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Vitamin D3 Supplementation for patients with type 2 diabetes. Clinical study

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Dedication

I hope this message finds you all in good health and high spirits. Today, I want to take a moment to express my heartfelt gratitude and extend my deepest appreciation to each one of you.

You have been the unwavering pillar of support in my life, standing by me through thick and thin. Your love, encouragement, and presence have made all the difference, shaping me into the person I am today. I am truly blessed to have such an incredible group of individuals who have walked this journey with me.

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Amira

Abstract

Objective

Type 2 diabetes (T2D) is characterized by insulin resistance and elevated blood glucose levels. Recent studies suggest that vitamin D3 might influence glucose metabolism and insulin sensitivity. This study aimed to evaluate the impact of vitamin D3 supplementation on hemoglobin A1c (HbA1c) and glucose levels in patients with type 2 diabetes.

Materials and Methods

This case-control study was conducted at Dr. Benzerjeb Hospital in Ain Temouchent and included adult patients with type 2 diabetes from February 18, 2024, to June 6, 2024. Patients' HbA1c and glucose levels were measured before and after oral vitamin D3 supplementation, with dosage adjustments based on initial vitamin D levels.

Results

The results showed a significant reduction in HbA1c, decreasing from 8,74% ± 1.40 to 8,51% ± 1.02 in patients. Glucose levels also showed a slight decrease, from 1,64 ± 0.46 g/L to 1,63 ± 0.59 g/L

Conclusion

The study revealed a high prevalence of hypovitaminosis D in the studied population, highlighting a potential need for supplementation.

Keywords

Type 2 diabetes, Type 1 diabetes, Vitamin D3 supplementation, Prohormone, Fasting blood glucose, Glycated hemoglobin (HbA1c).

Résumé

Objectif

Le diabète de type 2 (T2D) se caractérise par une résistance à l'insuline et des niveaux élevés de glucose sanguin. Des études récentes suggèrent que la vitamin D3 pourrait influencer le métabolisme du glucose et la sensibilité à l'insuline. L'objectif de cette étude était d'évaluer l'impact de la supplémentation en vitamin D3 sur les niveaux d'hémoglobine A1c (HbA1c) et de glucose chez les patients diabétiques de type 2.

Matériel et méthodes

Cette recherche cas-témoin a été menée à l'Hôpital Dr. Benzerjeb à Ain Temouchent et a inclus des patients adultes atteints de diabète de type 2, du 18 février 2024 au 06 juin 2024. Les niveaux d'HbA1c et de glucose des patients ont été mesurés avant et après une supplémentation orale en Vitamin D3, avec des ajustements de dosage basés sur les niveaux initiaux de Vitamin D.

Résultats

Les résultats ont montré une réduction significative de l'HbA1c, passant de 8,74% ± 1.40 à

8,51% ± 1.02 chez les patients. Les niveaux de glucose ont également montré une légère diminution, passant de 1,64 ± 0.46 g/L à 1,63 ± 0.59 g/L.

Conclusion

L'étude a révélé une prévalence élevée d'hypovitaminose D dans la population étudiée, soulignant un besoin potentiel de supplémentation.

Mots-clés

Diabète de type 2, Diabète de type 1, Supplémentation en Vitamin D3, Prohormone, Glucose sanguin à jeun, Hémoglobine glyquée (HbA1c).

الملخص

الهدف

يتميز مرض السكري من النوع 2 (T2D) بمقاومة الأنسولين وارتفاع مستويات الجلوكوز في الدم. تشير الدراسات الحديثة إلى أن فيتامين D3 قد يؤثر على استقلاب الجلوكوز وحساسية الأنسولين. هدفت هذه الدراسة إلى تقييم تأثير مكملات فيتامين D3 على مستويات الهيموغلوبين A1c (HbA1c) والجلوكوز لدى مرضى السكري من النوع 2.

المواد والأساليب

أجريت هذه الدراسة الحالة والشواهد في مستشفى الدكتور بن زرجب في عين تموشنت وشملت مرضى بالغين مصابين بداء السكري من النوع 2 في الفترة من 18 فبراير 2024 إلى 6 يونيو 2024. تم قياس مستويات HbA1c والجلوكوز لدى المرضى قبل وبعد مكملات فيتامين D3 عن طريق الفم، مع تعديل الجرعات بناءً على مستويات فيتامين D الأولية.

النتائج

أظهرت النتائج انخفاضًا ملحوظًا فيHbA1c ، حيث انخفضت % 8.74 ± 1.40 إلى % 8.51 ± 1.02 لدى المرضى. كما أظهرت مستويات الجلوكوز انخفاضًا طفيفًا، حيث انخفضت من 1.64±0.59 جم/ل إلى 1.63 ± 0.64 جم/ل.

الخلاصة

كشفت الدراسة عن انتشار مرتفع لنقص فيتامين D في السكان المدروسين، مما يبرز الحاجة المحتملة للمكملات.

الكلمات المفتاحية

داء السكري من النوع 2، داء السكري من النوع 1، مكملات فيتامين D3، الهرمون الأولي، جلوكوز الدم الصائم، الهيموغلوبين الجليكوزيلاتي (HbA1c).

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List of abbreviations

ADP: Adenosine di-phosphate

APCI: Atmospheric Pressure Chemical Ionization

ATP: Adenosine Triphosphate

BPD: Blood Pressure Diastolic

BPS: Blood Pressure Systolic

CDC: Centers for Disease Control and Prevention

CHOD-PAP: Cholesterol Oxidase-Phenol Aminophenazone

DBP: Diastolic Blood Pressure

ESI: Electrospray Ionization

GIP: Glucose-dependent Insulinotropic Polypeptide

GK: Glycerol Kinase

GLP-1: Glucagon-like Peptide-1

GPO: Glycerophosphate Oxidase

HBA1c: Hemoglobin A1c

HD: Heart Disease

IDF: International Diabetes Federation

IU: International Unit

LC: Liquid Chromatograph

LLE: Liquid-Liquid Extraction

mcg: Micrograms

MRM: Multiple Reaction Monitoring

MS: Mass Spectrometry

NAFLD: Non-Alcoholic Fatty Liver Disease

NIDDK: National Institute of Diabetes and Digestive and Kidney Diseases

NIDDM: Non-Insulin-Dependent Diabetic Mellitus

OAD: Oral Antidiabetics

PP: Protein Precipitation

PRRs: Pathogen-Recognition Receptors

RDA: The Recommended Dietary Allowance

SBP: Systolic Blood Pressure

SD: Standard Deviation

SPE: Solid-Phase Extraction

T1D: Type 1 Diabetes

T2D: Type 2 Diabetes

TLRs: Toll-Like Receptors

TNF-α: Tumor Necrosis Factor-alpha

UHPLC: Ultra-High Performance Liquid Chromatography

UVA: Ultraviolet A

UVB: Ultraviolet B

VDRs: Vitamin D Receptors

Vit D3: Vitamin D3

WHO: World Health Organization

µg: Microgram

µL: Microliter

25(OH)D: 25-hydroxy cholecalciferol

Introduction

Type 2 diabetes (T2D) is a concerning disease experiencing a constant increase in prevalence worldwide. In 2021, over 537 million people were affected by diabetes, approximately 1 in 10 individuals, with 61 million cases reported in Europe according to the 2021 Atlas of the International Diabetes Federation **(Sun *et al*., 2022)** A study conducted in Algeria by the Ministry of Health and the WHO between 2016 and 2017 revealed a prevalence of 14.4% among Algerians aged 18 to 69 **(Belhadj *et al*., 2019)**. In the Sidi Bel Abbès region in the west of the country, **(Chami *et al*., 2015)** we estimated the prevalence of T2D at 26.7% among individuals aged 65 and older. This underscores the magnitude of the public health issue posed by this chronic disease characterized by chronic hyperglycemia due to insulin resistance and insufficient insulin production. Cardiovascular, neurological, and renal complications associated with T2D can significantly impact patients' quality of life **(American Diabetes Association, 2020)**.

Over the years, studies have highlighted the potential beneficial effects of vitamin D in T2D. Vitamin D plays a crucial role in regulating glucose and insulin metabolism **(Bouillon *et al*., 2019)**, However, the results of studies on the efficacy of vitamin D and chromium supplementation in T2D have been mixed. Some studies have reported significant beneficial effects **(Dipasquale, Presti, *et al*., 2022; D.-D. Li *et al*., 2022; Talaei *et al*., 2013)** Others have observed no or minimal effects **(Nezhad *et al*., 2013; Sadiya *et al*., 2014).**

In this study, our focus revolves around one objective, exploring the impact of vitamin D supplementation on patients with type 2 diabetes, specifically assessing changes in glycemic parameters such as fasting blood glucose and glycated hemoglobin before and after supplementation. Additionally, we evaluate the level of vitamin D25OH in this population.

This research was conducted at Dr. Benzerjeb Hospital in Ain-Temouchent from February 18, 2024, to June 06, 2024. Our study is structured into four comprehensive chapters: literature synthesis, materials and methods, results, and discussion. Through this meticulous investigation, we aim to provide valuable insights into the role of vitamin D in managing diabetes. We hope that our findings will contribute to informing future therapeutic strategies and ultimately improving patient outcomes.

Chapter I: LITERATURE REVIEW

I. Diabetes

I.1. Definition

Diabetes is a long-term illness (chronic condition) that occurs when the pancreas does not produce enough insulin or the body has problems with using the insulin (struggles to use it effectively) **(*Who: World Health Organization*, 2022).**

The pancreas also releases insulin, a hormone that works to enable glucose to enter cells, which can then be used for energy. **(*NIDDK: National Institute of Diabetes and Digestive and Kidney Diseases*, 2023)**, Insulin deficiency occurs when the body fails to generate or utilize insulin efficiently, and glucose remains in your bloodstream and does not reach your cells (Figure 1), This can lead to such catastrophic conditions as heart stroke, vision impairment, and renal disease **(*NIDDK*, 2023).**

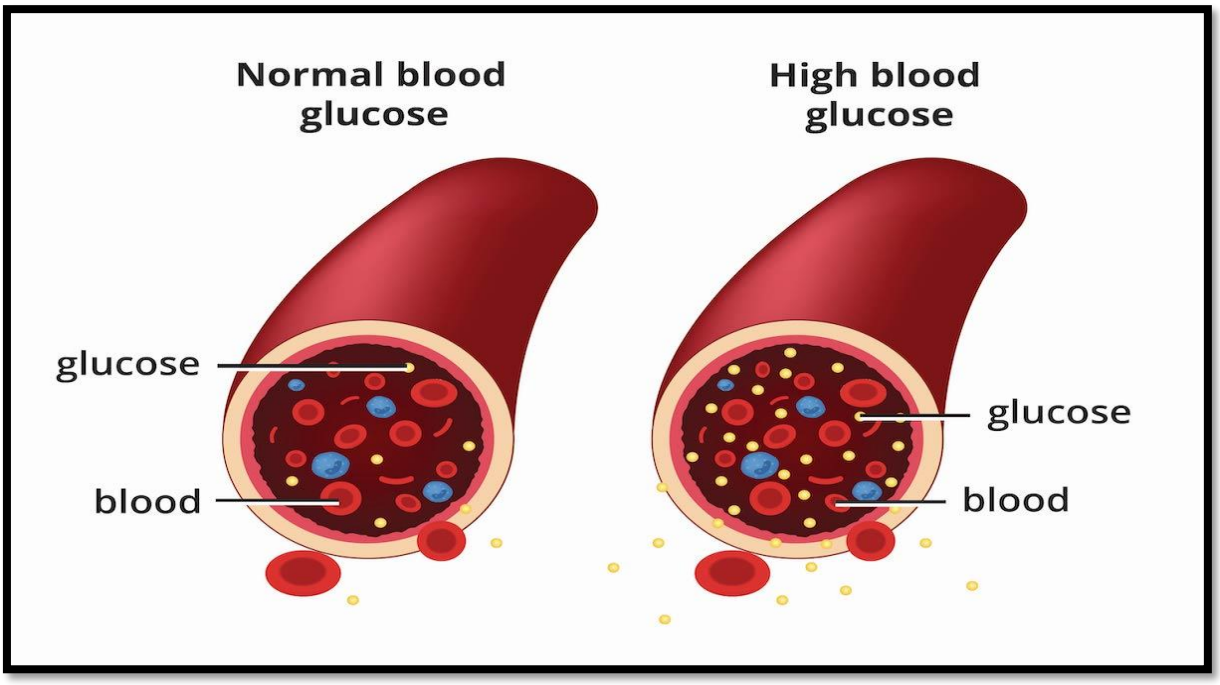

Figure 1: Comparing Normal and High Blood Glucose Levels: A Visual Representation

(*NIDDK*, 2023)

