition, there is growing evidence that depression is an important risk factor for developing T2D.

The relative risk was found to be 1.17 for women with depressed mood and 1.25 for women taking antidepressants (Tang, 2021). Based on that, insulin secreted by pancreatic beta cells normally reduces glucose output from the liver and increases glucose uptake in skeletal muscle and adipose tissue. Hyperglycemia occurs when there is beta cell dysfunction in the pancreas and/or insulin resistance in the liver, skeletal muscle or adipose tissue, resulting in too much glucose circulating in the blood. Various fac-tors listed below **(Fig. 3)** influence insulin secretion and insulin action (Zheng *et al.,* 2018).

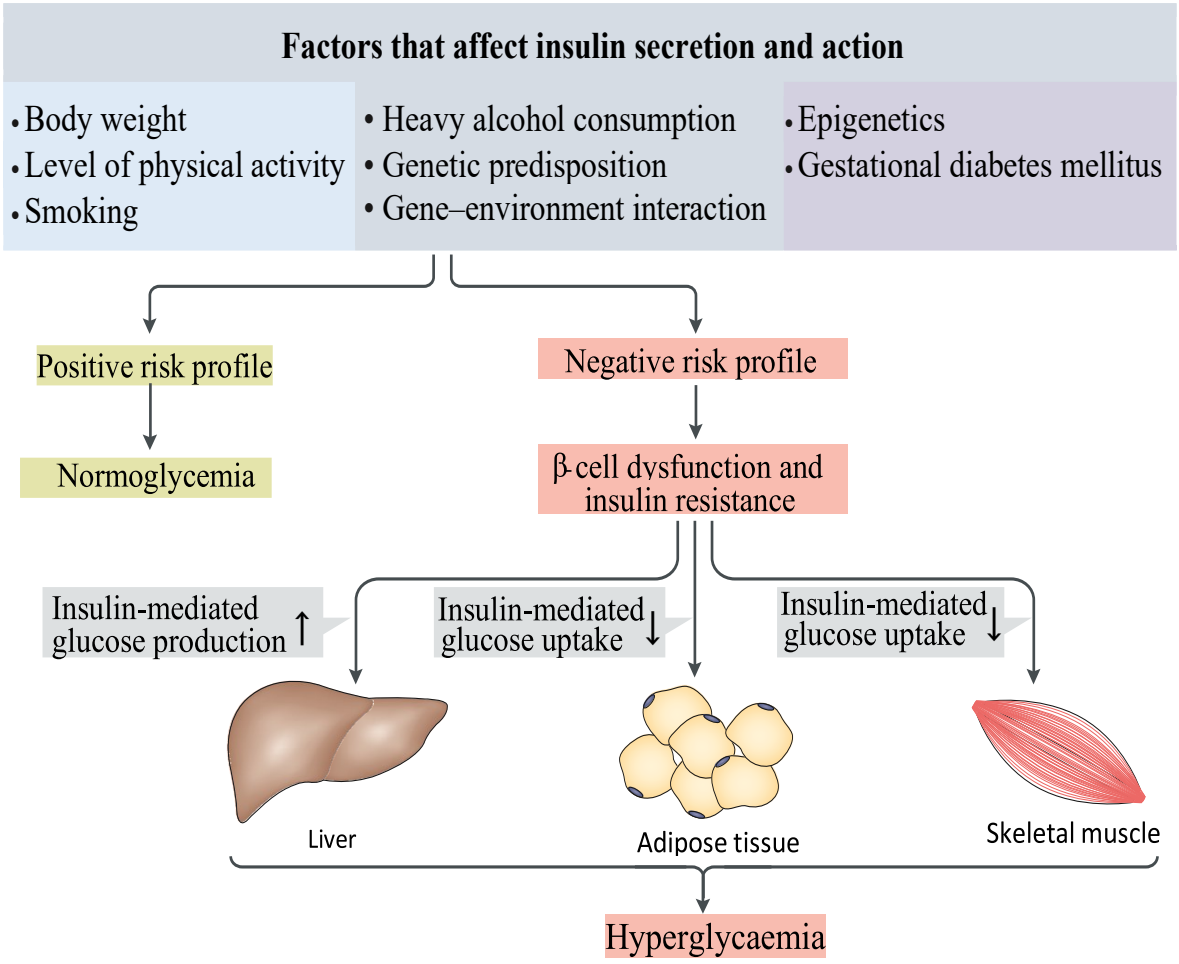



**Figure 3** | Pathophysiology of hyperglycaemia in T2DM (Zheng *et al.,* 2018)**.**

## 1.6 Diagnostic Criteria of Diabetes Mellitus

According to (ADA), the most widely accepted tests to investigation of DM are the fasting plasma glucose (FPG), post-prandial glucose (PPG), the oral glucose tolerance test (OGTT) and (HbA1c) glycated hemoglobulin **(Fig. 4)**.

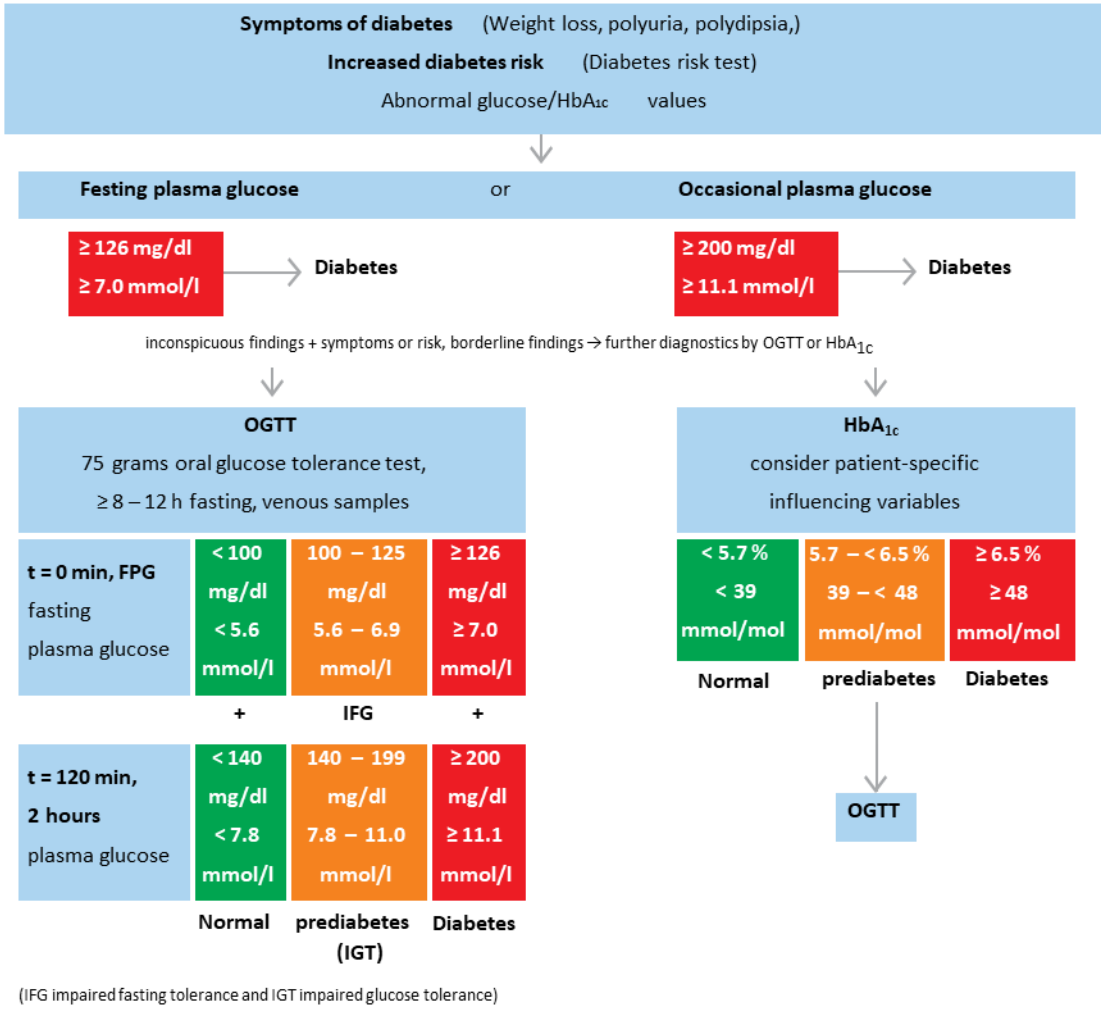



**Figure 4** | Algorithm for the diagnosis of diabetes **(**Petersmann *et al.,* 2019**).**

## 1.7 Complications

Diabetes complications are the leading cause of morbidity and death linked with this chronic metabolic condition. Diabetic complications can be divided into acute and chronic complications, as summarized in **(Fig. 5).** Acute complications include hypoglycemia, diabetic ketoacidosis, and hyperosmolar hyperglycemia syndrome, while chronic complications include microvascular and macrovascular disease (Nambam *et al.,* 2017).

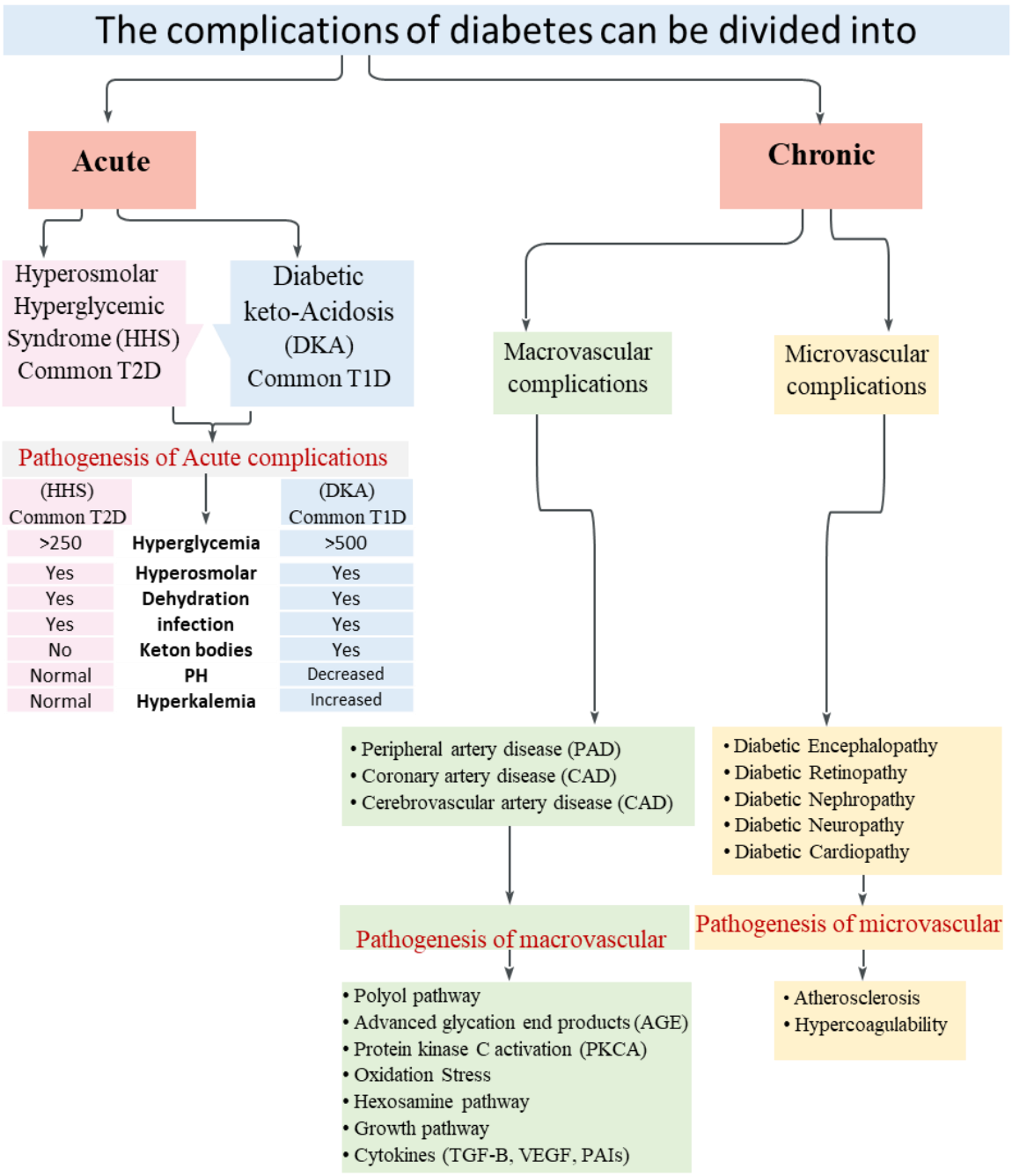



**Figure 5** | Algorithm for the complications of DM. *(VEGF) Vascular Endothelial Growth Factor;(TGF-β) Transforming Growth Factor-β; (PAIs) Plasminogen Activator Inhibitor*