There are many types of diabetes, but there are three major forms of diabetes

- Type 1 diabetes is classified as an auto-immune disease where the body destroys the insulin-producing cells within the pancreas (Beta cells) And causes a shortage of insulin. **(CDC: Centers for Disease Control and Prevention and Prevention, 2023)**.
- Second gestational diabetes is considered a form of diabetes that can occur only in pregnant women and usually disappears after delivery. It occurs in women who were previously non-diabetic but experience a condition called gestational diabetes where their blood sugar levels are very high during pregnancy. Women suffering from this disease have higher chances of experiencing pregnancy complications and complications during childbirth, and may further develop type 2 diabetes. **(American Diabetes Association, 2020).**
- The most common type of diabetes is type 2 diabetes (T2D). T2D is a chronic metabolic disease involving persistent hyperglycemia due to insulin resistance and variable impairment of pancreatic β cell function (Figure 2). Type 2 diabetes accounts for the majority of the cases globally pegged at 90-95%. T2D commonly occurs in people with high body mass index levels and low physical activity levels. Also formerly called non-insulin-dependent diabetes mellitus (NIDDM), it requires the use of pills in the first instance and not injections as in the case of type 1 diabetes. **(American Diabetes Association, 2019).**

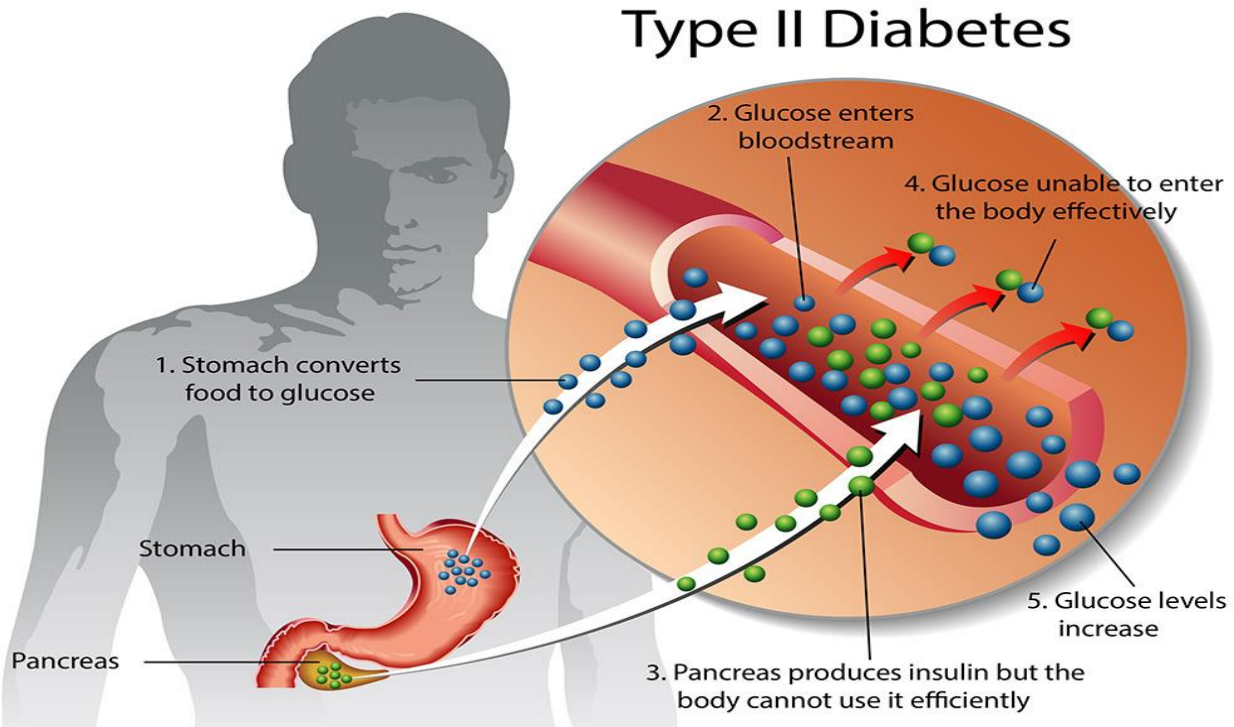

Figure 2: Type 2 diabetes **(Australia, 2024).**

I.2. Epidemiology and global burden of diabetes type 2

Notably, type 2 diabetes is an epidemic all over the world, and it has affected many patients. T2D has remained a prevalent factor, particularly in the last few decades, owing to causes including changes in lifestyle and an increase in the average age data from the International Diabetes Federal indicated that in the year 2019, there were about 463 million adults aged 20-79 years, suffering from diabetes. Yet, the number of cases is predicted to increase to 700 million in 2045 (Figure 3), if enough preventive steps are not taken **(Saeedi *et al*., 2019).**

The regional studies in Algeria confirmed similar results to the national research on diabetes prevalence and distribution. In the 2007 Tlemcen study, 14.2% of individuals in western Algeria had type 1 or type 2 diabetes, with type 2 diabetes at 10.5% and type 1 diabetes at 3.7%. Diabetes was more prevalent in urban areas (15.3%) compared to rural regions (12.9%) and among men (20.4%) compared to women (10.7%). The 2001 Setif study reported a type 2 diabetes prevalence of 8.2%, increasing with age, with no significant difference based on gender or urban/rural distribution in eastern Algeria (7.3% urban, 9.7% rural); half of the diabetes cases in this study were previously undiagnosed **(Lamri *et al*., 2014).**

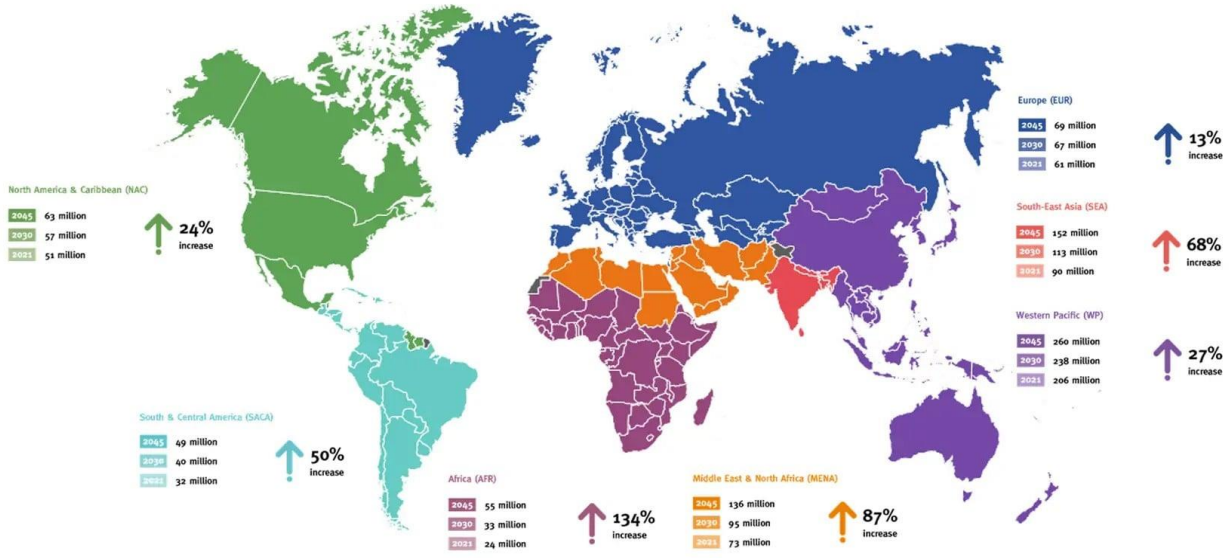

Figure 3: world map of Diabetes around the world. **(Magliano *et al*., 2021)**

I.3. Etiology of type 2 diabetes

There are numerous genetic variations, which have diverse extents of impact on type 2 diabetes (T2D) which is a complex disease. The ultimate planning of treatment, evaluating the risk and early intervention for type 2 diabetes all require certain knowledge of the genetics of the disease **(Fuchsberger *et al*., 2016)**. T2D is also caused by factors coming from the environment such as obesity and the sedentary nature of work and life. Obesity is associated with high insulin resistance as well as Type 2 Diabetes while a sedentary lifestyle which involves times of energetic sparing activity increases the chances of T2D; lack of regular exercise decreases insulin sensitivity and glucose metabolism efficiency. People who are prone to poor diets, foods in cans and bags, sugary beverages, and meals that are calorie-laden are highly likely to suffer from type 2 diabetes. These aspects thus pose significant and crucial challenges to the formulation of strategies for the prevention and treatment of this disorder **(McCarthy, 2010)**.

A pregnant woman with gestational diabetes is more likely to develop Type 2 Diabetes in the future. The symptoms of gestational diabetes are, that the lady suffers from high blood glucose when she is pregnant. The children born of mothers with gestational diabetes mellitus are also more vulnerable. Other factors that explain the prevalence of T2D comprise an individual's socioeconomic features, including income, education, and healthcare. This may affect that people with lower SES experience restricted access to healthy foods, health care, and PA opportunities. **(Hu, 2011).** Stress, trauma, or the like all influence insulin response and glucose tolerance, which in effect enhances the risk of T2D **(Tamayo *et al.,* 2010).** Other aspects of sleep comprising low quality, duration, and sleep disorders like sleep apnea may also cause T2D. Nevertheless, the above-discussed environmental changes are modifiable by adopting behavioral interventions like exercising, obtaining a balanced diet, controlling stress, and ensuring sleep **(Dutil & Chaput, 2017).**

I.4. Pathophysiology of type 2 diabetes

I.4.1. Insulin resistance

Insulin resistance is a key feature of type 2 diabetes (T2D) Closely connected with the pathophysiology of T2D, insulin resistance is commonly accompanied by other metabolic disorders: obesity, dyslipidemia, and hypertension. It is a complex disease that has some relation with both heredity and the environment **(Galicia-Garcia *et al*., 2020).**

Several mechanisms contribute to the development of insulin resistance:

Adipose Tissue Dysfunction: In obesity, particularly visceral adiposity (fat stored around the abdominal organs), adipose tissue releases pro-inflammatory substances called adipokines. These substances interfere with insulin signaling in the body, leading to insulin resistance. Adipose tissue dysfunction is also associated with increased free fatty acid release **(Richard *et al*., 2000)**, which further impairs insulin action: Genetic Factors, Excessive Caloric Intake and Physical Inactivity

Insulin resistance can lead to compensatory hyperinsulinemia, where the pancreas produces and releases more insulin to overcome the reduced sensitivity of cells. Over time, the pancreatic beta cells may become exhausted and unable to produce enough insulin to maintain normal blood glucose levels, resulting in the development of T2D **(Samuel & Shulman, 2012).**

I.4.2. Beta-cell dysfunction

Beta cells are particular cells in the pancreas that generate and secrete insulin. Beta-cell dysfunction refers to impaired beta-cell function, which can contribute to the development and progression of type 2 diabetes (T2D). In T2D, beta-cell dysfunction is characterized by reduced insulin secretion and impaired regulation of blood glucose levels **(Galicia-Garcia *et al*., 2020).**

I.4.3. Incretin dysfunction

Incretins are hormones released by the digestive system in response to food intake. They play a critical role in regulating blood glucose levels by stimulating insulin secretion and suppressing glucagon release. Incretin dysfunction refers to abnormalities in the secretion or action of incretin hormones, particularly: Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) **(Michałowska *et al*., 2021).**

I.5. Risk factors that caused diabetes type 2

I.5.1. Obesity

is a health issue caused by excessive body fat accumulation, leading to chronic diseases like type 2 diabetes. The imbalance between energy intake and expenditure, unhealthy diets, and sedentary lifestyles influence it. Genetics, environmental, and socioeconomic factors contribute to obesity. Managing it involves lifestyle modifications, behavioral changes, and medical interventions, including portion control and physical activity **(Panuganti *et al*., 2024).**

I.5.2. Sedentary lifestyle

Sedentary behavior can lead to alterations in body composition, including increased body fat percentage, decreased muscle mass, and bone mineral density, all of which are linked to adverse health outcomes in individuals with Type 2 diabetes (D.-D. Li et al., 2022)**,** A sedentary lifestyle is identified as one of the major risk factors for Type 2 diabetes, significantly increasing the likelihood of developing the condition **(Yuan *et al*., 2023),** Prolonged sitting and lack of exercise are key factors leading to obesity and insulin resistance, which are precursors to Type 2 diabetes (D.-D. Li et al., 2022), behavior is associated with a greater relative risk for Type 2 diabetes and metabolic syndrome, emphasizing the importance of reducing sedentary time to mitigate these risks **(Hamilton *et al*., 2014).**

I.5.3. Family history

A family history of type 2 diabetes (T2D) increases an individual's risk of developing the condition. While it doesn't guarantee the condition, it is a significant risk factor. Genetic factors, such as certain variants, can influence an individual's risk. Family history also includes shared lifestyle and environmental factors, such as dietary habits, physical activity levels, and socioeconomic status. These factors can contribute to the development of T2D, as families may have similar eating patterns, activity levels, and exposure to obesogenic environments **(InterAct Consortium *et al*., 2013).**

The CDC lists a family history of diabetes as a known risk factor for T2D, along with age, weight, physical activity level, and gestational diabetes**(CDC, 2022).** The American Diabetes Association also recognizes a family history of diabetes as a risk factor for T2D.