### 1.7.1 Acute Complications

#### 1.7.1.1 Diabetic ketoacidosis (DKA)

Patients with diabetic ketoacidosis typically have metabolic acidosis with blood glucose levels below 500 mg/dL. DKA is caused by very low levels of effective circulating insulin and concurrently increased levels of counter-regulatory hormones such as glucagon, catecholamines, cortisol, and growth hormone. This combination results in catabolic changes in carbohydrate, fat and protein metabolism. Impaired glucose utilization and increased glucose production by the liver and kidneys leading to hyperglycemia. Lipolysis leads to raise production of ketones, particularly (beta-OHB), ketosis, and metabolic acidosis, which is exacerbated by persistent fluid and electrolyte loss (Joseph *et al.,* 2016).

#### 1.7.1.2 Hyperglycemic Hyperosmolar syndrome (HHS)

Hyperosmolar hyperglycemia syndrome was defined as extremely elevated blood glucose > 600 mg/dL (> 33.30 mmol/L) and serum osmolarity > 320 mosm/kg without overt ketosis and acidosis. Small amounts of ketone bodies may be present in blood and urine (Nambam *et al.,* 2017).

### 1.7.2 Chronic complications

#### 1.7.2.1 Macrovascular complications

Macrovascular are affecting medium and large blood vessels. Macrovascular complications include accelerated atherosclerosis leading to increased coronary artery disease, cerebrovascular disease and stroke, and increased peripheral arterial disease leading to critical limb ischemia. There is also strong evidence that diabetes can cause direct cardiac side effects leading to diabetic cardiomyopathy (Joseph *et al.,* 2016).

#### 1.7.2.2 Microvascular complications

Microvascular complications affecting small blood vessels, such as arterioles. Diabetic retinopathy, diabetic nephropathy, diabetic heart disease, diabetic encephalopathy, and diabetic neuropathy are major microvascular complications of chronic hyperglycemia through multiple mechanisms, such as the production of advanced glycation end products (AGEs), the development of pro-inflammatory microenvironments, and the Generates and induces oxidative stress (Nambam *et al.,* 2017).

# Chapter Two: Vitamin D

## 2.1 History

Vitamin D has a long history before Nobel Prize winner Adolf Otto Reinhold Windaus determined its chemical structure in 1930. Rickets is a bone disease caused by vitamin D deficiency, known in ancient times and described in detail by Glisson in 1650.

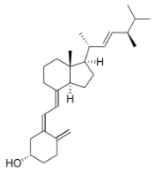
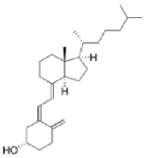
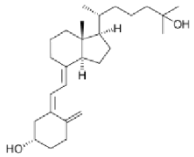
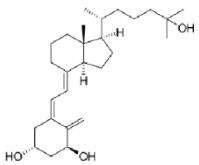
A number of causes and treatments for rickets have been proposed. Although cod liver oil has been used medicinally for a long time, Schutte first used it to treat rickets in 1824 (Wolf**,** 2004). The greatest experience was made by McCollum and his colleagues in 1922, when they found that heat-oxidized cod liver oil did not prevent dry eye, but cured rickets in rats. "This suggests that oxidation destroys fat-soluble A without destroying another substance that plays an important role in bone growth. "Vit. A" and another are a newly discovered factor that fights rickets. Because the water-soluble factor discovered at the time was called vitamin B, and the well-known ascorbic factor was called vitamin C, they named the new factor vitamin D. At the same time, under the influence of UV light, a very different treatment for rickets has emerged. There is a long tradition that fresh air and sunlight are beneficial in preventing rickets (McCollum, 1922).

## 2.2 Background

Vitamin D deficiency currently affects more than half of the population of all ages. The role of vitamin D in bone health is well known. In addition, vitamin D may also play a role in extra skeletal function. Vitamin D is a fat-soluble hormonal steroid with endocrine, autocrine and paracrine functions. Vitamin D acts as a chemical messenger and is involved in regulating transcription in about 3% of the human genome. Most tissues and organs have receptors for vitamin D, which appear to be involved in many biological functions (**Tab. 2**).

In fact, several studies have shown that low 25(OH)D levels are associated with other pathological conditions, such as autoimmune disease, hypertension, cardiovascular disease, and cancer. Insulin resistance is also associated with vitamin D deficiency. In addition, insulin resistance is associated with various diseases such as obesity, T2D and its complications, metabolic syndrome (MS) and polycystic ovary syndrome (PCOS). In this context, all of these diseases may be associated with vitamin D deficiency (Contreras-Bolívar *et al.,* 2021).

**Table 2** | Chemical structure and pharmacokinetic of vitamin D (Tripkovic *et al.,* 2012).

| | **Ergocalciferol** | **Cholecalciferol** | **Calcifediol** | **Calcitriol** |
|---|---|---|---|---|
| Chemical Structure |  |  |  |  |
| Absorption | Intestine (bile required) | Intestine (bile required) | Intestine, readily absorbed [a] | Intestine, readily absorbed [b] |
| Vitamin D-Binding Protein dissociation constant | 10−7 | 10−7 | 10−9 | 10−7 |
| Volume of distribution | very limited in plasma compartment; rapidly stored in fat tissue | very limited in plasma compartment; rapidly stored in fat tissue | larger than plasma volume | plasma compartment |
| Tissue distribution for long-term | adipose tissue, muscle | adipose tissue, muscle | blood, adipose tissue, muscle | blood and tissues |
| Circulating half-life | 2 days | 2 days | 3 weeks | 4–8 h |
| Functional half-life | 2–3 months | ≤2 months | 2–3 months | 4–8 h |

[a, b] *Faster absorption than ergocalciferol and cholecalciferol.*

## 2.3 Sources of Vitamin D

The main source of vitamin D is skin synthesis. The contribution of dietary sources is less pronounced, as foods containing vitamin D are generally not a daily part of most diets. Therefore, vitamin D supplementation is often required due to limited sun exposure or reduced skin vitamin D synthesis, such as vitamin D deficiency. e.g., Prescribing in the elderly. (Dominguez *et al.,* 2021).

### 2.3.1 Endogenous Vitamin D Synthesis

Endogenous vitamin D3 hormone production occurs when 7-dehydrocholesterol is exposed to UV light within the skin's microvessels, as a result of which it is converted to cholecalciferol. However, since vitamin D3 is a fat-soluble oxysterol, it must be carried in the circulation via DBP, a liver-derived apoprotein and albumin gene family member. In people with fair skin, only 20 minutes of sun exposure on the face and arms per week can produce 10000 UI of cholecalciferol. For centuries, the majority of humanity depended significantly on spontaneous production from sunlight. The reality that sun-derived vitamin D3 is taken by DBP separates it from exogenous vitamin D3 due of its potential influence on biodistribution. (Linda, 2018).

### 2.3.2 Exogenous Vitamin D Sources

Exogenous sources of vitamin D include diet (eggs, fish, liver, and marine mammal fats) and dietary supplements. as shown in **(Tab.3).** While food sources can be in the D3 or D2 form, dietary supplements are usually derived from the plant hormone ergocalciferol (D2). A key feature of dietary or supplemental sources is that orally ingested D3 is absorbed from the gut via chylomicrons, which enter the lymphatic system and then return to the central venous system via the thoracic duct. Ultimately, about 35% of ingested D3 is transported as lipoprotein rather than DBP (Linda *et al.,* 2018).

**Table 3** | Content of vitamin D in some foodstuff [a] (Dominguez *et al.,* 2021)

| Vitamin D | Foodstuff | µg / Serving | IU / Serving |
|---|---|---|---|
| **Vitamin D2** | Mushrooms, raw, exposed to UV light, 1/2 | 9.2 | 366 |
| **Vitamin D3** | Cod liver oil, 1 tablespoon | 34.0 | 1360 |
| | Trout (rainbow), cooked, 3 ounces | 16.2 | 645 |
| | Salmon (sockeye), cooked, 3 ounces | 14.2 | 570 |
| | Sardines, canned in oil, drained, 2 sardines | 1.2 | 46 |
| | Egg, 1 large, scrambled | 1.1 | 44 |
| | Tuna fish, canned in water, drained, 3 | 1.0 | 40 |
| | Cheese, cheddar, 1 ounce | 0.3 | 12 |
| | Chicken breast, roasted, 3 ounces | 0.1 | 4 |
| | Beef, ground, 90% lean, broiled, 3 ounces | traces | 1.7 |
| | Broccoli, raw, chopped, 1/2 cup | 0 | 0 |

*[a] To convert from mcg (µg) to UI, multiply by 40.*

## 2.4 Vitamin D Status Evaluation

Determination of vitamin D status is not based on the measurement of serum 1,25-(OH)2D concentrations. Vitamin D status was measuring by the prohormone 25(OH)D, which is the most stable, suitable indicator and abundant metabolite of vitamin D in human serum. It is an indicator rather than function with a half-life about 3 weeks **(Tab. 4)**.

**Table 4**| Classification of Vitamin D Status by 25(OH)D Concentration[a] (Tom *et al.,* 2011).

| Classification | ng/mL | nmol/L |
|---|---|---|
| Sever deficient | <10 | <25 |
| Deficient | 10 To 20 | 25 To 50 |
| Insufficient | 20 To 30 | 50 To 75 |
| Optimal level (sufficient) | 30 To 70 | 75 To 175 |
| Toxicity possible | >100 | >250 |

*[a] to convert from ng/mL to nmol/L, multiply by 2.496.*

## 2.5 Vitamin D Needs

Most of vitamin D needs are obtained through sun exposure. However, there is no point in recommending a one-size-fits-all sun exposure dose that is adequate for ever-yone's indispensable annual vitamin D needs (**Tab. 5**). In fact, many parameters come into play, including age, physical characteristics, exposure time, season (Priemel, 2009).

**Table 5 |** Recommended intakes according to age and sun exposure (Amstutz *et al.,* 2011).

| **Sun exposure** | Adequate | | | Inadequate | | |
|---|---|---|---|---|---|---|
| **Age** | <50 years | 50-70 years | >70 years | <50 years | 50-70 years | >70 years |
| **µg/day** | 5 | 10 | 15 | 15 | 20 | 30 |
| **UI/day** | 200 | 400 | 600 | 600 | 800 | 1000 |

## 2.6 Vitamin D3 metabolism

Synthesis of D3 occurs in the skin where (7-DHC) is converted to previtamin D3, bound to DBP in the bloodstream and transported after exposure to sunlight or intestinal absorption of natural and fortified foods and supplements D2 and D3 to liver. D2 and D3 are hydroxylated by hepatic 25-hydroxylase. The resulting (25(OH)D) is 1-hydroxylated in the kidney by 1-α-hydroxylase. This produces the active secosteroid 1,25(OH)2D, which has different effects on different target tissues (Dominguez *et al.,* 2021).