I.5.4. Ethnicity

Ethnicity can significantly impact the risk of developing type 2 diabetes (T2D). Ethnic groups with higher prevalence rates, such as African, Hispanic/Latino, Native American, Asian American, and Pacific Islander, have a higher risk. Genetic and environmental factors, as well as obesity rates and insulin resistance, also contribute to the increased risk. Cultural factors, such as dietary preferences and traditional lifestyle practices, can also influence the risk. Healthcare providers should consider ethnicity when assessing an individual's risk for T2D and tailor prevention and management strategies to individual needs **(Chan *et al*., 2009).**

I.5.5. Age

Age is a significant risk factor for type 2 diabetes, with prevalence increasing with advancing age. Factors include insulin resistance, lifestyle changes, cumulative exposures, and coexisting health conditions(E. S. Huang et al., 2014). Regular health screenings and lifestyle modifications are crucial for early detection and management **(American Diabetes Association, 2019).**

I.6. Type 2 diabetes's effects

Diabetes significantly affects various organs and cells in the body (Figure 4), leading to a wide range of complications. the primary mechanisms by which diabetes causes damage if a person does not control the condition **(Dresden, 2023)**.

- **Brain**

Cognitive Impairment: Diabetes, especially in older persons, has been associated with cognitive deterioration and an increased risk of dementia **(Dresden, 2023)**.

Neurodegeneration: Disorders like Alzheimer's disease can result from neurodegeneration, which is exacerbated by high blood sugar levels **(Dresden, 2023)**.

- **Eyes**

Diabetic Retinopathy: Elevated blood sugar can harm retinal blood vessels, resulting in impaired vision and possibly blindness.

Cataracts: Diabetes raises the chance of cataract development, which can result in vision loss **(Dresden, 2023)**.

- **Kidneys**

Diabetic Nephropathy: If unchecked, high blood sugar levels can harm the kidneys and cause renal failure.

Kidney Damage: Diabetes increases the risk of renal disease and failure by causing damage to the kidneys **(Dresden, 2023)**.

- **Liver**

Non-Alcoholic Fatty Liver Disease (NAFLD): Diabetes raises the chance of developing NAFLD, which can cause scarring and damage to the liver.

Liver Damage: Cirrhosis and liver fibrosis are two disorders that can result from high blood sugar levels damaging the liver **(Dresden, 2023)**.

- **Glands**

Pancreas: Damage to the insulin-producing cells of the pancreas results in decreased insulin release and synthesis, which is a hallmark of diabetes.

Thyroid: Diabetes has been associated with a higher chance of thyroid conditions, including hyperthyroidism and hypothyroidism **(Dresden, 2023)**.

- **Heart**

Diabetes raises the risk of peripheral artery disease, heart attacks, and strokes, among other cardiovascular conditions **(Dresden, 2023)**.

- **Nerves**

Damage to the nerves brought on by high blood sugar levels can result in tingling, numbness, or pain in the hands and feet **(Dresden, 2023)**.

- **Skin**

Diabetes can lead to several skin issues, including dry skin, sluggish wound healing, and a higher risk of infection **(Dresden, 2023)**.

- **Cellular mechanism**

Inflammation: Chronic inflammation is linked to diabetes and may have a role in the emergence of problems in different organs and tissues. **(Dresden, 2023)**.

Oxidative Stress: Excessive blood sugar can result in oxidative stress, which can harm tissues and cells. **(Dresden, 2023)**.

- **Prevention and Management**

Blood Sugar Control: Preventing and managing diabetes complications requires maintaining appropriate blood sugar control through medication and lifestyle modifications. Regular Check-Ups: Monitoring and resolving any issues early on depends on routine medical check-ups and screenings **(Dresden, 2023)**.

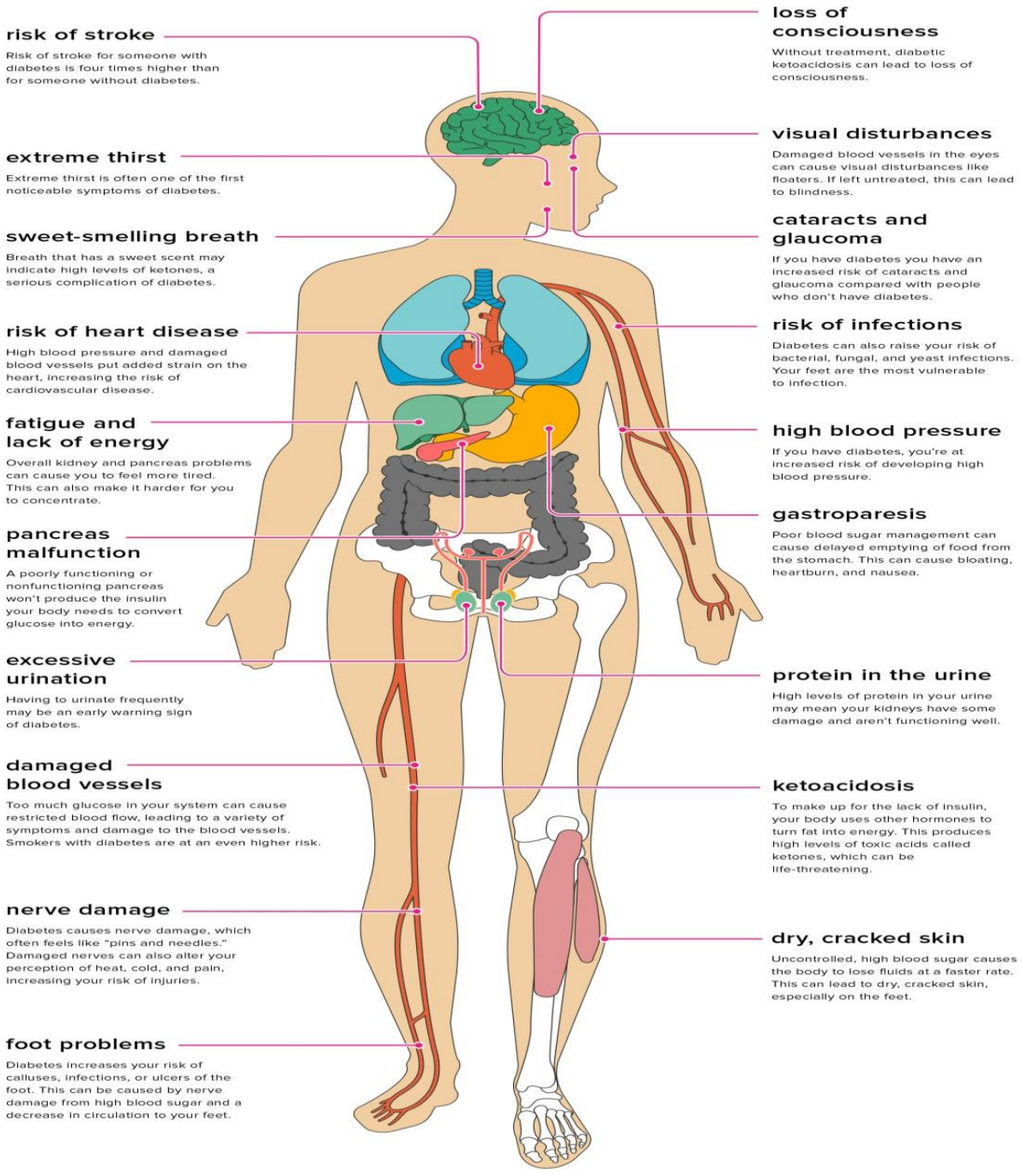

Figure 4: diabetes's long-term effects **(Pietrangelo, 2023)**

II. Vitamin D

II.1.Definition

In any individual, vitamin D also known as a group of fat-soluble secosteroids is significant in the nutritional worth considering its roles in enhancing the body's intake of calcium for structural development of bones, proper coordination of the nervous system, and bolstering the immune system. This is a type of vitamin that can be received through the exposure to Sun, in foods, or in the form of supplements **(Holick *et al*., 1987)**. Vitamin D is usually available in two forms. The first is vitamin D2 also called ergocalciferol other is vitamin D3 also called cholecalciferol, They have different molecular structures (Figure 5). However, Vitamin D3 is considered to be more effective at raising the levels of vitamin D in the blood compared to Vitamin D2 **(Jillian, 2021) .**This is because Vitamin D3 is the form of vitamin D that is naturally produced in the body, and it has a higher bioavailability **(Tsiaras & Weinstock, 2011).**

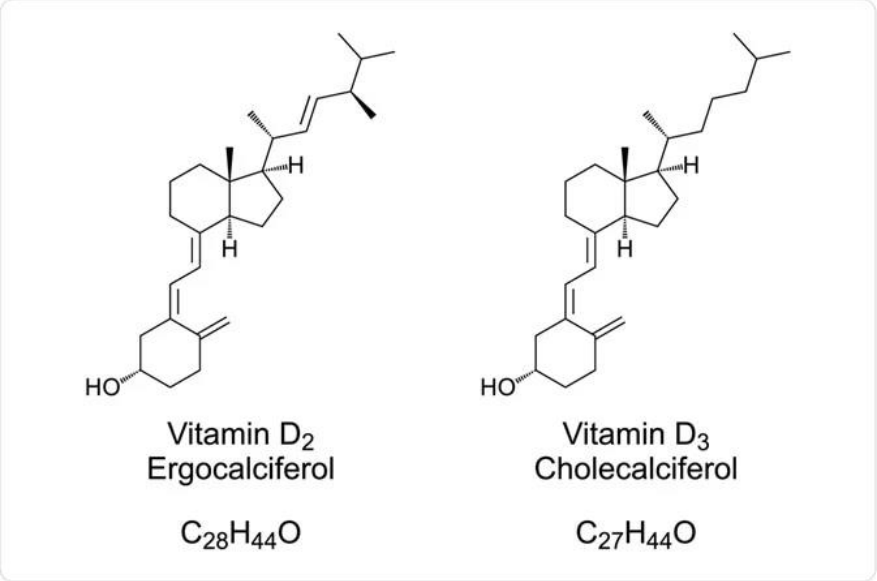

Figure 5: Chemical structure of vitamin D2 and vitamin D3. **(Atli, 2018)**

The primary distinction lies in the sources of vitamin D: D2 is derived from plants, while D3 is sourced from animals, including humans **(Yvelette, 2024).**

Vitamin D is the collective name for cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), which are precursors of hormones with an important role in the regulation of the metabolism of calcium and phosphates **(Demer *et al*., 2018).**

❖ **The recommended dietary allowance for vitamin**

The Recommended Dietary Allowance (RDA) for vitamin D varies based on age, health status, and other factors (Table I). Here's a summary of the RDA and factors affecting vitamin D needs Average daily recommended amounts are listed below in micrograms (mcg) and International Units (IU) **(Office of Dietary Supplements - Vitamin D, 2024).**

Table I: Recommended Dietary Allowance Across Various Age Stages **(Office of Dietary Supplements - Vitamin D, 2024).**

Life Stage	Recommended Amount
Birth to 12 months	400 IU (10 mcg)
Children 1-13 years	600IU (15 mcg)
Teens 14-18 years	600IU (15 mcg)
Adults 19-70 years	600IU (15 mcg)
Adults 71 years and older	800IU (20 mcg)

II.2.Factors Influencing Vitamin D Requirements

- Age: The skin's ability to produce vitamin D decreases with age, necessitating higher doses for older adults **(MacLaughlin & Holick, 1985)**
- Sex: Women may have a higher risk of deficiency, potentially due to hormonal differences and other physiological factors **(Hars *et al*., 2020).**
- Health Status: Conditions like osteoporosis and multiple sclerosis may increase vitamin D needs **(Ware *et al*., 2019).**
- Sun Exposure: Geographic location, time spent outdoors, and skin exposure to sunlight affect vitamin D synthesis **(Tsiaras & Weinstock, 2011)**

- Diet: Dietary sources include fatty fish, liver oils, and fortified foods, but it may be difficult to meet RDA through diet alone **(Tsiaras & Weinstock, 2011)**

II.3.Production in the skin

Cholecalciferol, the third form of vitamin D, is formed in the skin from a compound, 7-dehydrocholesterol. This synthesis occurs in the presence of the sunlight's ultraviolet B (UVB) light that ranges from 290-315 nm. The skin is relatively rich in 7-dehydrocholesterol and on exposure to UVB light, the substance directly turns into Vitamin D3. This process does not involve enzymes and is directly proportional to the time an individual has been exposed to UVB radiation **(Wacker & Holick, 2013).**

For instance, the amount of Vitamin D3 synthesized depends on the extent of skin exposure to the bright UVB rays as well as the skin color. As an end product of the melanocyte, Tan stimulates the natural sun protection mechanism that minimizes the production of Vitamin D3 in response to absorbed UVB rays. Although this protective mechanism is deemed to be favorable, the outcomes could be relatively severe in that people with skin of color have lower circulating levels of Vitamin D3 **(Wacker & Holick, 2013).**

Additionally, other extrinsic agents such as clothing and sunscreen can also inhibit the penetration of UVB radiation, which in turn, restricts the skin's capacity to synthesize Vit D3. Access also has its influence; people living in regions close to the geographical equator may experience an even tougher time in catalyzing their bodies to produce enough Vitamin UVB has less power than UVA and is normally responsible for producing the burn in D3 due to its weak strength in the winter time **(SunSmart, 2024).**

II.4.Transport to the liver

Once Vitamin D3 is produced in the skin or ingested through the diet, it enters the bloodstream and is transported to the liver. In the liver, Vitamin D3 undergoes a crucial transformation into the prohormone calcifediol, also known as 25-hydroxycholecalciferol or 25(OH)D **(Wang *et al*., 2022).**

This conversion is facilitated by the enzyme vitamin D 25-hydroxylase (Figure 6), which is encoded by the CYP2R1 (Figure 7) gene and expressed in hepatocytes, and the liver cells.

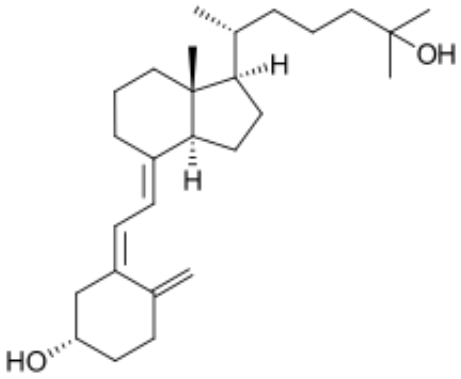

Figure 6: 25-hydroxycholecalciferol or 25(OH)D or Calcidiol

(PubChem, 2023)

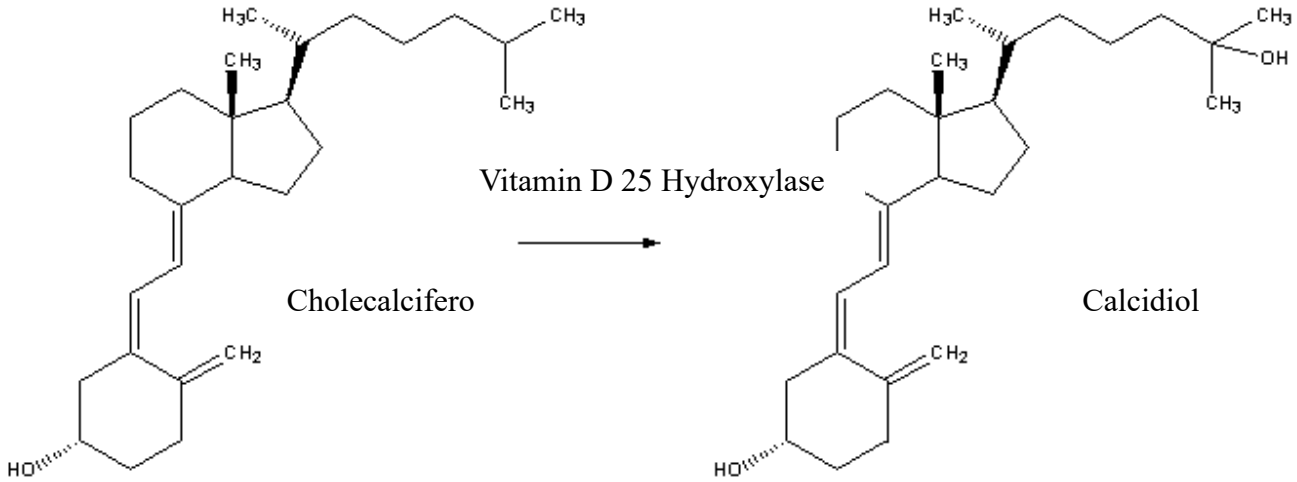

Figure 7: Enzymatic Conversion of Cholecalciferol to Calcidiol by Vitamin D 25-Hydroxylase

(Robert.Allen, 2010)

The newly formed calcifediol is then released back into the bloodstream, where it binds to a specific α-globulin carrier protein called the vitamin D-binding protein. This binding is essential for the transport of calcifediol throughout the body, ensuring that it reaches its target tissues, including the kidneys, where further activation occurs **(Zhu *et al*., 2013).**

II.5.Activation in the kidneys

The second step in Vitamin D synthesis takes place in the kidneys. In the last step of this process, there is another hydroxylation of calcifediol that forms what is chemically known as calcitriol or 1,25-dihydroxycholecalciferol or 1,25(OH)2D (Figure 8). This compound is considered the active form of Vitamin D which triggers several responses in the biological systems of the body **(Charoenngam & Holick, 2020).**

The kidneys help in the conversion of calcifediol through the enzyme known as 1-α - hydroxylase. This enzyme activity is well regulated by factors such as levels of parathyroid hormone, serum calcium concentration, phosphate levels, and calcitriol. Calcitriol synthesis is central; this

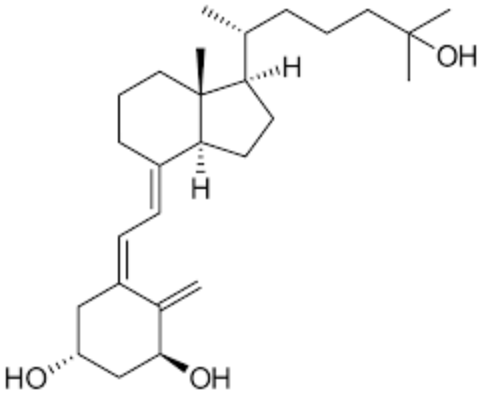

Figure 8: The chemical structure active form of vitamin D **(PubChem, 2023).**

represents the final step after Vitamin D activation to allow it to perform its roles effectively **(Negrea, 2019).**

II.6.Clinical applications

Calcitriol, the active form of Vitamin D, is a potent regulator of several key biological processes. It plays a vital role in the immune system, helping to modulate both innate and adaptive immune responses. Calcitriol also maintains calcium balance and supports bone health by facilitating the

absorption of calcium in the small intestines, promoting the reabsorption of calcium in the kidneys, and regulating the release of calcium and phosphorus from the bones. **(Panichi *et al.*, 2003)**

Moreover, calcitriol contributes to the maturation of osteoblasts, the cells responsible for bone formation. This function is particularly important for the development and maintenance of a strong skeletal structure **(Palmer *et al.*, 2006).**

In the bloodstream, Vitamin D metabolites are predominantly bound to proteins, such as the vitamin D-binding protein and albumin. Only a small fraction circulates as free calcitriol. The liver is the primary site for the production of these binding proteins. However, in certain diseases affecting the liver, intestines, or kidneys, the levels of these proteins may decrease (Figure 9), leading to reduced total levels of Vitamin D in the body **(Palmer *et al.*, 2006).**

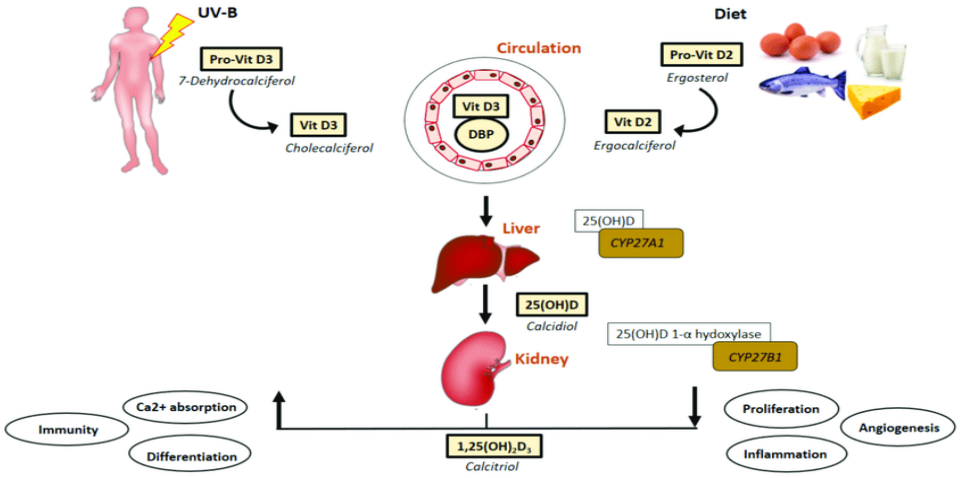

Figure 9: Schematic illustration of vitamin D synthesis pathway and signaling mechanisms **(Anghel *et al.*, 2023)**

The metabolism of Vitamin D, from its initial synthesis in the skin to its final activation in the kidneys, underscores its importance in human health. The active form, calcitriol, is essential for numerous physiological functions, including maintaining calcium balance, ensuring bone health, and supporting the immune system **(Lu *et al.*, 2018).**

II.7.The effect of vitamin D3 on diabetics

II.7.1. The effect of vitamin D on the immune systems

Many studies reported that low vitamin D levels are associated with an increased risk of infections and autoimmune diseases due to molecular mimicry. Calcitriol is a pluripotent regulator of the innate immune system. The bacterial infection triggers the activation of toll-like receptors(TLRs) that regulate VDRs expression and 25 (OH) D-1α-hydroxylase activities. TLRs are a class of non-catalytic transmembrane pathogen-recognition receptors (PRRs) that interact with specific pathogen-associated molecular patterns (PAMPs) **(Sîrbe *et al.*, 2022)** (Figure 10).

- **Vitamin D's immune system mechanisms**

Autocrine Regulation: Vitamin D modulates the function and responsiveness of immune cells, including dendritic cells, monocytes, macrophages, T cells, and B cells, locally.**Immune Cell Function Modification:** Vitamin D affects the expression of genes that are involved in the activation, proliferation, and differentiation of immune cells, which results in modifications to the immunological response **(Ao *et al.*, 2021).**

Inhibition of Pro-Inflammatory Cytokines: Vitamin D inhibits the synthesis of pro-inflammatory cytokines, which are implicated in the development of autoimmune disorders, including TNF-α and IL-1β **(Ao *et al.*, 2021).**

- **The cells involved in vitamin D-immune system interactions**

Dendritic Cells: Dendritic cells are essential for the presentation of antigens to T cells and the start of immunological responses. Dendritic cells' capacity to activate T lymphocytes is influenced by the regulation of their function by vitamin D **(Ao *et al.*, 2021).**

Macrophages and monocytes: Both types of cells are important in the identification and eradication of pathogens. These cells' capacity to release pro-inflammatory cytokines and stimulate T cells is modulated by vitamin D **(Ao *et al.*, 2021).**

T cells: T cells are essential for the development of autoimmune illnesses and are in charge of cell-mediated immunity. T cell differentiation and activation are influenced by vitamin D, which controls T cell function **(Ao *et al.*, 2021).**

B cells: B cells make antibodies and are involved in humoral immunity. The function of B cells is

modulated by vitamin D, which affects their capacity to specialize and generate antibodies ***(Ao et al., 2021).***