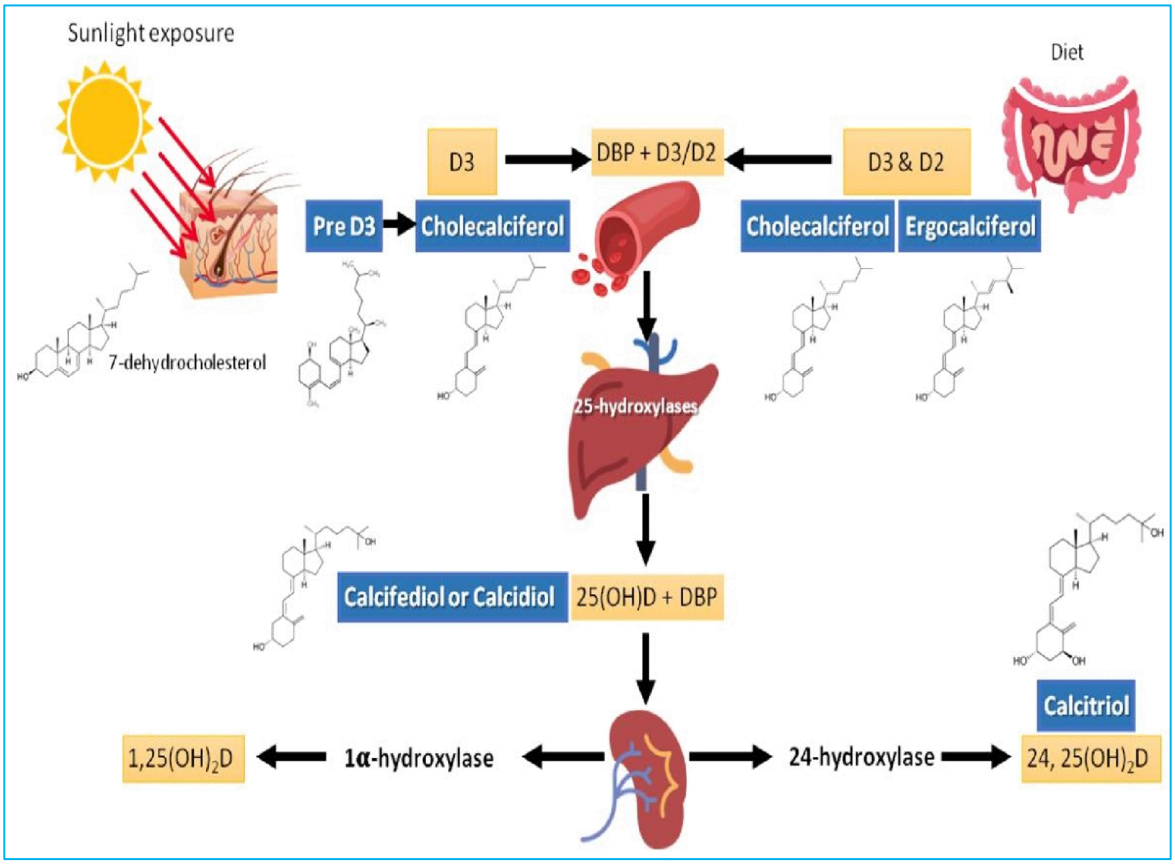



**Figure 6 |** The synthesis of vitamin D (Dominguez *et al.,* 2021).

### 2.6.1 Cutaneous Production of Vitamin D3

The precursor of vitamin D (7-DHC) is located on the Kandutsch-Russell cholesterol pathway **(Fig. 7)**. The final enzymatic reaction is mediated by 7-dehydrocholesterol reductase (DHC-R7), which converts 7-DHC to cholesterol, and is regulated by multiple factors, including vitamin D and cholesterol, which promote its breakdown, resulting in 7-DHC levels raised. Allows conversion to vitamin D during UVB exposure to sunlight (Prabhu, 2016).

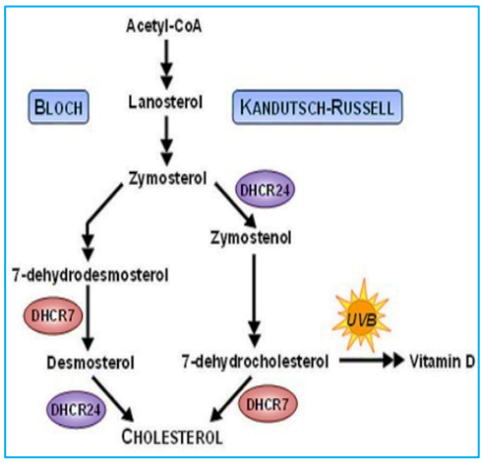



**Figure 7** | The Kandutsch-Russell pathway *(*Mitsche *et al.,* 2015*)*.

### 2.6.2 Hepatic Production of 25(OH)D

The next step, bioactivation of D2 and D3, hydroxylation to 25OHD, occurs primarily in the liver, although many other tissues express this enzymatic activity. The resulting 25-hydroxycholecalciferol (25(OH)D). Therefore, 25OHD is the predominant circulating form of vitamin D. However, 25-hydroxylase (25-OHase) activity was found in both liver mitochondria and endoplasmic reticulum, and the enzymatic activity appeared to be different, reflecting different proteins. At this time, mitochondrial CYP27A1 and microsomal CYP2R1 received the most attention. These enzymes are widely distributed in various tissues, with the highest concentrations in liver and muscle, and also in kidney, intestine, lung, skin and bone (Thacher *et al.,* 2015).

### 2.6.3 Renal Production of 1,25(OH)2D

The most powerful metabolite of 1,25(OH)2D, is responsible for the majority of its hormonal effects. The enzyme 25(OH)D-1 hydroxylase (CYP27B1) produces the product 1,25(OH)2D from 25(OH)D. The brain, placenta, testes, gut, lung, breast, macrophages, lymphocytes, parathyroid gland, osteoblasts, and chondrocytes all express this enzyme in the renal tubules (Fu *et al.,* 1997). However, the kidney is usually thought to be the primary source of circulating 1,25(OH)2D, with extrarenal CYP27B1 activity meeting local demands under normal conditions. The parathyroid hormone (PTH) stimulates the production of 1,25(OH)2D from 25(OH)D in the kidney, whereas calcium, phosphate, and 1,25(OH)2D itself decrease it (Dominguez *et al.,* 2021).

### 2.6.4 Renal Production of 24,25(OH)2D

The kidney is also a major producer of the second important metabolite of 25OHD, 24,25(OH)2D, and the responsible enzyme is (CYP24A1) 25OHD-24-hydroxylase (Shimada *et al.,* 2001). CYP24A1 and CYP27B1 are homologous enzymes that coexist in the mitochondria of the tissues in which they are found. Although CYP24A1 is highly expressed in renal tubules, its tissue distribution is rather widespread. Generally speaking, as long as the VDR is found, CYP24A1 can be found. In addition, the affinity of 1,25(OH)2D is higher than 25(OH)D, making this enzyme an efficient means of eliminating 1,25(OH)2D. Therefore, CYP24A1 may play an important role in protecting the body from excess 1,25(OH)2D (Schlingmann *et al.,* 2011).

## 2.7 Vitamin D transport in blood

For endogenous and exogenous sources, because its lipophilicity, vitamin D is transported in the bloodstream by DBP, albumin or lipoprotein. Endogenously produced D3 is transported in the aqueous environment of the bloodstream via DBP and albumin, while exogenous (diet and supplemental) D3 and D2 absorbed from the gut are transported within chylomicrons, which are further metabolized to lipoproteins (e.g, VLDL and LDL) deal with many of them continue to carry exogenous D. The common sites of vitamin D hormone metabolism are the proximal tubules of the liver and kidneys, where hydroxylases convert it to the active form. But hydroxylase activity is also present in parenchymal and immune cells of other tissues, including vascular smooth muscle cells and monocytes (Linda *et al.,* 2018).

## 2.8 Storage and cell distribution

Vitamin D, unlike other fat-soluble vitamins, is stored mostly in adipose tissues and muscles in the form of 25-hydroxyvitamin D (25(OH)D, which can be mobilized if dietary intake and/or endogenous production (cutaneous) are reduced. Vitamin D's distribution in the body differs depending on the molecule. Cholecalciferol (25(OH)D3), which accounts for 65 % of all vitamin D in the body, is primarily stored in adipose tissue (75 %), whereas 25(OH)D2 (35 % of vitamin D in the body) is more evenly distributed throughout the body (20 % in muscle, 30 %% in serum, 35 % in adipose tissue, and 15 % in all other tissues) (Heaney *et al.,* 2009).

## 2.9 Mechanism of action

Because the VDR mediates the activity of calcitriol, it is critical to understand either genomic or non-genomic activities when studying the processes that are triggered by 1,25(OH)2D.

### 2.9.1 Genomic actions

The VDR-mediated modulation of target gene expression is determined by the genomic action of calcitriol (1,25(OH)2D). The 1,25(OH)2D interacts to the target cell's nuclear vitamin D receptor (VDRn). The VDR then forms a heterodimer with the retinoid X receptor (RXR), a vitamin 'A' receptor, increasing the VDR/RXR complex's affinity for the vitamin D response element (VDRE), a nucleotide sequence in the promoter region of the vitamin D responsive gene. The VDR/RXR complex binds to the VDRE, attracting a group of proteins known as coactivators to the VDR/RXR complex. The DRIP (Mediator) coactivator complex connects the VDRE and RNA polymerase II, as well as other proteins in the initiation complex, to the TATA box (or other transcription-controlling elements) Histone acetyl transferases (HAT) are recruited by SRC coactivators to the gene, facilitating the opening up of its structure to allow the transcription machinery to operate **(Fig. 8).** The gene's transcription is started to create the matching mRNA, which then exits the nucleus to be translated into the protein (Daniel and Bikle, 2021).