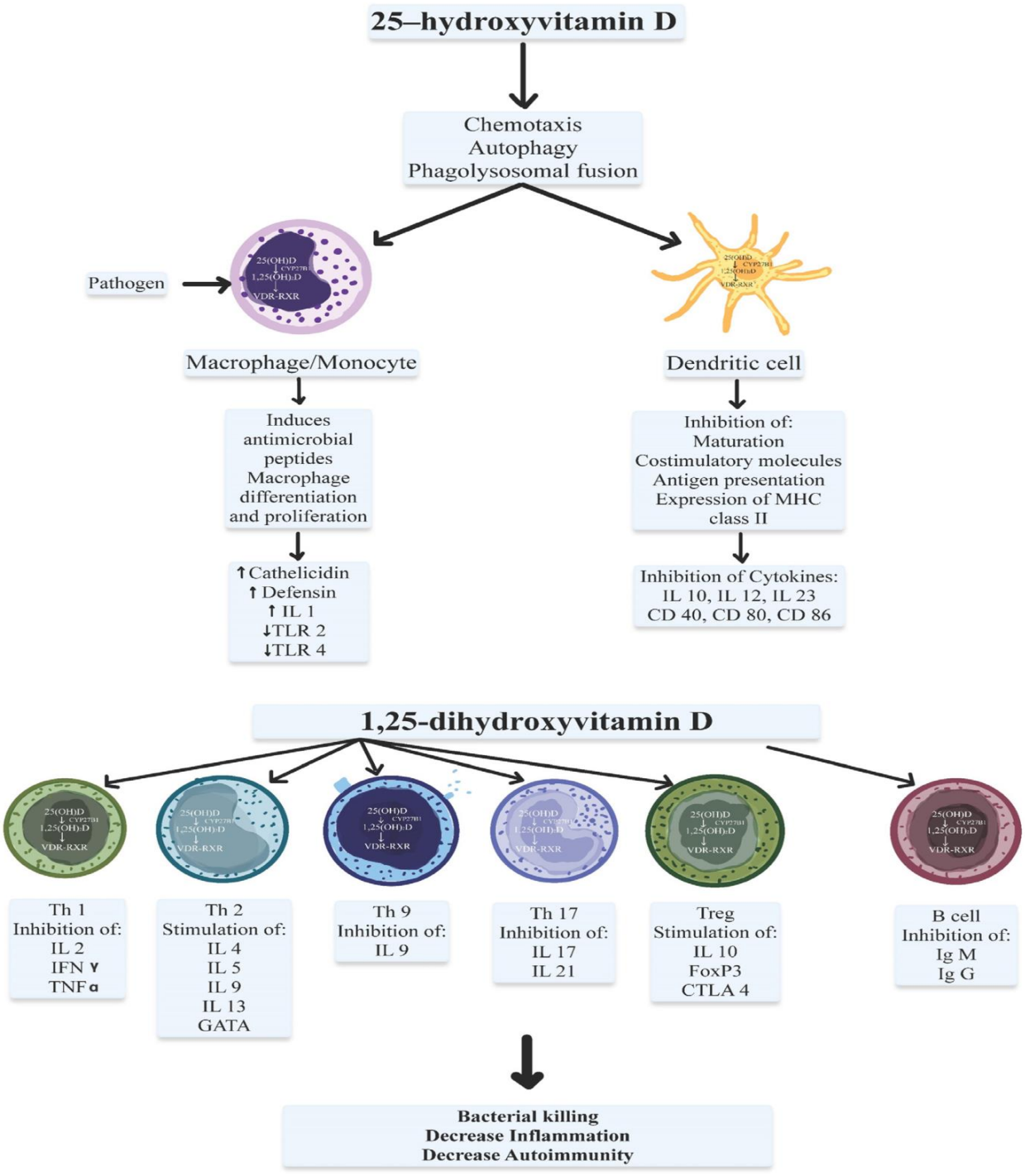

Figure 10:Immunomodulatory actions of active vitamin D (1,25-dihydroxyvitamin D3; 1,25-(OH)2D3) **(Sîrbe *et al.*, 2022).**

II.7.2. The effects of vitamin D on the organs and cells

- **Cognitive Function of the Brain:** Vitamin D insufficiency, especially in older persons, has been associated with cognitive deterioration and an increased risk of dementia. This could be because vitamin D controls the expression of genes related to synaptic plasticity and neuroprotection **(Wu *et al*., 2023).**

- **Cardiovascular Disease:** Studies on the impact of vitamin D on cardiovascular health have shown that it lowers the risk of peripheral artery disease, heart attacks, and strokes. This could be a result of its function in lowering inflammation, enhancing lipid profiles, and controlling blood pressure **(Abugoukh *et al*., 2022).**

- **Diabetic Retinopathy:** Research has demonstrated that vitamin D can help prevent diabetic retinopathy, a major side effect of diabetes. This could be because of its function in controlling the expression of genes related to retinal cells **(Abugoukh *et al*., 2022).**

- **Thyroid:** The function of the thyroid has been related to vitamin D, and a lack of it may aggravate hyperthyroidism and hypothyroidism. This could be because of its function in controlling the expression of genes related to the production and control of thyroid hormones **(Abugoukh *et al*., 2022).**

- **Heart Health:** Research has shown that vitamin D can improve blood vessel function and lower the risk of heart disease, among other positive impacts on heart health. This could be a result of its function in lowering inflammation, enhancing lipid profiles, and controlling blood pressure **(Abugoukh *et al*., 2022).**

- **Diabetic Nephropathy:** Research has demonstrated the preventive role of vitamin D against diabetic nephropathy, a frequent diabetic consequence. This could be because of its function in controlling the expression of genes related to the survival and differentiation of

kidney cells **(Abugoukh *et al*., 2022).**

- **Insulin Sensitivity:** Research has shown that vitamin D enhances insulin sensitivity, which may help control blood sugar levels and lower the chance of complications from diabetes. This could be because of its function in controlling the expression of genes related to glucose metabolism and insulin signaling **(Abugoukh *et al*., 2022).**
- **Inflammation:** The anti-inflammatory qualities of vitamin D can aid in lowering the long-term inflammation linked to diabetes and its aftereffects. This could be because of its function in controlling the expression of genes involved in inflammation and immune response **(Abugoukh *et al*., 2022).**
- **Non-Alcoholic Fatty Liver Disease (NAFLD):** Vitamin D has been shown to positively affect liver health, including lowering the risk of non-alcoholic fatty liver disease (NAFLD) and enhancing liver function. This could also be because of its function in controlling A9Ithe expression of genes related to the survival and differentiation of liver cells **(Abugoukh *et al*., 2022).**

Chapter II: MATERIAL & METHOD

I. Study description

I.1. Description of the human sample

This is a case-control clinical study, that was conducted at the internal medicine department of Dr. Benzerjeb Hospital of Ain Temouchent. The study was dispersed across almost four months, from February 18, 2024, to June 06, 2024. The study aimed to assess the effects of vitamin D3 supplementation on patients with type 2 diabetes, specifically by measuring changes in Hemoglobin A1c and glucose levels before and after oral supplementation. Consent was obtained from all patients before participation (Annex). All cases had confirmed type 2 diabetes diagnosed by their attending physician. Following the medical consultation, patients underwent a strict 8-hour fasting blood draw for various biochemical assays. Before the blood draw, they completed our questionnaire (Annex), and we conducted anthropometric measurements including blood pressure, weight, height, waist, and hip circumference. All the information is reported in the anthropometric results.

I.2. Inclusions and exclusions factors

Inclusion factors: patients with confirmed type 2 diabetes aged between 40 and 80 years.

Exclusion factors: pregnancy, Complex Type 2 Diabetes with Complications such as Renal Issues, Stroke, Cardiovascular Problems, Amputation, and Severe Vision Impairment.

II. Biological test

As part of this study, a biological assessment was carried out on all diabetic patients, after an 8H fasting blood sample was taken in a heparin tube for vitamin D and glucose, we used an EDTA tube for HbA1c measure. This assessment included several dosages:

II.1.Glucose measure

measured by kit There are many types of kits but the principle for all of them is one:

We need 3 tubes 1 for the blank and 1 for standard, and the third depends on the number of patients. In the blank tube we need just 1000µl of glucose reagent and in the tube of the standard we need 1000µl of glucose reagent + 10µl standard(100mg/dl_1M) and for the third we need 1000µl of glucose reagent + 10µl patient sample (just serum) after we incubate the assay mixture for 10 min

at 37° or 15 min in room temperature in that time we program the machine mindray ba-88a (Annexe V) when we choose the test (glucose) it asks us to enter the blank (absorbs) and after that, she will ask to absorb from standard and now she will ask to read the sample when it absorbs from the test tube it will give us the result.

Principle method:

Glucose is oxidized by GOD (Glucose Oxidase) to gluconic acid and hydrogen peroxide (H2O2) which in conjunction with POD (Peroxidase), reacts with chloro-4-phenol and PAP (4-Aminoantipyrine) to form a red quinoneimine:

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + PAP + \text{Chloro-4-Phenol} \xrightarrow{POD} \text{red Quinone} + H_2O$$

$$\text{Glucose} + O_2 + PAP + \text{Chloro-4-Phenol} \xrightarrow{GOD,\ POD} \text{Gluconic Acid} + \text{Red Quinoneimine} + H_2O$$

II.2.Vitamin D measure

The technique used for measuring vitamin D levels is it employs an automated quantitative test using the Enzyme-Linked Fluorescent Assay (ELFA) technique on instruments from the VIDAS family. This method is used for the determination of 25-hydroxyvitamin D (25-OH Vitamin D) Total in human serum or plasma.

Principle

The assay combines an enzyme immunoassay competition method with final fluorescent detection. The Solid Phase Receptacle (SPR®) serves as both the solid phase and the pipetting device. All assay steps are automated by the instrument, which measures fluorescence to determine vitamin D levels.

Sample Preparation

- Serum or plasma samples are pre-treated to separate vitamin D from its binding proteins.
- The pre-treated sample is then mixed with an alkaline phosphatase-labeled anti-vitamin D antibody (conjugate).

- Vitamin D in the sample competes with vitamin D coated on the SPR for binding sites on the antibody.

Detection and Measurement

- The substrate (4-methyl-umbelliferyl phosphate) is introduced, and the enzyme catalyzes its hydrolysis into a fluorescent product.
- Fluorescence is measured at 450 nm, with intensity inversely proportional to the concentration of vitamin D in the sample.
- Results are automatically calculated using calibration curves stored in the instrument and printed out.

Calibration and Quality Control

- Calibration is performed using a provided calibrator, and quality control checks ensure the accuracy and reliability of the assay.

II.3.HBA1c measure:

The standard A1c care system is based on immunoassay and reflectometry technology. STANDARDtm A1cCare (Annexe V) test kit uses an anti-HbA1c(%) antibody which is specific for the first few amino acid residues of the glycated N-terminus of the B-chain of hemoglobin A0. STANDARDtm A1ccare Test contains the test panel (nitrocellulose membrane), latex-tablet (bleu-dyed latex micro-particles conjugated to specific antibodies), and the buffer solution (hemolysis reagent).

HbA1c+Anti-HbA1c antibody-latex conjugate ⟶ HbA1c-Antibody complex

This product is prepared from human or animal origin and contains preservatives and stabilizers.

Test panel

Anti HBA1c antibody 0.86 ug

Anti-chicken antibody 0.86 ug

Bovine serum albumin 0.004 ug

Sacarose 0.035 ug

We add first the blood to the buffer solution and mixed with a later tablet, the erythrocyte is instantly lysed to release glycated hemoglobin(hereafter, Hba1c). when a simple mixture is loaded onto the simple port of the test panel, the mixture fluid migrates along the membrane of the test panel by capillary action and then HbA1c has been immobilized onto the anti-HbA1c antibody coated line. The amount of bleu conjugates on the anti-HbA1c line reflects the amount of HbA1c in the sample. For measuring total hemoglobin in the simple, the intensity of hemoglobin color measured from the desired area on the membrane of the test panel is measured. The chemical and immune reaction that occurs on the test panel is measured by an optical system in STANDARDtm A1cCare Analyzer. This kit measures both fractions and an algorithm that converts the result into the percentage of HbA1c in the sample.

II.4.Urea measure

By the kit diagno_uree enz. First of all, urea the product of protein metabolism is synthesized in the liver and excreted in the kidneys. The serum lure level is used in conjunction with creatinine for the assessment of renal function. The hydrolysis of urea present in the sample is catalyzed by urease, producing ammonium and carbonate ions, in the presence of nitro prosside, the ammonium ions formed.

Preparation of the reagent plus the operating mode.

Standard: aqueous solution duration equivalent to 40 mg/dl (6.6 mmol/l).

A. Dissolve the contents of a durease/salicylate vial with the volume of deionized water indicated on the label. shake gently until completely dissolved.

B. dilute 3 ml of the contents of the alkaline hypochlorite vial in 100 ml of deionized water. Etalon is ready for use. Bring the working reagent and the analyzer to 37C mix standard, sample and reagent A, then incubate either 3 minutes or 5 minutes at room temperature after reagent B and incubate again for 5 minutes or 3 minutes at the same temperature, and reading by wavelength: 578nm, 600 nm. the operation of the product depends on both the reagent and the manual or automatic Mindray BA-88A (Annexe V) reading system used.

II.5.Cholesterol measure

The diagno kit uses the CHOD-PAP method to measure cholesterol levels in blood samples. The kit includes a reagent calibration solution, and control to ensure accurate results. the test provides a direct measure of cholesterol levels, which is one used to assess the risk of cardiovascular disease. The CHOD-PAP method focused on simple preparation, reagent addition, enzymatic hydrolysis, Oxidation, and colorimetric detection. Also, calibrate and control for calibrate the calorimeter and ensure the accuracy of the test. The reference range for cholesterol levels is typically 100-200 mg/dl for total cholesterol (the automat Mindray BA-88A).