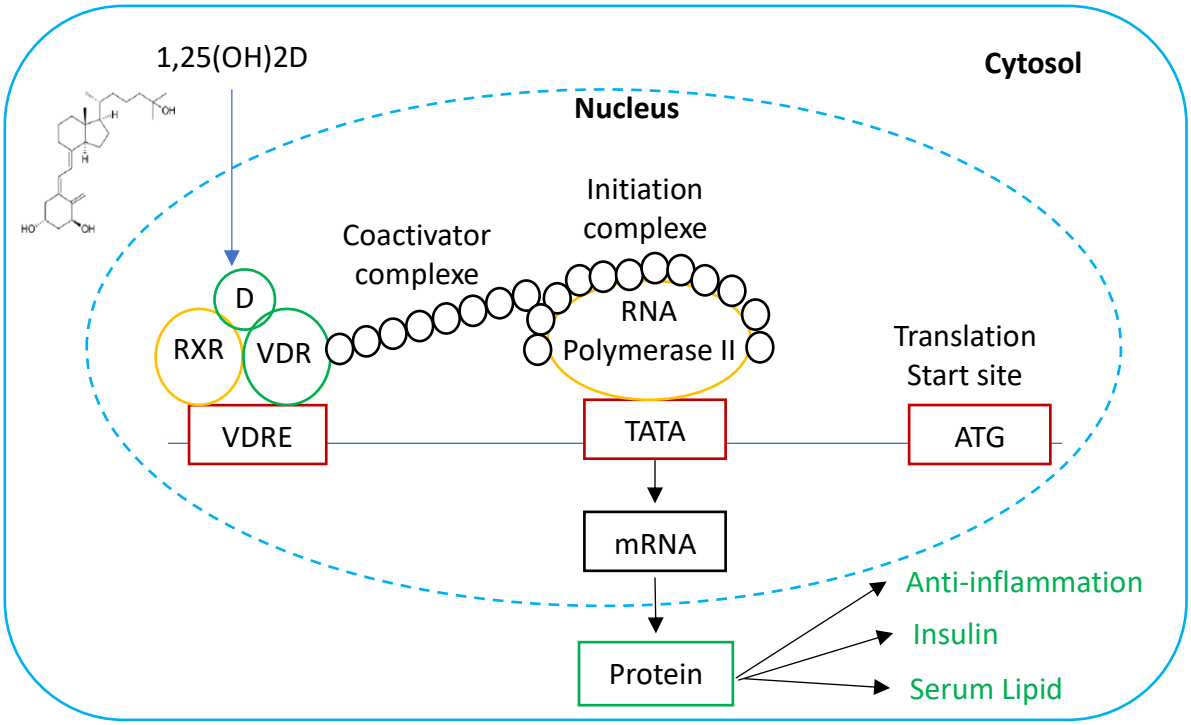



**Figure 8** | Genomic actions of 1,25(OH)2D, Modification **(**Daniel and Bikle, 2021**).**

### 2.9.2 Non genomic actions

1,25(OH)2D is known to have non-genomic effects, such as activating kinases, phosphatases, and ion channels (Mizwicki and Norman, 2009*);* hence, calcitriol interacts to a putative plasma membrane vitamin D receptor (VDRm). GTP displacement of GDP and dissociation of the and subunits from the newly active subunit result in the activation of a G protein. Then G-GTP activates phospholipase C (PLC) ((β or γ) which hydrolyzes phosphatidyl inositol bisphosphate (PIP2) to inositol trisphosphate (IP3) and diacyl glycerol (DG). IP3 activates protein kinase C and releases calcium from intracellular reserves via the IP3 receptor in the endoplasmic reticulum (PKC). Calcium and PKC may control calcium influx across the plasma membrane via a variety of calcium channels, including L-type calcium channels (Daniel and Bikle,2021).

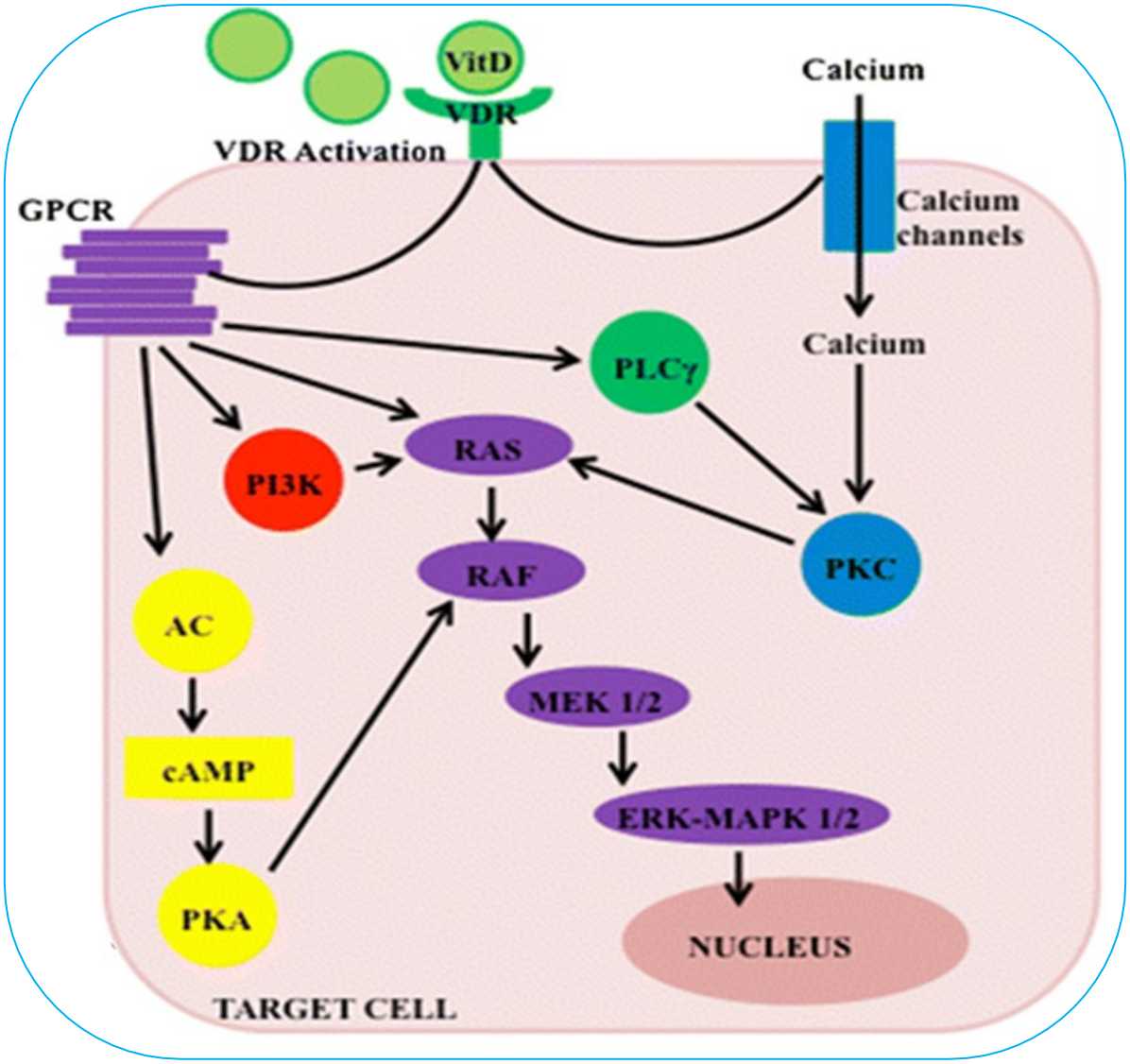



**Figure 9 |** Model for non-genomic actions of 1,25(OH)2D **(**Zheng *et al.,* 2018**).**

### 2.10.1 Target tissue responses: Calcium regulating organs

Calcium is the human body's fifth most prevalent element. Calcium is required for bone production as well as a variety of physiological activities such as blood clotting, muscular contraction, nerve transmission, and hormone secretion **(Fig. 14)**. Vitamin D3 is a key component in the maintenance of appropriate calcium homeostasis (Puneet *et al.,* 2017).

#### 2.10.1.1 Intestine

Vitamin D helps to maintain calcium homeostasis by boosting calcium absorption in the intestine, as summarized in **(Fig. 10).** 1,25(OH)2D promotes saturable (transcellular) transport in the proximal small intestine when dietary calcium levels are low. 1,25(OH)2D enters the cell, binds to VDR in the nucleus, and stimulates gene transcription of the TRPV6 (transient receptor potential vanilloid 6) apical membrane calcium channel, resulting in enhanced calcium absorption from the intestinal lumen into the cell. The 1,25(OH)2D/VDR system increases calbindin 9 k (calcium-binding protein) gene expression, which can then shuttle calcium from the apical membrane to the basolateral membrane, and upregulate the basolateral plasma membrane protein PMCA1b, allowing calcium to be extruded from the intestinal cell into the blood via an ATP-dependent process. 1,25(OH)2D/VDR signaling can also boost gene expression of tight junction proteins such as claudin 2 and 12, cadherin-17, and aquaporin 8, allowing for passive, diffusional, and paracellular calcium fluxes across the gut, especially in the jejunum and ileum (Goltzman, 2018).

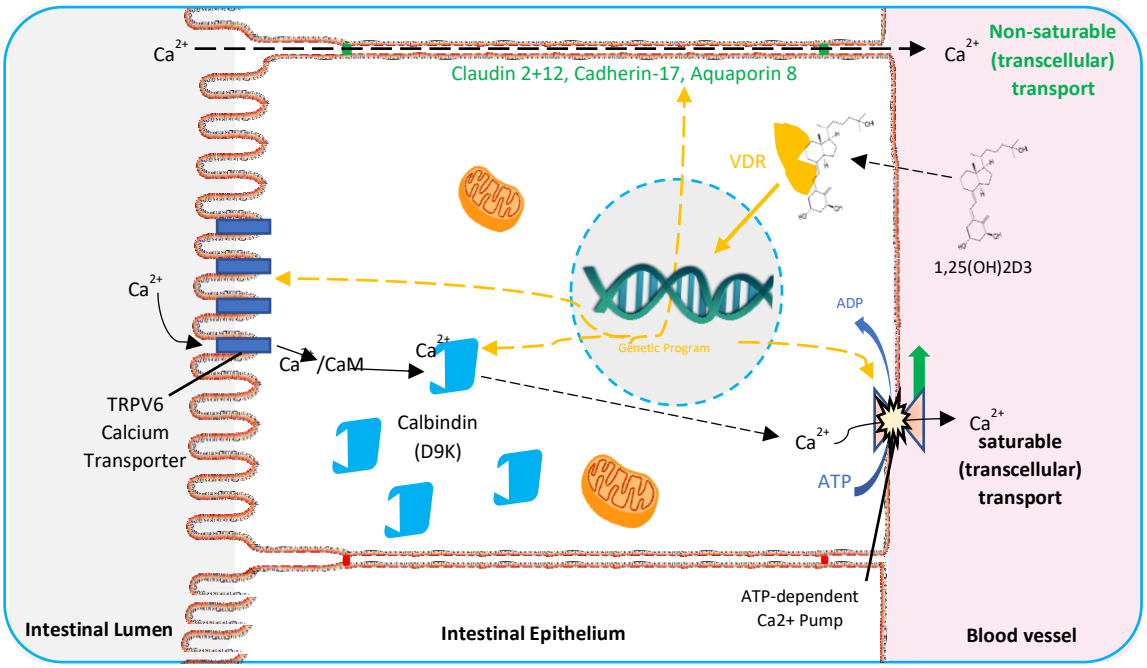



**Figure 10 |** 1,25(OH)2D/VDR-mediated calcium ($Ca^{+2}$) transport in intestine

### 2.10.1.2 Kidney

In the kidney, distal tubular calcium reabsorption involves an active transcellular mechanism in which calcium enters distal tubular cells via TRPV5, as summarized in **(Fig. 11).** calcium is transported to the cytoplasm by binding to calbindin-D9k and calbindin-D28k, and is transported to the blood vessel by NCX1 (sodium/calcium exchanger 1) and plasma membrane calcium pump PMCA1b 1b. The 1,25(OH)2D/VDR system is known to enhance the gene expression of several calcium transporters, including TRPV5, Calbindin-D9k, CalbindinD28k and possibly NCX1b (Van Abel *et al.,* 2003; Song *et al.,* 2003).

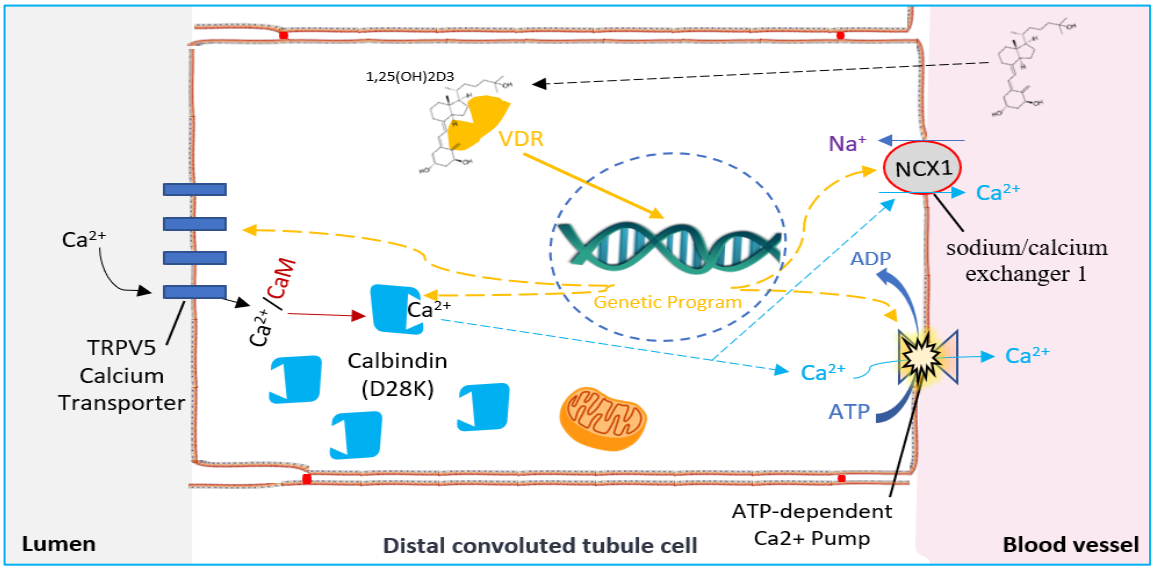



**Figure 11**| Calcium reabsorption at the distal tubule in the kidney

### 2.10.1.3 Bone

The vitamin D receptor and the enzyme CYP27B1 are found in all cells of the skeleton, including chondrocytes, osteoblasts, and osteoclasts, and are essential for the production of the active metabolite of vitamin D, 1,25 (OH)2D3 (*Bikle,2012*). Normal levels of 25(OH)2D suppress osteoclastic bone resorption by decreasing the ratio of RANKL/OPG (Receptor Activator of Nucleus Factor-kapa B Ligand / osteoprotegerin) in mature osteoblasts through the VDR. In mature osteoblasts, 1,25(OH)2D activity via the VDR also accelerates the pace of bone production (BFR). As a result, there is more cortical and trabecular bone **(Fig. 12).** In less developed osteoblasts, increased levels of 1,25(OH)2D acting through the VDR may enhance RANKL/OPG, accelerate osteoclastic bone resorption, and diminish trabecular bone. As a result, high levels of 1,25(OH)2D in mature osteoblasts and osteocytes might enhance local and systemic osseous mineralization inhibitors and impair bone mineralization, resulting in osteomalacia (Goltzman, 2018).

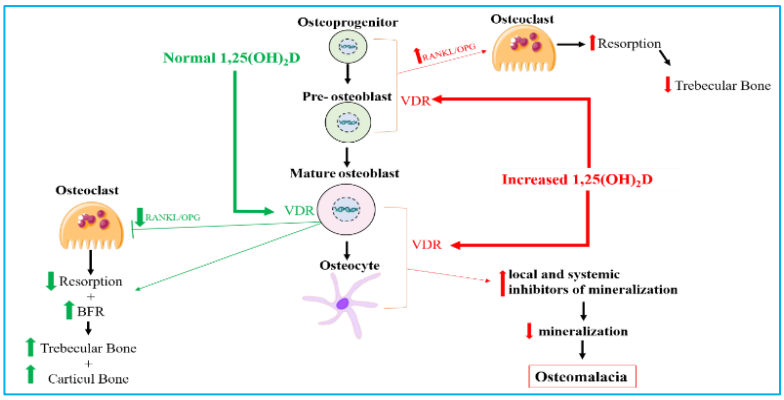



**Figure 12** | Direct actions of 1,25(OH)2D/VDR on bone (Goltzman, 2018).

## 2.10.2 Target Tissue Responses: Non-Calcium Transporting Tissues

1,25(OH)2D regulates the function of a wide number of tissues that all contain the VDR (Regulation of differentiation, proliferation, hormone secretion and immune function).

### 2.10.2.1 Regulation of hormone secretion

The existence of calcitriol receptors in the parathyroid, pancreas, and other glands has raised the possibility of a direct involvement for 1,25(OH)2D in hormone production and secretion control. Following are a few instances.

**A. Parathyroid gland (PTH Secretion)**

PTH promotes the activity of 1-hydroxylase in renal proximal tubular cells, resulting in the production of 1,25(OH)2D, which mediates some of PTH's activities. In turn, 1,25-(OH)2D suppresses PTH production in parathyroid cells (Juppner and Kronenber, 2004).

**B. Fibroblast growth factor (FGF23)**

FGF23 is predominantly generated by bone, namely osteoblasts and osteocytes. This process is stimulated by 1,25(OH)2D3, although the mechanism is unknown (Kolek *et al.,* 2005). FGF23 suppresses 1,25(OH)2D synthesis by the kidney, therefore this feedback loop, like the one for PTH secretion, keeps the levels of these critical hormones in check (Fukumoto and Yamashita, 2007).

**C. Pancreatic β-cells (Insulin Secretion)**

VDR, CYP27B1, and calbindin-D28k are all detected in pancreatic B-cells (Clark *et al.,* 1999), and experiments using calbindin-D28k null animals have revealed that calbindin-D28k can influence depolarization-stimulated insulin release via modulating intracellular calcium (Sooy *et al.,* 1999). As a result of the opening and closure of calcium channels, 1,25(OH)2D increases insulin secretion (Leung, 2016).

### 2.10.2.2 Regulation of proliferation and differentiation

**A. Immune System**

It is now well accepted that the vitamin D endocrine system plays a role in cell differentiation, cell growth inhibition, and immunomodulation. The enzyme of 1,25(OH)2D is involved in both adaptive and innate immunity. Activated dendritic cells, macrophages, and lymphocytes all have VDR. These cells (which express CYP27B1, which converts 25(OH)D to 1,25(OH)2D3) and create 1,25(OH)2D3. Their growth and function are controlled by 1,25(OH)2D3 (Bikle, 2021).

**B. Cancer**

For almost 40 years, 1,25(OH)2D has been studied in animal and cell investigations for its possible anti-cancer action (Eisman *et al.,* 1979). The list of cancer cells that express VDR is rising, and the list of cancer cells that express CYP27B1 is also growing. The anti-proliferative, pro-differentiating properties of 1,25(OH)2D on most cell types are the acknowledged basis for its promise in the prevention and treatment of cancer (Fleet et al., 2012).

**C. Skin**

The only cells in the body possessing the whole vitamin D metabolic pathway are epidermal keratinocytes. Vitamin D3 is produced in the epidermis from 7-DHC, as previously stated. The epidermis, on the other hand, includes CYP27A1, a mitochondrial enzyme that 25-hydroxylates vitamin D, and CYP27B1, a mitochondrial enzyme that converts 25OHD to 1,25(OH)2D. Calcitriol (1,25(OH)2D) controls keratinocyte differentiation in part by altering calcium's capacity to do so. At least part of 1,25(OH)2D's capacity to promote differentiation is due to its ability to raise Cai ((Eisman *et al.,* 1979).

### 2.10.2.3 Other tissues

Many non–calcium–regulating cell types, including as dermal fibroblasts and keratinocytes of the skin, immunological cells, certain cardiovascular cell types, and cellular components of numerous other tissues, are now known to express the VDR (Wang *et al.,* 2012) Foll-owing are a few instances (**Fig. 13**).

**A. Heart**

Vitamin D deficiency has been linked to a decrease in contractility in animals (Weishaar *et al.,* 1990). This might be attributed to a deficiency in vitamin D, as well as hypocalcemia and hypophosphatemia. 1,25(OH)2D, on the other hand, promotes calcium absorption by heart muscle cells in vitro (Selles and Bolan, 2013***).*** Furthermore, 1,25(OH)2D suppresses the production of atrial naturetic factor, which is one of the rare genes having a negative VDRE in its promoter (Wu*,* 1995). Hypertrophy and fibrosis are caused by the deletion of the VDR in cardiac muscle. Low levels of 25OHD in the blood have been linked to an increased risk of myocardial infarction in males (Gardner *et al.,* 2013). However, a big randomized clinical trial failed to establish that vitamin D supplementation protects people with normal levels of 25OHD from developing cardiovascular disease (Manson *et al*., 2019).

**B. Skeletal muscle**

Reduced high energy substrates (ATP, creatinine phosphate) have been found with vitamin D insufficiency, as well as proximal muscle weakness (Boland*,* 1986). VDR is found in myoblasts, however its expression in adult muscle cells is debatable. Muscle weakness might be due to reduced calcium and phosphate levels rather than a drop in 1,25(OH)2D. However, there is growing evidence that 1,25(OH)2D and VDR have a direct role in muscle function (Girgis *et al.,* 2013). Furthermore, 1,25(OH)2D may have effects on muscle that are independent of the VDR, at least in terms of its genetic activities. The Boland laboratory has discovered that 1,25(OH)2D has an immediate influence on calcium uptake, PLC, PLA2, PLD, PKC, and adenylate cyclase activities, all of which might affect muscle performance (Boland, 2011).

**C. Pituitary**

In vivo, the VDR has been detected in thyrotropes, while in vitro, it has been found in GH and prolactin-secreting cell lines. Increased intracellular calcium (Cai) and Inositol Triphosphate IP3 synthesis promote Thyrotropin-releasing hormone (TRH) induced (TSH) secretion via 1,25(OH)2D. (Tornquist*,* 1992).

**D. Breast**

VDR is found in the breast (Narbaitz *et al.,* 1981), and vitamin D is important for appropriate breast growth. Furthermore, VDR is found in breast cancer cells, and 1,25(OH)2D and its analogs inhibit cell growth in vivo and in vitro (Eisman *et al.,* 1989).

**E. Liver**

In the liver, low amounts of VDR have been discovered, mainly in stellate cells Even when blood calcium is adjusted by a high calcium diet, vitamin D deficient mice have poor hepatic regeneration, showing that 1,25(OH)2D plays a function in hepatic cell development and the prevention of hepatic fibrosis (Cao *et al.,* 2020).

**F. Lungs**

In type II epithelial pneumocytes, VDR has been discovered (Nguyen *et al.,* 1990). Their maturation is stimulated by 1,25(OH)2D, which results in enhanced phospholipid synthesis and surfactant release (Hanchette *et al.,* 1992). The aberrant alveolar development seen in pups born to vitamin D deficient mothers is consistent with these findings (Gaultier *et al.,* 1984). Furthermore, 1,25(OH)2D promotes the innate immune response in bronchial epithelial cells and may give protection in cystic fibrosis patients with recurrent lung infections, as well as individuals with Covid-19 infections (Bilezikian *et al.,* 2020).