II.6.Triglyceride measure

By the kit diagno-TG. The triglycerides present in the sample are hydrolyzed enzymatically by the action of lipases, leading to the formation of the fatty acid glycerol. in the presence of glycerol kinase (GK), the phosphorylation of glycerol occurs in the presence of ATP to give glycerol-3-phosphate and the corresponding ADP. Using glycerophosphate oxidase (GPO), glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate and hydrogen peroxide.

In the final step, with peroxidase as a catalyst, hydrogen peroxide reacts with 4-aminopyrine and 4-chlorophenol to give rise to quinoneimine. The intensity of the color produced is proportional to the quantity of triglycerides present in the samples. the operating mode.

the reagent and the ethanol are ready for use, mix the test standard and reagent well then incubate at 37C for 3 minutes or 10 minutes, and then take the reading (the automat Mindray BA-88A).

III. Protocole of vitamin D supplementation

The selection of controls and patients was done in a randomized manner. The patients received their usual diabetes medications and were given vitamin D3 supplementation, while the controls received only the medication. The patients supplemented with vitamin D3 received their doses based on their level of 25-hydroxy vitamin D. We followed this supplementation protocol, Patients with a 25-hydroxy vitamin D level ≤10 ng/ml receive 10 ampoules of cholecalciferol (vitamin D3) at 200,000 IU/ml. Patients with levels between 10 and 20 ng/ml receive 8 ampoules. Those with levels between 20 and 30 ng/ml receive 6 ampoules, and patients with levels between 30 and 70 ng/ml receive 3 ampoules. The patients took the ampoules once weekly by oral intake.

IV. Statistical analysis

The mean and standard deviation of the quantitative variables were used to express them in the descriptive section (mean standard deviation). The qualitative variables numbers and frequencies (%) were used to express them. Sex, medical history, current therapy, marital status, waist circumference, and hip turret were all considered qualitative factors. Age, weight, height, BPS, BPD(verifier ces termes dans abbreviation), fasting blood glucose level, HbA1c level, and vitamin D level are examples of quantitative variables (for both Patients and control). The Student's t-test is utilized to compare means between two groups. A p-value is considered significant if it is less than 0.05; otherwise, it indicates no significance.

Data entry was done on the Excel version 2019. The analysis was carried out on Excel version 2019.

Chapter III: RESULTS & DISCUSSIONS

I. Results before vitamin D supplementation

We conducted an interventional study in the Internal Medicine Department with the main objective of investigating the impact of vitamin D supplementation on diabetes management, focusing on reducing blood sugar levels and altering HbA1c. To achieve this, we selected 19 patients who received vitamin D3 ampoules based on their 25(OH) vitamin D levels and a control group who did not receive any vitamin D3 supplementation. Both groups continued to receive their medication for type 2 diabetes.

I.1. Patients results

I.1.1. Anthropometric measures results

Nineteen diabetic patients were selected as patients to receive Vitamin D3 supplementation, 68% are women (Figure 11).

Our population has an average age of 59.11 years. Women have an average age of 57,62 years, while men have an average age of 62,33 years. In Figure 12, we categorized our population by gender and age. We observed that women predominate between the ages of 40 and 80; after this decade, men become predominant. In reviewing their medical history, we found that approximately half (42%) had type 2 diabetes alone. About 11% of patients had both diabetes and arterial hypertension(AHT), while 5% had concurrent cardiovascular disease and prostate issues. Another 5 % were diagnosed with diabetes along with thyroid conditions and almost a fourth (26%) of patients had both diabetes and obesity and for diabetes and obesity with AHT we have 11% (Figure 13).

The classification of our patients according to medication type reveals that 79% use oral antidiabetic drugs (OAD), 5% use insulin, and 16% use both insulin and OAD (Figure 14).

The classification of the patients according to the BMI (Figure 15) shows us that 47% of the patients are overweight. About 11% of patients 37% are obese, and just 16 % the healthy weight (Figure 15).

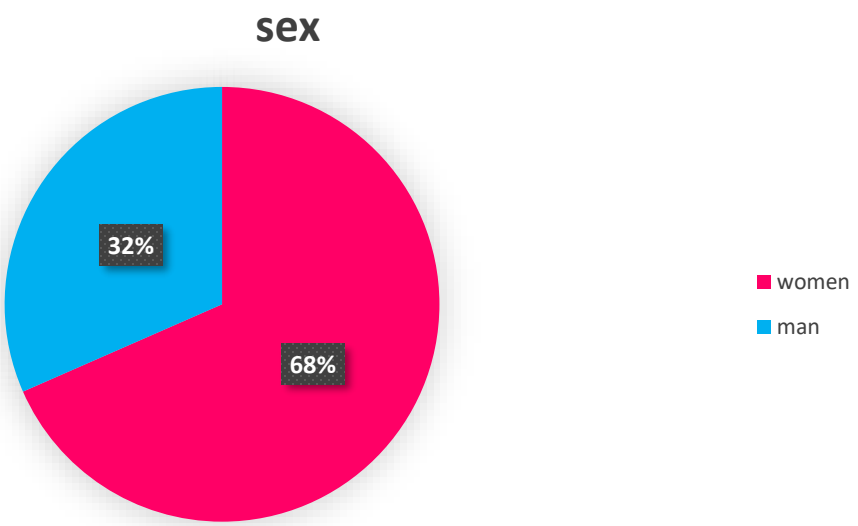

Figure 11: Patients' gender classification

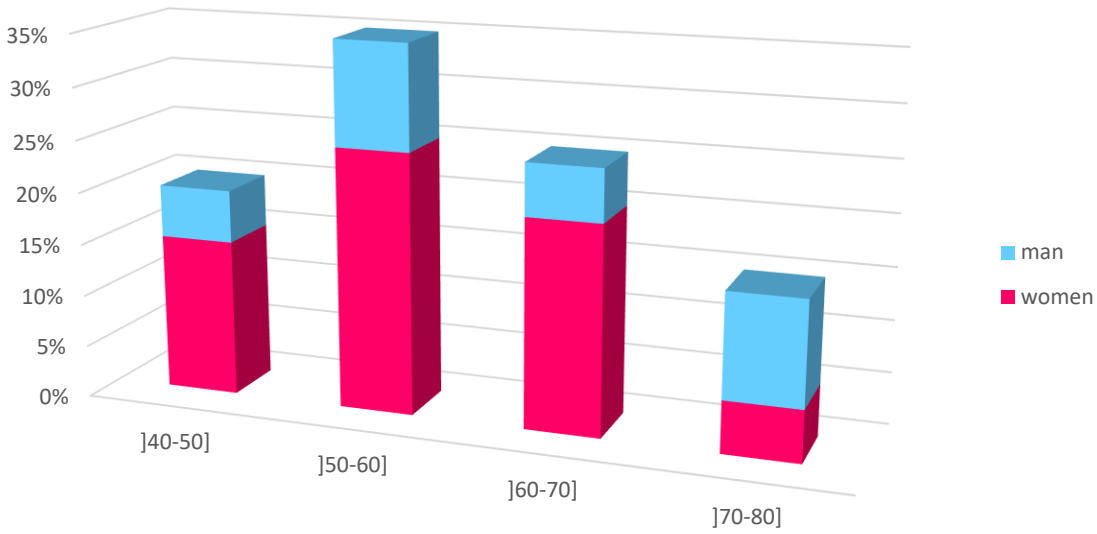

Figure 12: Age and Gender Column Chart

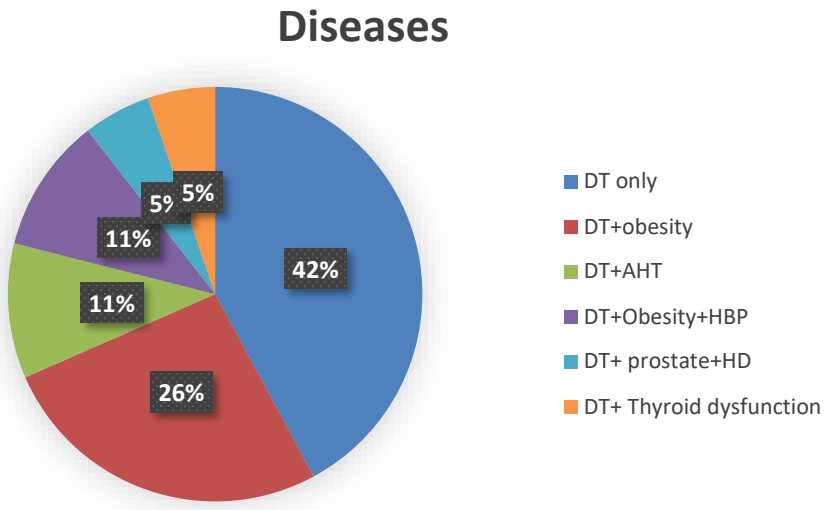

Figure 13: Patients classification based on medical history

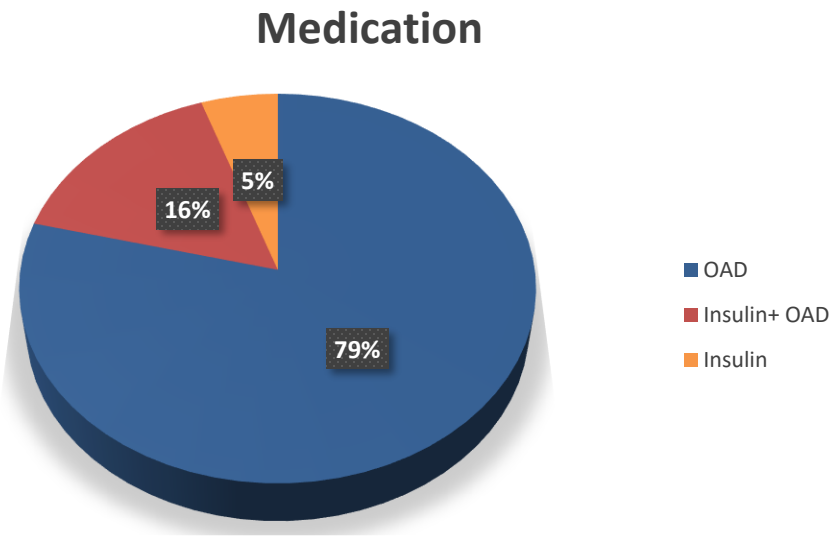

Figure 14:Patients classification according their medication

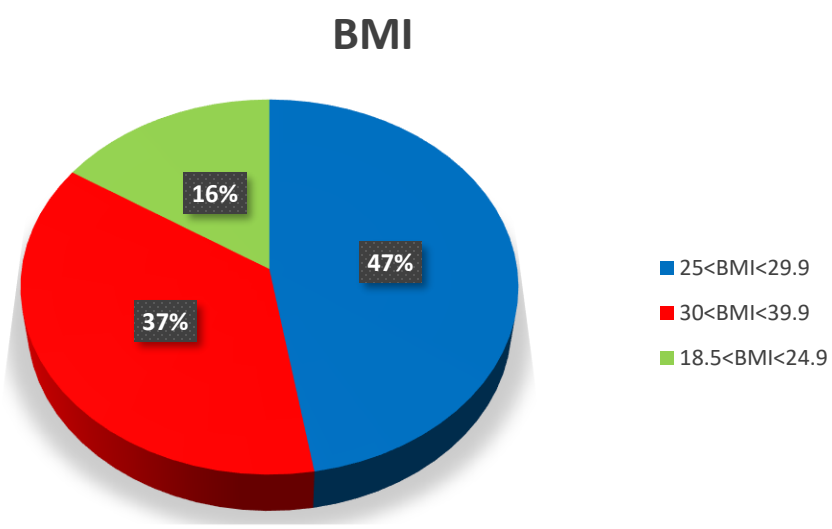

Figure 15: Patients BMI classification

I.1.2. Biological measures results

According to the criteria set by WHO and IDF for fasting glycemia classification, 11% of patients exhibit normal blood glucose levels (less than 1.1 g/L). Meanwhile, 17 % fall within the range of 1.10 to 1.25 g/L, and 72% have hyperglycemia (blood sugar levels exceeding 1.26 g/L) (Figure 16). The study population has an average blood sugar of 1.64 ± 0.59 g/ l.

The study's HbA1c threshold distribution shows that 100% of patients had values over 6.5 %, The average rate of HbA1c is 8.74 ± 1.40 %.