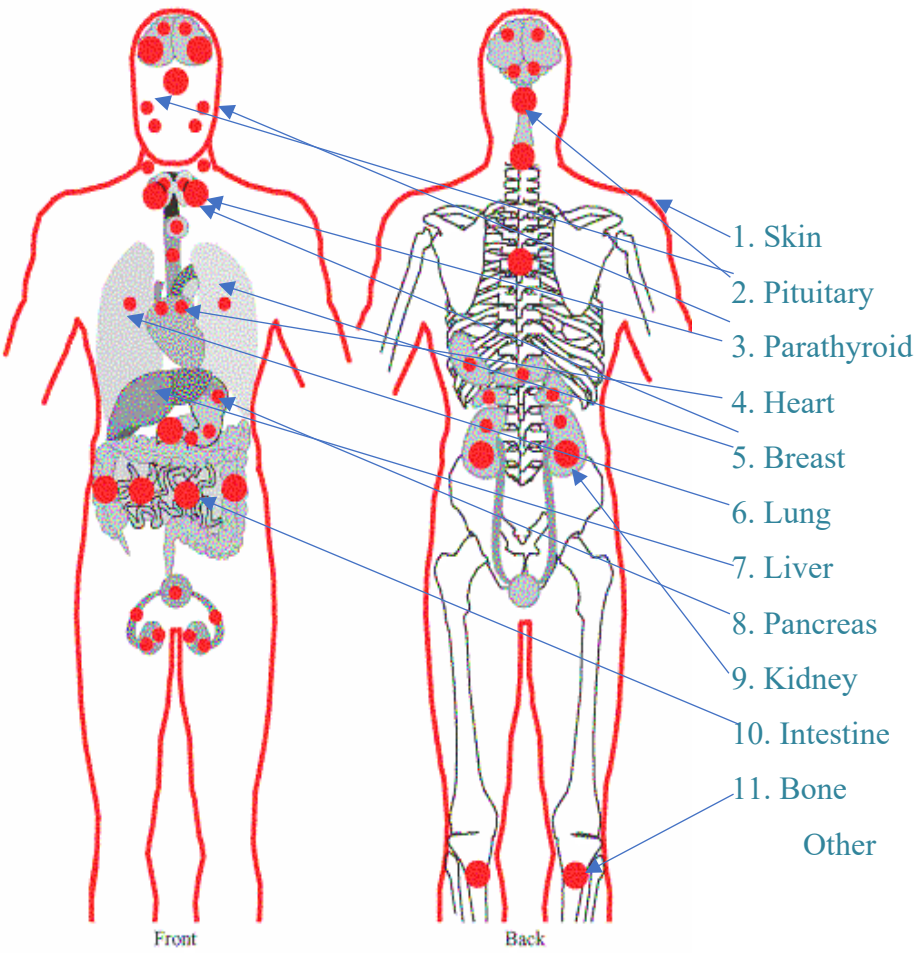



**Figure 13** | Vitamin D target tissues (Daniel and Bikle, 2021).

## 3.1 Vitamin D and Type 2 Diabetes Mellitus

Substantial evidence supports the link between vitamin D deficiency and T2D (**Tab.6**). This association is mediated through the direct and indirect effects of vitamin D on insulin secretion, insulin sensitivity, and systemic inflammation (Giustina and Bilezikian, 2018).

### 3.1.1 Vitamin D and Pancreatic β-cell function

In vitro and in vivo research have reported that vitamin D may play an important role in maintaining pancreatic β-cell function. There may be different explanations for this effect. It can be induced by activating VDR located in pancreatic beta cells. This is demonstrated by the results of the study, which showed that mice without VDR impair insulin secr-etion, and that adding calcitriol to the medium stimulates islets and leads to an increase in insulin secretion (Araceli *et al.,* 2019).

### 3.1.2 Vitamin D and insulin secretion

First, vitamin D can affect insulin secretion by regulating the opening and closing of calcium channels, as summarized in (**Fig. 14**). Calcitriol acts as a chemical messenger, inter-acting with various receptors that regulate calcium flux in beta cells. They are located on the phospholipid layer of the plasma membrane. Therefore, calcium is essential for adequate insulin secretion by pancreatic beta cells. Thus, vitamin D insufficiency alters normal insulin secretion by alter-ing calcium flux in beta cells. In this context, regulation of calbindin, a type of calcium-bin-ding protein, by vitamin D may be another mechanism affecting insulin secretion. In addition, preclinical studies have shown that vitamin D can reduce overactivity of the renin-angiotensin system, thereby improving β-cell function **(**Leung, 2016**)**.

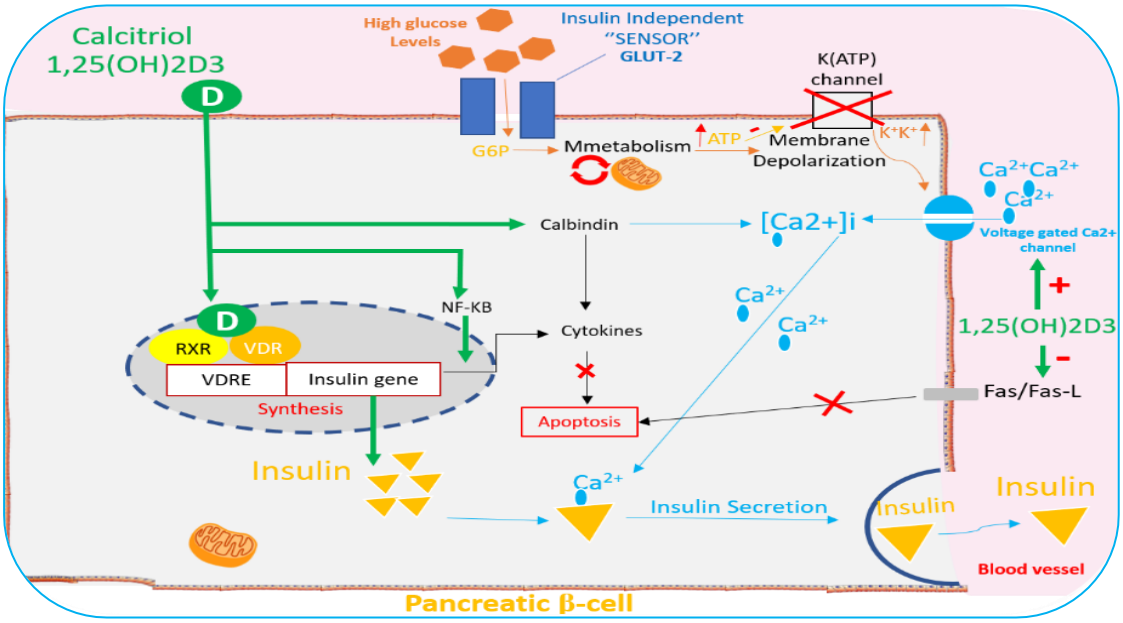



**Figure 14** | The action of calcitriol in insulin secretion

### 3.1.3 Vitamin D and insulin sensitivity

Adequate vitamin D levels also improve insulin resistance pathways associated with diabetes, as summarized in (**Fig. 15)**. It is mainly caused by changes in calcium flux and concentration on the cell membrane of insulin-responsive tissues. Regulation of extracellular and intracellular calcium concentrations may promote dephosphorylation of glucose transporter 4 (GLUT-4), thereby reducing insulin-stimulated glucose transport. 1,25-(OH)2D stimulates the expression of insulin receptors, thus stimulating insulin sensitivity. In addition, calcitriol can improve insulin sensitivity by activating peroxisome proliferator-activated delta receptor (PPAR-*δ*), a transcription factor that regulates fatty acid metabolism in adipose tissue and skeletal muscle. Another interesting study showed that insulin resistance can also be reduced by calcitriol's specific effects on hepatic lipid synthesis and glucose output as well as skeletal muscle (Araceli *et al.,* 2019).

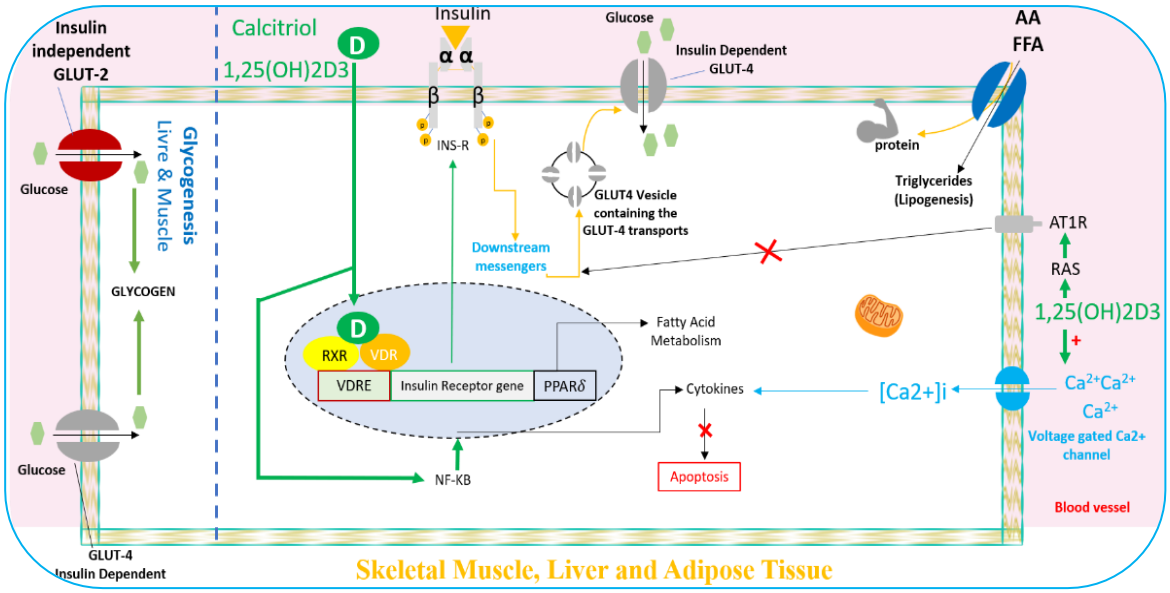



**Figure 15** | The action of calcitriol in insulin sensitivity

### 3.1.4 Vitamin D and systemic inflammation

Vitamin D also shortens the effects of chronic inflammation and is known to play a key role in the pathogenesis of T2D. Thus, 1,25(OH)2D can prevent cytokine-induced β-cell apoptosis by directly regulating cytokine activity and expression, an effect that counteracts cytokine-induced Fas expression (Chun *et al.,* 2014). As shown in (**Fig. 14).** In addition, vitamin D has been shown to inactivate inflammatory cytokines associated with insulin resistance and promote the expression of calbindin, including preventing apoptosis. Other immunomodulatory effects of vitamin D include blockade of dendritic cell differentiation, inhibition of lymphocyte proliferation, enhanced regulation of T lymphocytes, development and downregulation of cytokine expression **(**Araceli *et al.,* 2019).

**Table 6**| Mechanisms & evidence to support a benefit of vitamin D in T2D (Chittari, 2014).

| Mechanism | Evidence |
|---|---|
| **Vitamin D and β-cell function / insulin secretion** | |
| **Direct Actions** | 1.25 $(OH)_2D$ + VDR<br>↓<br>RXR<br>VDR + RXR<br>↓<br>Vitamin D response elements (VDRE)<br>↓<br>Enhances transcriptional activation of insulin gene<br>↓<br>Insulin Synthesis |
| **Indirect Actions** | 1. Calcium flux through the β-cells and intracellular calcium $Ca^{2+}$.<br>2. Regulates calbindin.<br>3. " Calcium Paradox. |
| **Vitamin D and insulin sensitivity** | |
| **Direct Actions** | 1. Stimulates expression of INS-R.<br>2. Activation of PPAR- $\delta$ (transcription factor) implicated in fatty acid metabolism skeletal muscles and adipose tissues. |
| **Indirect Actions** | 1. Regulating extracellular calcium flux though the cells.<br>2. Anti-apoptotic effect (by modulating through interaction of nuclear K - B (NF-KB) and effects on cytokines.<br>3. Through RAAS. |
| **Vitamin D and Systemic Inflammation** | |
| **Effects of D3 on cytokines** | Anti-apoptotic effect by:<br>1.Modulating the generation and effects of cytokine.<br>2. Down-regulating Fas-related pathways.<br>3. Via calbindin by its ability to buffer intracellular calcium. |

# Chapter Four: Materials and Methods

## 4.1 Material

### 4.1.1 Justification of the study

In Algeria, there is a lack of information on the status of vitamin D in T2D and its relationship to blood glucose levels. Work in this field may be helpful in boosting the research in diabetic care and management.