And for the study's vitamin D threshold distribution shows that 37 % of patients had values between 10 and 20, Meanwhile, 32 % fall within the range of 20 to 30 ng/mL, 26% is less than 10, and just 5% of patients had values between 30 and 40 (Figure 17). The average rate of Vitamin D is 17.64± 9.58 ng/mL

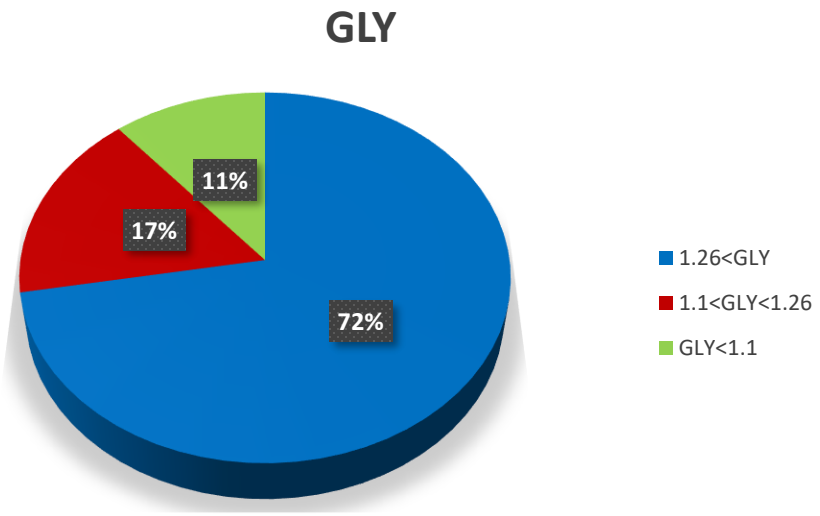

Figure 16: Distribution of patients according to fasting glycemia

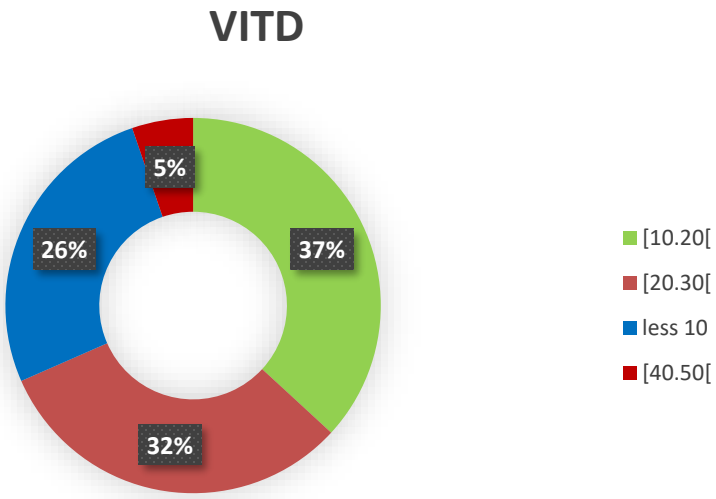

Figure 17: Distribution of patients according the levels of vit D

I.2. Control results

Table II provides a detailed summary of the anthropometric and biological measures of the control group in the study. The anthropometric measures include the distribution of controls by gender, average age, and BMI classifications. Additionally, the table lists the average values and standard deviations for key biological measures such as blood sugar, HbA1c, cholesterol, triglycerides, urea, and creatinine levels. This information highlights the health status and physical characteristics of the control population, emphasizing the prevalence of conditions such as diabetes, hypertension, and obesity within the group.

Table II: Summary of Anthropometric and Biological Measures of Control Group

Description of Population by Anthropometric Measures			
Measure	Men	Women	Total
Number of Controls	28%	72%	100%
Average Age	55.6	55.08	56
BMI: 29.22 ± 5.74			
Underweight (<18.5)	_	_	6%
Healthy Weight (18.5-24.9)	_	_	17%
Overweight (25-29.9)	_	_	33%
Obese (≥30)	_	_	44%

Biological Measures

Measure	Average ≠ standard deviation(SD)
Blood Sugar (g/L)	1.95 ± 0.75
HbA1c (%)	9.25 ± 1.78

Cholesterol (g/L)	1.75 ± 0.45
Triglycerides (g/L)	1.30 ± 0.58
Urea (g/L)	0.84 ± 2.37
Creatinine (mg/L)	8.85 ± 2.30

I.2.1. Anthropometric characteristics between controls and patients

Table III presents a detailed comparison of the anthropometric parameters between the control group and the patient group. It includes the mean values and standard deviations for various measurements such as age, weight, height, BMI, waist circumference, hip circumference, and blood pressure (both systolic and diastolic).

Table III: Anthropometric Parameters of Controls and Patients

Anthropometric Parameters	**Controls Mean ± standard deviation(SD) (n: 18)**	**Patients Mean ± standard deviation(SD) (n: 19)**
Age	56 ± 10.73	59.11 ± 10.55
Poids (Kg)	75.92 ± 12.16	78.78 ± 11.07
Taille(cm)	162.82 ± 38.50	163.37 ± 6.40
BMI (kg/cm^2)	29.22 ± 5.58	29.79 ± 4.04
Waist Circumference (cm)	104.75 ± 24.12	100.50 ± 10.50
Hip Circumference (cm)	109.19 ± 12.49	106.57 ± 9.43
BPS	111.33 ± 16.28	118.13 ± 10.14

BPD	65.71 ± 8.21	69.88 ± 9.54

I.2.2. Biological characteristics between controls and patients

Table IV compares the biological characteristics of the control group and the patient group. It includes the mean values and standard deviations for various biological parameters such as fasting glycemia, HbA1c, vitamin D, creatinine, total cholesterol, triglycerides, and urea levels.

Table IV: Biological Characteristics of Controls and Patients

Paramètres biologiques	**Controls** **Mean ± standard deviation(SD)** **(n: 18)**	**Patients** **Mean ± standard deviation(SD)** **(n: 19)**
Fasting glycemia (g/l)	1.95 ± 0.75	1.64 ± 0.59
HbA1c %	9.25 ± 1.87	8.74 ± 1.40
Vitamin D (ng/ml)	_	17.64 ≠ 9.58
Créatinine (mg/l)	8.85 ± 2.30	8.66 ± 2.15
Cholestérol total (g/l)	1.75 ± 0.45	1.68 ± 0.39
Triglycérides (g/l)	1.30 ± 0.58	1.25 ± 0.44
urea	0.84 ± 2.37	0.3 ± 0.08

II. Results after vitamin D supplementation

II.1.Patient results

II.1.1. Anthropometrics characteristics of patients before and after vitamin D supplementation

Table V: Anthropometrics characteristics patients before and after vitamin D supplementation

Anthropometric Parameters	**Patients before supplementation** **Mean ± standard deviation(SD)** **(n: 19)**	**Patients after supplementation** **Mean ± standard deviation(SD)** **(n: 19)**
Age	59.11 ± 10.55	59.11 ± 10.55
Poids (Kg)	78.78 ± 11.07	78.78 ± 11.07
Taille(m)	163.37 ± 6.40	163.37 ± 6.40
BMI (kg/m^2)	29.79 ± 4.04	29.79 ± 4.04
Tour de taille (m)	100.50 ± 10.50	100.50 ± 10.50
Tour de Manche (m)	106.57 ± 9.43	106.57 ± 9.43
BPS mm de Hg	118.13 ± 10.14	118.13 ± 10.14
BPD mm de Hg	69.88 ± 9.54	69.88 ± 9.54

II.1.2. Biological characteristics of patients before and after vitamin D supplementation

Table VI: Biological characteristics of patients before and after vitamin D supplementation

Paramètres biologiques	**Patients before supplementation** **Mean ± standard deviation(SD)** **(n: 19)**	**Patients after supplementation** **Mean ± standard deviation(SD)** **(n:19)**
Fasting glycemia (g/l)	1.64 ± 0.59	1.63 ± 0.64

HbA1c %	8.74 ± 1.40	8.51 ± 1.02
Vitamin D (ng/ml)	17.64 ± 9.58	_
Créatinine (mg/l)	8.66 ± 2.15	7.51 ± 3.16
Cholestérol total (g/l)	1.68 ± 0.39	1.52 ± 0.42
Triglycérides (g/l)	1.25 ± 0.44	1.07 ± 0.56
urea	0.3 ± 0.08	0.31 ± 0.09

Comparaison between two parameters with T-Test . P>0.05

II.2.Control results

II.2.1. Anthropometrics characteristics

Table VII: Control anthropemetric parameters: Initial to Two Months

Anthropometric Parameters	**controls before supplementation mean ± standard deviation(SD) (n: 18)**	**controls after supplementation mean ± standard deviation(SD) (n: 18)**
Age	56 ± 10.73	56 ± 10.73
Poids (Kg)	75.92 ± 12.16	75.92 ± 12.16
Taille(m)	162.82 ± 38.50	162.82 ± 38.50
BMI (kg/m^2)	29.22 ± 5.58	29.22 ± 5.58
Tour de taille (m)	104.75 ± 24.12	104.75 ± 24.12

Tour de Manche (m)	109.19 ± 12.49	109.19 ± 12.49
BPS mm de Hg	111.33 ± 16.28	111.33 ± 16.28
BPD mm de Hg	65.71 ± 8.21	65.71 ± 8.21

II.2.2. Biological characteristics between controls and patient supplementation

The average levels of various biomarkers in patients and control groups before and after supplementation. After supplementation, the average Blood glucose levels in patients remained almost unchanged, with a minor decrease from 1.64 g/L to 1.63 g/L (Figure 18), HbA1c levels in patients decreased slightly from 8.74% to 8.51% (Figure 19). Urea levels exhibited no change in patients, remaining at 0.31, and Cholesterol levels in patients decreased from 1.68 g/L to 1.52 g/L, Triglyceride levels went from 1.25 to 1.51 g/L. Overall, supplementation resulted in a stabilizing or slightly improving effect on the measured biomarkers, particularly in reducing HbA1c and cholesterol levels in patients (Table VIII).

Table VIII: Biological characteristics of controls and Patients after vitamin D supplementation

Comparaison between two parameters with T-Test . P>0.05

Paramètres biologiques	**Controls after two months** **Mean ± standard deviation(SD)** **(n: 18)**	**Patients after two months** **Mean ± standard deviation(SD)** **(n: 19)**
Fasting glycemia (g/l)	1.96 ± 0.97	1.63 ± 0.64
HbA1c %	8.58 ± 0.82	8.51 ± 1.02
Vitamin D (ng/ml)	_	_
Créatinine (mg/l)	7.67 ± 2.97	7.51 ± 3.16

Cholestérol total (g/l)	1.86 ± 0.88	1.52 ± 0.42
Triglycérides (g/l)	1.09 ± 0.48	1.07 ± 0.56
urea	0.31 ± 0.07	0.31 ± 0.09
creatinine	4.29 ± 0.31	1.63 ± 0.64

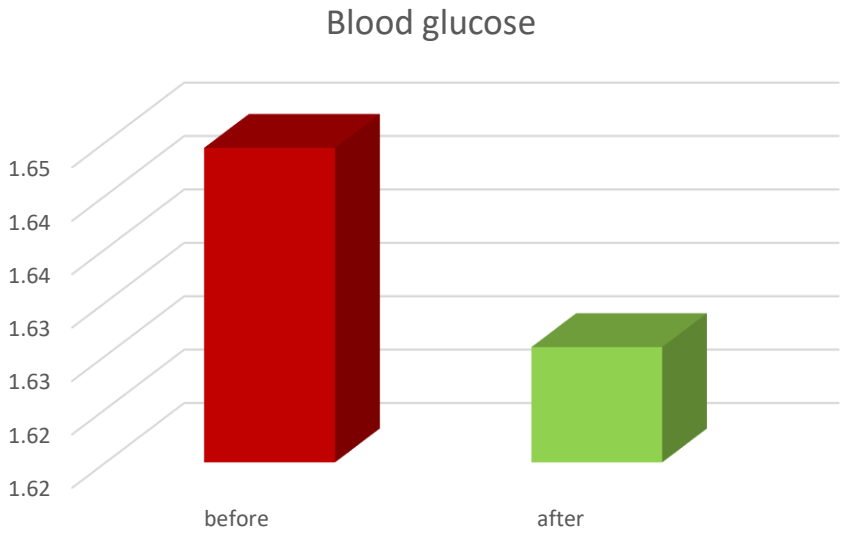

Figure 18: column charts for classifying patient Blood glucose before and after vitamin D3 supplementation

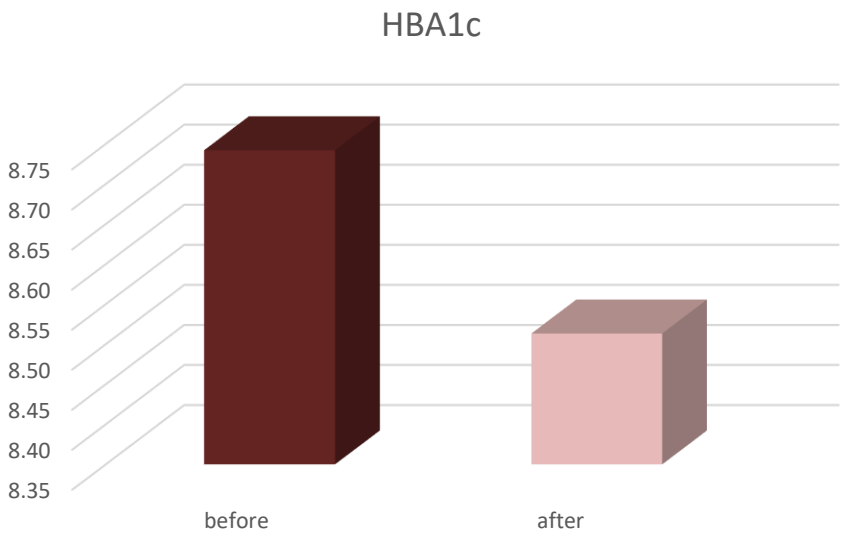

Figure 19: column charts for classifying patient HBA1c before and after vitamin D3 supplementation

DISCUSSIONS

To assess vitamin D levels and examine the effect of vitamin D supplementation on glycemic profiles in type 2 diabetic patients at the Internal Medicine Department of Dr. Benzerdjeb Hospital in Ain Temouchent, a total of 19 diabetic patients were collected. We analyzed glycemic parameters (blood glucose and glycated hemoglobin) before and after supplementation. Examining the vitamin D norms in our population, we found that 40% of participants had vitamin D deficiency (≤20 ng/ml), while 30 % had insufficient vitamin D levels (between10 and 20 ng/ml) and 30 % hed severe vitamin D deficiency (less than 10). The baseline average for vitamin D was 17.64 ng/ml. Our patients exhibited vitamin D deficiency or insufficiency, consistent with other studies confirming hypovitaminosis in diabetic patients **(Fuchsberger *et al*., 2016; Huang *et al*., 2012; Nobécourt *et al*., 2010; Oueslati *et al*., 2015; Safi *et al*., 2015; Suzuki *et al*., 2006; Ait-Aberahmane *et al*., 2016; Fondjo *et al*., 2017**).