### 4.1.2 Study objectives

#### 4.1.2.1 General objective

To investigate the status of vitamin D and its correlation to glycated hemoglobin control in Mostaganem city's patients with T2D.

#### 4.1.2.2 Specific objectives

To achieve this general objective, the following specific objectives should be accomplished to measure the plasma level of vitamin D, determine the HbA1c and glycemia of T2D patients and to relate the vitamin D levels with HbA1c control of the patients.

### 4.1.3 The Population Study

The studied population was T2D patients attending the selected during the study period between March and June 2022 in Mostaganem city, Algeria. This study was carried out at the private medical analysis laboratory of Dr. Ettalhi. There were 31 (56.4%) males **(M)** and 24 (43.6%) females **(F)**.

### 4.1.4 The Equipment

#### 4.1.4.1 Sampling tools

The tools that were considered as a starting point {No-sterile gloves, Tourniquet, Antiseptic (alcohol), Cotton, Needle (syringe) and Anticoagulant tubes (EDTA & Heparin)}.

#### 4.1.4.2 Centrifuge TDZ4WS (Bioridge)

An apparatus with a fast-circulating bowl that applies centrifugal force to blood, typically to separate it into Serum (supernatant) and the pellet. As the blood is divided, the heavier particles go down to the bottom of the tube and the liquid plasma rises.

#### 4.1.4.3 HumaMeter A1c (Humma)

It allows for quick testing of HbA1c, a long-term diabetes metric, at the point-of- care. Only 4 µl of blood is required and results are accurate and exact in only 5 minutes.

#### 4.1.4.4 PC-VIDAS (bioMérieux)

It's an ELFA (Enzyme Linked Fluorescent Assay)-based automated quantitative test for determining 25-hydroxyvitamin D total in human blood or plasma. For an accurate measure of vitamin D status, it represents vitamin D generated cutaneously as well as vitamin D received through diet and supplementation.

#### 4.1.4.5 Spectrophotometer BA88A (Mindray)

It's a device that determines how much glucose is in the blood. Also known as the semiautomatic biochemistry tester, is integrated with easy-to-use software.

### 4.1.5 Reagents

#### 4.1.5.1 Vitamin D reagents

|a| Anti-vitamin D Antibody Marked with Alkaline Phosphatase Conjugate;

|b| Substrate 4-Methyl-ombillfery;

|c| 4-Methyle-ombilliferol.

#### 4.1.5.2 HbA1c reagents

**|a| Test cartouches**

| | | | |
|---|---|---|---|
| CAR | 2*25 | Fluorescent boronate conjugate<br>Ammonium chloride buffer<br>Sodium azide<br>Sodium deoxycholate monohydrate | $8,2*10^{-9}$ %<br>0,535 %<br>0,05 %<br>0,4 % |

**|b| Sample sticks**

| | | |
|---|---|---|
| SAMPS | 1*50 | (Treated with EDTA and surfactants) |

#### 4.1.5.3 Glycemic reagents

**|a| Enzymatic reagent**

| | |
|---|---|
| Phosphate buffer (pH 7.5) | 100 mmol/l |
| 4-aminoantipyrine | 0,25 mmol/l |
| Phenol | 0.75 mmol/l |
| Glucose oxidase | ≥ 15 KU/l |
| Peroxidase | ≥ 1.5 KU/l |
| Mutarotase | ≥ 2.0 KU/l |
| Sodium Azide | 0,095 % |

**|b| Standard**

| | |
|---|---|
| Glucose | 1g/L (5,55 mmol/l) |

## 4.2 Methods

### 4.2.1 Sampling

By a well-trained nurse, drew venous blood samples from T2D patients in the laboratory to measure the plasma concentration of (Fasting glucose, Glycated hemoglobin and Vitamin D). We needed to get 3-5 mL of blood by venipuncture. At this point**,** the EDTA and heparin tubes have received the greatest attention among the anticoagulant tubes. The EDTA tube used for the HbA1c analysis, which is done on whole blood without centrifugation, while the heparin tube used for the analyzes of 25(OH) Vitamin D and fasting blood glucose parameters, which will be carried out on the serum. Thus, before performing this test, the blood is centrifuged at 3000 rpm for 5 minutes.

### 4.2.2 Measurement of glycemia

The glycemia is measured by the spectrophotometry (Mindray). The glucose in the samples is oxidized to gluconic acid and hydrogen peroxide by the enzyme glucose-oxidase (GOD). Thus produced H2O2 aggressively combines with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to form red-colored quino-neimine dye, which is colorimetrically quantified at 540 nm. The ferocity of the coloration is dependent on the amount of glucose in the samples.

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{glucose oxidase GOD}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase POD}} \text{red quinoneimine dye} + H_2O_2$$

### 4.2.3 Measurement of HbA1c

The HbA1c is measured by HumaMeter A1c device. Which allows an immediate and very precise determination of glycated hemoglobin and reported in (%). HbA1c is a better reflection of average blood glucose over the last three months. This test needs calibration and control steps. In order for the device to start working. First, we scan barcode to get started and carefully remove the cartouche's lid which gives rise to immediate use. Second, after insertion a cartouche, the device displays the message "Reagent Rehydration" at this moment we have to push the reagent ball into the cartouche by using the rounded end of a sample capillary. Third, by the sample capillary we just need 4 µl of blood taken from the fingertip using a single-use lancet or a venous whole blood sample that previously taken from an EDTA tube.

Finally, the sample inserted into the cartouche and closing of the analyzer cover. The total Hb and HbA1c content are determined after hemolyzed blood by the hemolyzing reagent. The time for measuring the HbA1c is 4-5 minutes and then the result displayed on the device's screen.

### 4.2.4 Measurement of 25(OH) Vitamin D

Vitamin D levels is measured with the PC-VIDAS (VITEK ImmunoDiagnostic Assay System). It is an automated enzyme-linked fluorescent Assay (ELFA). This assay is intended for quantitative of total 25-(OH) $D_3$ in human serum. There are a sequences steps to perform a 25-(OH) D test at the laboratory. All steps are fully automated from incubation to washing and final reading. First, by a micropipette we took 100 µl of serum sample and dispense into cartouche, and place the tip and the cartouche in the system, Second, launch the reaction by clicking on the start button using the connected PC at VIDAS. Total duration of assay is 27-30 minutes at room temperature.

Finally, after the start of the test, the results are calculated automatically by the instrument in relation to a stored calibration curve then displayed and reported in (ng/ml) on the PC's screen connected to VIDAS.

## 5.1 Statistical analysis

Analysis of the data was performed by using SAS (Statistical Analysis System-9), It's a way of expressing statistical correlations between variables. we considered the essentials of data analysis and then looked at how to present descriptive statistics in writing and also in the form of tables, histograms and graphs that would be appropriate. The test used in this study is NEWMAN and KEULS at a confidence interval P< 0.05.

## 5.2 Results

This is a cross-sectional descriptive study, titled " Association between vitamin D and HbA1C control in T2D". A total of 55 patients were examined. The findings and observations, as well as the final results, are listed below. Patients' ages varied from 40 to 84 years; the greatest number of instances were in the age range of between 50 and 70 years ($AR_2$), with 29 patients (52.7%), under 50 years ($AR_1$), 14 patients (25%) and over 70 years ($AR_3$), with 12 patients (21.8%) in each category.

### 5.2.1 HbA1c level

The findings of diabetes patients' glycated hemoglobin tests are depicted in (**Fig. 16**) based on two parameters age and gender that collected from (**Tab. 7**).

**Table 7** | The mean HbA1c levels based on factors age and gender

| | HbA1c % | | |
|---|---|---|---|
| | **$AR_1$** | **$AR_2$** | **$AR_3$** |
| M | 9.45±1.86 a | 7.80±1.37 b | 8.65±1.54 b |
| F | 7.65±1.76 b | 9.53±2.7 a | 7.63±0.67 b |

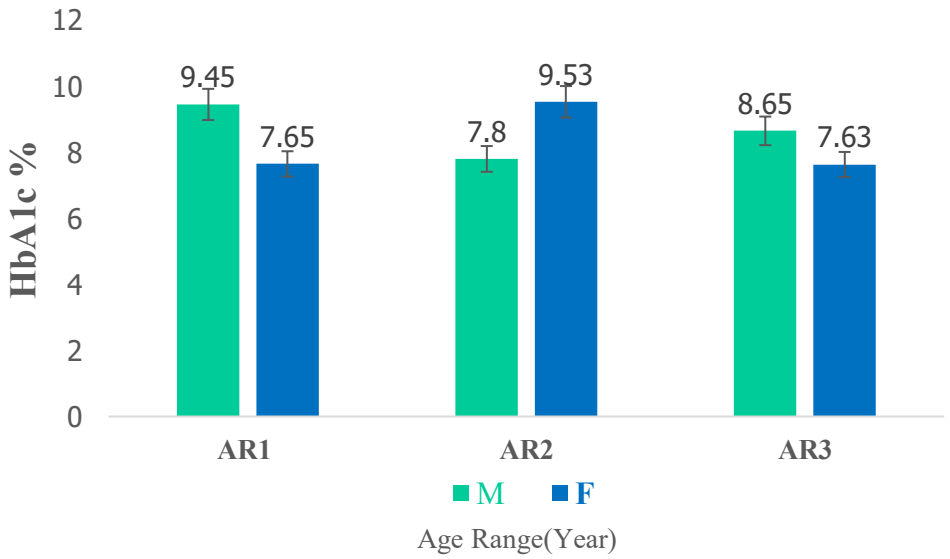



**Figure 16** | The mean HbA1c levels based on factors age and gender

Per the statistical study reported in **(Fig. 16)**, the results show that both of (M /F) in all ranges $AR_1$, $AR_2$, and $AR_3$ had HbA1c readings that are (9,45%; 7,80%; 8,65%) and (7.65 %; 9.53 %; 7.63) higher than the norm, respectively, whereas the norm recommended HbA1c level is 4-6%. We also contrasted genders and found $MAR_1$ and $MAR_3$ have considerably higher HbA1c rates than $FAR_1$ and $FAR_3$ (9.45%; 8,65 % VS 7.63%; 7,65%), respectively. while, $FAR_2$ have considerably higher HbA1c rates than $MAR_2$ (9.53 % VS 7,80%).

### 5.2.2 Glycaemia

The outcomes of our diabetes patients' glycaemia tests are depicted in (**Fig. 17**) based on two parameters age and gender that collected from (**Tab. 8**).

**Table 8** | Average glycemic values based on factors age and gender.

| | Glycemic | | |
|---|---|---|---|
| | **$AR_1$** | **$AR_2$** | **$AR_3$** |
| M | 2.07±0.52 a | 1.70±0.35 b | 1.96±0.87 a |
| F | 1.41±0.28 c | 2.06±0.92 a | 1.48±0.41 b |

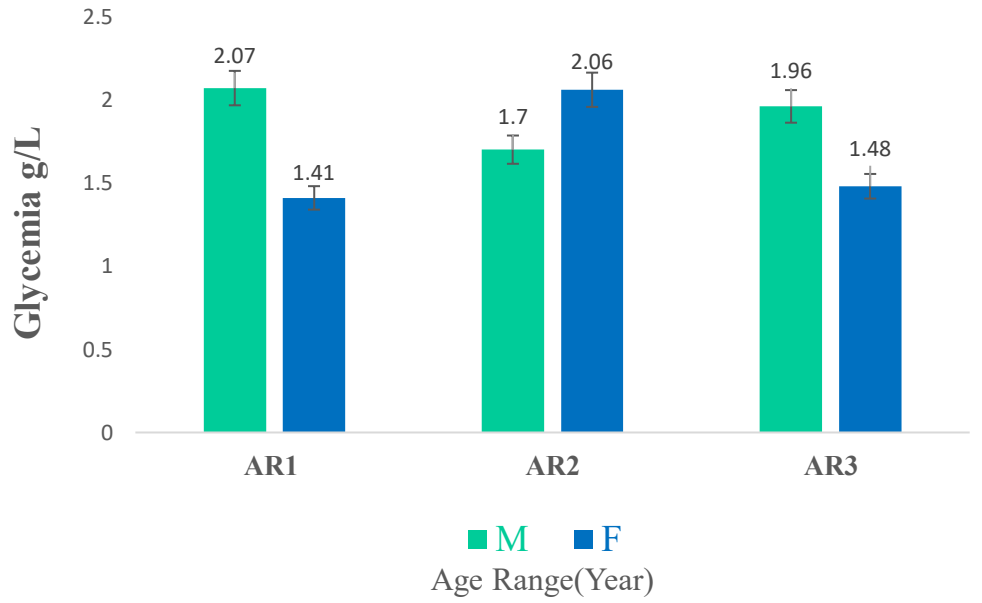



**Figure 17** | Average glycemic values based on factors age and gender.

From the statistical analysis in (**Fig. 17**), glycemia is increased in both sexes (M/F) in all groups $AR_1$, $AR_2$, and $AR_3$ (2,07g/l; 1,70 g/l; 1,96g/l and 1.41g/l; 2.06g/l; 1.48g/l) respectiv-ely. Whereas, normal glycemia is (<1,26g/l). We also analyzed genders and observed that $MAR_1$ and $MAR_3$ have significantly higher glycemic rates than $FAR_1$ and $FAR_3$ (2,07g/l; 1,96g/l VS 1.41g/l; 1.48g/l), respectively. while, $FAR_2$ have significantly higher glycemic levels than $MAR_2$ (2.06g1/l VS 1,70 g/l) respectively.

### 5.2.3 Vitamin D level

Vitamin D levels of our diabetic patients are illustrated in (**Fig. 18)** based on two parameters age and gender that collected from (**Tab. 9**).

**Table. 9** | The mean vitamin D levels based on factors age and gender.

| | 25-(OH)D level ng/ml | | |
|---|---|---|---|
| | **$AR_1$** | **$AR_2$** | **$AR_3$** |
| M | $12.76^{d} \pm 4.72$ | $16.41^{d} \pm 4.90$ | $20.35^{b} \pm 15.39$ |
| F | $17.30^{b} \pm 9.54$ | $14.93^{c} \pm 7.58$ | $23.86^{a} \pm 32.00$ |


**Figure 18 |** The mean vitamin D levels based on factors age and gender.

According to the statistical analysis in **(Fig. 18)**, the result shows the serum 25(OH) D concentration of (M/F) are deficient in $AR_1$ and $AR_2$ with an average of (12.76; 16.41ng/ml and 17.30; 14.93 ng/ml) respectively. while, insufficient is observed in $AR_3$ (20.35ng/ml and 23.86ng/ml) respectively. We also observed that $FAR_1$ and $FAR_3$ were higher than $MAR_1$ and $MAR_3$ (12.76; 20.35 ng/ml VS 17.30; 23.86 ng/ml) respectively. while, $MAR_2$ were higher than $FAR_2$ (16.41ng/ml vs 14.93 ng/dl), respectively.

The prevalence of hypovitaminosis D is high in T2D population, as evidenced by a study of 500 diabetic individuals, in which 81% have lower-than-normal vitamin D levels, with 57 % and 24 % suffering from deficient and insufficient, respectively (Hannah *et al.,* 2021).

## 5.3 General Discussion

T2D management is difficult for health care personnel, patients and their families. Current management guidelines emphasize on glycemic control to lessen the risk of long-term problems. The primary goal of our study was to determine whether there is a link between vitamin D levels and glycated hemoglobin in T2D patients (**Fig. 19**). In this study, we looked at vitamin D levels with T2D and compared it to HbA1c levels (**Tab. 10**).

**Table 10** | Association between vitamin D and T2D based on two factors age and gender

| | $AR_1$ | | | $AR_2$ | | | $AR_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Glycemic g/l** | **HbA1c %** | **VitD ng/ml** | **Glycemic g/l** | **HbA1c %** | **Vit D ng/ml** | **Glycemic g/l** | **HbA1c %** | **Vit D ng/ml** |
| **M** | 2.07 | 9.45 | 12.76 | 1.70 | 7.80 | 16.41 | 1.96 | 8.65 | 20.35 |
| **F** | 1.41 | 7.65 | 17.30 | 2.06 | 9.53 | 14.93 | 1.48 | 7.63 | 23.86 |

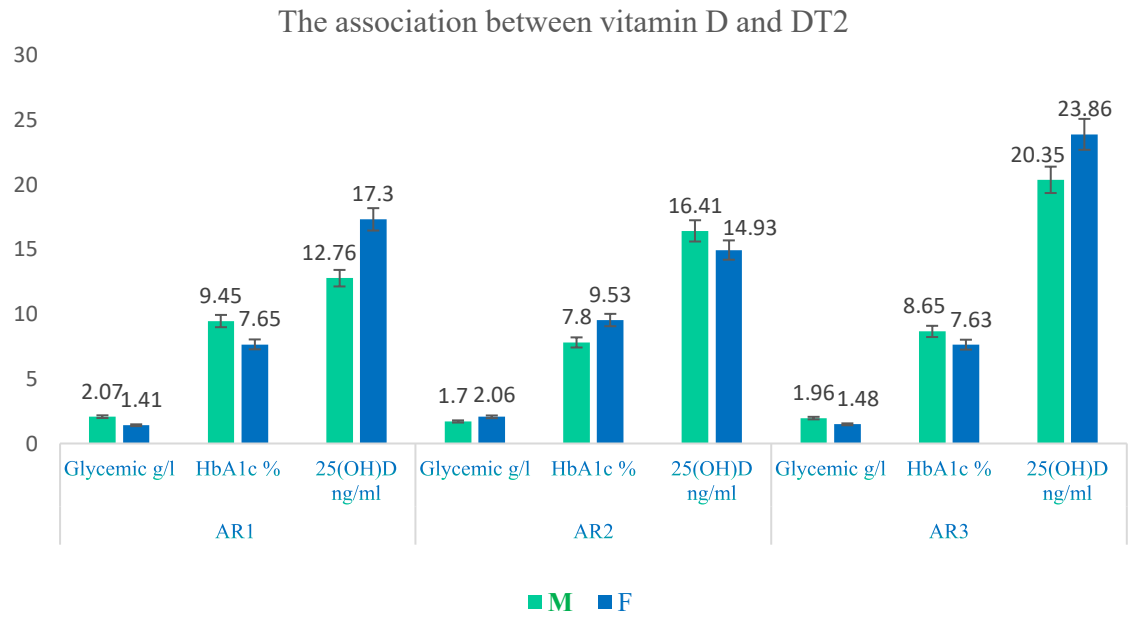



**Figure 19** | Association between vitamin D and DT2 based on two factors age and gender

As far as we know**,** this is not the first study to look into the three-way relationship between gender, vitamin D, and HbA1c. This research found three major results.

Firstly, males had lower vitamin D status than females, and both of (M/F) were lower than the normal value. The results showed that 72.7% of the 55 patients had a vitamin D level in the deficiency ranges ($AR_1$/ $AR_2$) and 27.3% had a vitamin D level in the insufficiency range ($AR_3$). A study by Anita et al., (2011), they indicated the average blood 25-(OH) $D_3$ concentration was significantly lower in diabetes males than diabetic females.

Second, lower levels of serum 25(OH)D3 were linked to glycosylated hemoglobin of T2D patients. In our simple correlation study found a strong association between serum 25(OH)D3 concentration and HBA1c levels in T2D patients. We observed the averages of vitamin D levels in each age ranges were 100 % lower than the normal value of 30 ng/ml, whereas, 72.7% was deficiency and 27.3% was insufficient in our patients. The mean vitamin D levels among three age ranges were 17.60 ng/ml. In compare**,** the averages of HbA1c levels in each age ranges were also 100% higher than the normal value of 4-6%, whereas, 100% of the 55 patients had a poor glycemic control. The mean HbA1c levels among three age ranges were 8.45%. We also discovered in three age ranges; our patients had a fasting glucose abnormality (100%). Thus, hyperglycemia and HbA1c results are in line with vitamin D levels, and the prevalence of hypovitaminosis D is significant in our study population.

A study by Karau et al. (2019) to assess the prevalence of hypovitaminosis D among T2DM patients at Kenyatta National Hospital in Nairobi, Kenya. They observed that 151 participants were recruited, with 69.5 % of them being females and a mean age of 58.2 years. The average HbA1c level was 8.46 %, with 62.9 % having poor glycemic control. In compare, Vitamin D deficiency and insufficiency were discovered in 38.4% and 21.9% of the subjects, respectively. They discovered a significant adverse relationship between vitamin D and glycemic control. They determined that there is a significant incidence of hypovitaminosis D in the community (Karau et al., 2019).

Third, based on the findings of this study, there was an inverse relationship between vitamin D and HbA1C. Low vitamin D levels have been linked to poor glycemic management. That is, in the event of insufficient Vitamin D levels, the study showed a high HbA1C, because vitamin D insufficiency is common in T2D, vitamin D may play a significant role in insulin resistance. The association between vitamin D insufficiency and T2D is supported by large evidence. This relationship is mediated by vitamin D's direct and indirect effects on insulin secretion, insulin resistance, and systemic inflammation. on the one hand**,** vitamin D can stimulate pancreatic B-cell for insulin synthesis by its genomic action. vitamin D also can influence insulin secretion via altering the opening and closure of calcium channels, as detailed in **(Fig. 14).** On the other hand, vitamin D stimulates the expression of insulin receptors, leading to stimulating insulin sensitivity in skeletal muscles and adipose tissues. Thus, supplementing with vitamin D can improve glycemic control and diabetes complications can be reduced by increasing glycemic control in T2D patients.

## 5.4 Conclusion

The following conclusions may be derived from the study's findings, vitamin D was found to be deficient and insufficient, while HbA1C levels were found to be greater than normal in all T2D patients. As a result, Vitamin D was found to be strongly associated to glycemic control. A study by Rolim *et al.,* (2016) investigated the status at hypovitaminosis D level and its correlation with glycemic control in patient with T2DM. They evaluated 108 patients with mean duration of diabetes of 14.34 ± 8.05 years and HbA1 of 9.2 ± 2.1%. The mean age was 58.29 ± 10.34 years. The prevalence of hypovitaminosis D was 62%. They concluded that the prevalence of hypovitaminosis D in Brazilians with T2DM was high, and there is a relation between hypovitaminosis and glycemic control in patient with T2DM.

Other studies in this regard support that vitamin D is required by the human body and it is obtained through foods, vitamin D supplements, and sunlight, and given the massive prevalence of vitamin D deficiency in T2D patients in Mostaganem particular, and Algeria as a whole. It is recommended to take special measures to compensate for this vitamin deficiency in diabetic patients. Because T2D is an awful disease, an appropriate diet is required for its management. Exercise improves blood glucose management and lowers the risk of complications such as hyperlipidemia, hypertension, and coronary heart disease in people with T2D. As for hypovitaminoses D, it may be prevented by getting enough sunlight and eating vitamin D rich foods and supplements. Clinicians must be aware of the reasons of diabetic consultations; the diagnosis of vitamin D status also should be considered. Furthermore, due to the high frequency of vitamin D insufficiency among diabetics, the monthly and quarterly vitamin D doses should be specified. This study was primarily and limited in two factors (age and gender), the restriction was the use of patients' blood sample. For these reasons, more research into the association between glycemic management and vitamin D may be more advantageous.

# Chapter Six: Reference List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