A study at the Faculty of Medicine in Algiers examined the prevalence of vitamin D deficiency among type 2 diabetic patients. The study included 290 patients over 40 years old over 6 months, showing a high prevalence of vitamin D deficiency, with 87.2% of patients having levels below 30 ng/ml **(Ait-Aberahmane *et al*., 2016)**. A Korean team found, among 276 type 2 diabetic patients, an average vitamin 25OHD concentration of 12.9 ± 0.4 ng/ml, with a prevalence of vitamin D deficiency of 98% **(Wang *et al*., 2022)**. The average vitamin 25OHD concentration in diabetic patients was 17.0 ± 7.1 ng/ml, not statistically different from the general population (17.5 ± 3.6 ng/ml), with a prevalence of hypovitaminosis D < 20 ng/ml of 70.6% **(Suzuki *et al*., 2006)**. Vitamin D deficiency was observed in 92.4% of type 2 diabetes cases and 60.2% of non-diabetic controls. Vitamin D deficiency was not significantly associated with HOMA-β [type 2 diabetes: r2 = 0.0209, p = 0.1338 and controls: r2 = 0.0213, p = 0.2703] and HOMA-IR [type 2 diabetes: r2 = 0.0233, p = 0.1132 and controls: r2 = 0.0214, p = 0.2690] in both controls and diabetes cases **(Fondjo *et al*., 2017)**.

In another study, the mean vitamin 25OHD level was 10.95 ± 6.99 ng/ml, with 98.1% of patients having a deficiency or insufficiency in vitamin D **(Safi *et al*., 2015)**. In a Tunisian study **(Oueslati *et al*., 2015)**, the average vitamin D level was 9.31 ± 7.7 ng/ml, and 88% of patients had low vitamin

D levels. Other researchers also found vitamin D deficiency (< 30 ng/ml) in 93% of French diabetic patients **(Nobécourt *et al*., 2010)**.

Hypovitaminosis D can be explained by medication use; certain drugs such as long-term corticosteroids or antiretrovirals lead to the catabolism of Vit 25OHD and calcitriol. Others compete with its metabolism via CYP450, such as certain anticonvulsants, Rifampicin, lithium, and immunosuppressants. Age also plays a role, as UVB-induced cutaneous vitamin D synthesis decreases with age due to reduced 7-DHC concentration in the deeper layers of the epidermis **(Wang *et al*., 2022).**

Comparing our study with others, we found significant divergence; some studies confirmed the effect of vitamin D, while others did not.

In our study, Vitamin D3 supplementation was considered to correct the vitamin D deficiency and improve the glycemic status in these patients. The vitamin supplementation followed a protocol based on the measurement of Vit 25OH. After supplementation, a small improvement in glycemic control was observed. The percentage of patients with hemoglobin A1c above 6.5 % decreased from 100 % to 88.89%. Additionally, the mean HbA1c also showed a decrease from 8.74 % to 8.51 %. However, blood glucose levels only experienced a non-significant decrease, from 1.64 g/l to 1.63 g/l. (X. Li et al., 2018)**.**

The supplementation of vitamin D was good for reducing glycated hemoglobin without a significant change in fasting glycemia parameters in our study, which aligns with other clinical, systematic, and meta-analytic studies **(Dipasquale, Lo Presti, *et al*., 2022; Talaei *et al*., 2013)**.

Other systematics studies said there is insufficient evidence to suggest that vitamin D supplementation significantly reduces the incidence of Type 2 Diabetes despite its effects on insulin resistance **(Khan et al., 2023).**

An Iranian study evaluated the effect of weekly oral supplementation with 50,000 IU of vitamin D3 for eight weeks on insulin resistance in 100 patients aged 30 to 70 with type 2 diabetes **(Talaei *et al*., 2013)**. The results showed significant improvements in fasting glucose (138.48±36.74 to

131.02±39 mg/dl, P=0.05), insulin (10.76±9.46 to 8.6±8.25 μIu/ml, P=0.028), and HOMA-IR index (3.57±3.18 to 2.89±3.28, P=0.008).

Another study demonstrated that in type 2 diabetic patients with vitamin D deficiency, supplementation with vitamin D3 improved glycemic control and reduced the use of hypoglycemic medications (HbA1c decreased from 7.6% or 60 mmol/mol to 7.1% or 54 mmol/mol). Metformin, acarbose, and pioglitazone were significantly lower ($p = 0.037$, $p = 0.048$, and $p = 0.042$, respectively) in the vitamin D3 treatment group compared to baseline. Insulin units (Lispro, Aspart, Glargine) were also lower in the vitamin D3 group at the study end ($p = 0.031$, $p = 0.037$, and $p = 0.035$, respectively) compared to the placebo group **(Dipasquale, Presti, *et al*., 2022**).

In another systematic review and meta-analysis, authors found a modest but significant reduction in HbA1c of 0.32% following vitamin D supplementation, although with substantial heterogeneity likely due to varying doses and durations across studies. However, they found no benefit of vitamin D supplementation in improving fasting blood glucose in individuals with type 2 diabetes. Separate analyses by vitamin D dose, treatment duration, initial vitamin D status (i.e., insufficient/deficient vs. replete), and study bias risk (i.e., excluding studies with high bias risk) confirmed the null result in the pooled analysis **(Fuchsberger *et al*., 2016)**.

Conversely, another clinical study administering vitamin D3 supplementation for six months to obese patients with type 2 diabetes and vitamin D deficiency normalized vitamin D status and reduced the incidence of elevated parathyroid hormone levels, but showed no effect on metabolic parameters such as fasting glucose, HbA1c, C-peptide, creatinine, phosphorus, alkaline phosphatase, lipids, C-reactive protein, or thyroid stimulating hormone concentration. The dose was 6000 IU/ml of vitamin D3 for 3 months followed by 3000 IU/ml daily for another 3 months **(Sadiya *et al*., 2014).**

We did not find a significant improvement, which may be due to the lack of adherence to a dietary regimen in parallel. We must also emphasize that trace elements are necessary for the proper functioning of vitamin D. Additionally, there is the issue of resistance at the VDR receptor level, with several polymorphisms implicated in this resistance.

To achieve desired metabolic control goals, it is essential to actively involve patients in developing a personalized dietary plan in collaboration with healthcare professionals. This plan should be coupled with regular physical activity to achieve normal body weight and maintain good metabolic control **(Pedroni *et al*., 2021)**.

This holistic approach, which integrates nutrition, supplementation with vitamins and trace elements, physical activity, and a healing mindset, may be crucial for optimizing the management of type 2 diabetes. It is important to emphasize that each patient is unique, and a personalized plan takes into account individual needs and preferences, guided by appropriate medical recommendations.

GENERAL CONCLUSION

Chapter IV: GENERAL CONCLUSION

In conclusion, our study at Dr. Benzerdjeb Hospital in Ain Temouchent highlighted a high prevalence of vitamin D deficiency and insufficiency among type 2 diabetic patients, consistent with global trends reported in similar research. Despite supplementation aimed at correcting these deficiencies, our findings showed only modest improvements in glycemic control, specifically a reduction in glycated hemoglobin levels. This aligns with broader systematic reviews indicating mixed evidence on the efficacy of vitamin D supplementation in significantly improving fasting glucose levels in diabetic populations.

The complexity of managing type 2 diabetes underscores the importance of a comprehensive approach that includes personalized dietary plans, physical activity, and appropriate medical interventions. While vitamin D supplementation may offer some benefits, its effectiveness varies and must be tailored to individual patient needs and responses. Future research should continue exploring optimal strategies to enhance metabolic outcomes in diabetic patients through integrated care protocols that address both nutritional and medical considerations.

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Annexe

Annexe I

The ADA defines an HbA1c level of 5.7% or below as normal. This is based on their diagnostic criteria for diabetes, where an HbA1c level of 5.7% to 6.4% indicates prediabetes and a 6.5% or higher indicates diabetes. Reference: American Diabetes Association. (2021)

- Glycaemia (Normal fasting blood glucose concentrations are estimated to range from 70 mg/dL (3.9 mmol/L) to 100 mg/dL (5.6 mmol/L))
- The range for vitamin D is 30–100 ng/ml. A lower level at 20 ng/ml is deemed vitamin D insufficiency, but a blood level of 25(OH) vitamin D between 20 and 29 ng/ml is considered deficient in vitamin D.

Annexe II

Body Mass Index

Formula BMI= Weight/height ^{2}

- **BMI Ranges**
- Below 18.5 you are in the underweight range.
- Between 18.50 and 24.9 you are in the healthy weight range.
- Between 25 and 29.9 you are in the overweight range.
- Between 30 and 39.9 you are in the obese range.

Annexe III

Lettre du consentement éclairé

- Date :
- Ville:
- Je soussigné(e)……, demeurant à ……., donne par la présente mon consentement éclairé pour ma participation en tant que Cas, dans une etude Clinique, qui s'interesse a étude de la supplementation en vitamin D chez le diabetique type 2.
- Je comprends que la démarche de l'étude implique le recrutement de patients

- Je déclare avoir été informé(e) en détail sur les différents aspects de l'étude. J'ai eu l'occasion de poser des questions et d'obtenir des réponses satisfaisantes concernant l'action, le traitement ou la procédure qui seront mis en place. J'ai reçu toutes les informations nécessaires pour prendre une décision éclairée. J'ai également accepté volontairement de participer en tant que cas.
- Je m'engage à participer à l'étude en fournissant les informations adéquates et correctes au clinicien, en effectuant les analyses sanguines nécessaires, et en répondant au questionnaire
- Veuillez trouver ci-joint ma signature attestant de mon accord :
- NOM:
- PRENOM:
- ADRESSE PERSONNELLE:
- SIGNATURE:
-

Annexe IV

Questionnaire

The clinical parameters studied are:

- Name:
- Sex:
- Age:
- Medications taken
- Civil status
- Weight : Height: BMI
- Waist circumference: Hip circumference:
- BPS: BPD:

- Medications taken:

yes or no questions:

- Contraception:
- Medical history :
- Sports :
- Smoking:
- Sun exposure:

Annexe V

Description of the automats:

SDA1cCare

The SDA1cCare analyzer is designed to measure glycated hemoglobin (HbA1c) levels in blood samples. Here is a brief overview of its features and functionality:

Technology: Utilizes immunoassay and reflectometry to detect HbA1c levels.

Sample Preparation: Involves lysing red blood cells to release hemoglobin.

Detection: Captures HbA1c with specific antibodies and uses color intensity measurement to quantify HbA1c.

Accuracy: Provides precise readings of HbA1c, which are essential for monitoring long-term glucose control in diabetic patients.

Ease of Use: Designed for efficient processing and quick results.

Mindray ba-88a

The Mindray BA-88A is a fully automated benchtop hematology analyzer designed to analyze complete blood counts (CBC) and white blood cell differentials. It is commonly used in clinical laboratories and healthcare settings for routine blood testing. (mindray.com)