People's Democratic Republic of Algeria

The Ministry of Higher Education and Scientific Research

Saad Dahlab Blida1 University

Faculty of Natural and Life Sciences
Department of Biology

**Final Dissertation**

To obtain a master's degree in Natural and Life Sciences

**Branch:** Biological Sciences

**Option:** Pharmacotoxicology

**Theme**

# Impact of prolonged metformin intake on vitamin B12 levels in type 2 diabetic patients


Presented by :

**Mrs. Gaci Meriem and Mrs. Mazouz Mounia**


**In front of a jury composed of:**

| | | | |
|---|---|---|---|
| **Dr Douaouri NH.** | MCB | Chairman | USDB1 |
| **Dr Djairene N.** | DECTOR | Examiner | USDB1 |
| **Dr Settar A.** | MAB | Supervisor | USDB1 |
| **Pr Zitouni L.** | PROFESSOR | Co-supervisor | ESH Salim Zemirli |

**Academic year : 2024-2025**

## Acknowledgments

This work is the culmination of many years of study. First and foremost, we would like to express our deepest gratitude to Almighty God for granting us the health, strength, and determination to begin and complete this thesis.

We sincerely thank the Chairman, **Mrs Douaouri N** and the Examiner, **Mrs Djairene** N for their time, valuable insights, and constructive feedback, which greatly enriched this work

This work would not have been as rich, nor would it have come to fruition, without the guidance and support of **Mrs. Settar A.** We are sincerely grateful for her exceptional supervision, patience, rigor, and availability throughout the preparation of this thesis.

Our sincere gratitude goes to **Mrs. Khaldoun H**, Head of the Pharmacotoxicology Department, for her efforts, invaluable advice, and continued support.

We also warmly thank Professor **Zitouni A.**, Head of the Internal Medicine Department at Slim Zemirli Hospital in El Harrach, who welcomed us and provided all the necessary means for the successful completion of our practical training.

Our thanks also go to all our professors for their generosity and patience, despite their demanding academic and professional responsibilities.

Without forgetting all the diabetic patients we met during the consultations, whom we sincerely thank for their cooperation and trust. Their contribution was essential to the completion of this research

Finally, we would like to thank all those, near and far, who contributed in any way to the realization of this humble work.

## Dedication

*I dedicate this work to my beloved parents, whose unwavering and boundless support has been the foundation of all that I have achieved. Their unconditional love and constant sacrifices have paved the way for me to become who I am today. May this modest work be the fulfillment of their heartfelt wishes and a small tribute to their countless sacrifices ; although I know I can never truly repay them.*

*To my sisters, Meriem and Asma, for their help and encouragement.*
*To my dear grandmother and my aunt Amina, for their support.*
*To my husband, for standing by my side throughout this journey.*
*To all my friends, for their companionship.*
*To my research partner, Meriem, and her family, for their kindness and support.*
*And finally, to all those who are dear to me and who supported me, whether directly or indirectly ; this work is also yours.*

***''Mounia"***

## Dedication:

*To my beloved mother, the source of tenderness and unwavering prayers — your constant support has been the foundation of every step I have taken. I offer you this work as a token of love and deep gratitude that words can never fully express.*

*To the soul of my late father, who may be absent from sight but never from my heart… You were the silent strength behind every achievement. May this work be a continuous charity for your soul and a light that reaches your resting place.*

*To my dear sisters, Asma and Amal, for their constant encouragement and comforting words during moments of exhaustion.*

*To my fiancé, who walked by my side with love and patience — I am truly grateful for your presence in my life.*

*And finally, to myself — for every moment of perseverance, for every step taken through fatigue, and for every time I kept going when everything seemed impossible.*

**‘’ Meriem”**

**الملخص:**

يُعد الميتفورمين من الأدوية الشائعة الاستخدام في علاج داء السكري من النوع الثاني، وقد أظهرت دراسات حديثة أن العلاج طويل الأمد به قد يؤثر سلبًا على الحالة الفيتامينية للمرضى، ولا سيما فيما يتعلق بفيتامين B12. تهدف هذه الدراسة إلى تقييم الحالة البيوكيميائية والفيتامينية لدى مرضى السكري من النوع الثاني الخاضعين للعلاج بالميتفورمين.

أُجريت دراسة تحليلية مقطعية استعادية على عينة مكونة من 64 مريضًا مصابًا بداء السكري من النوع الثاني، وذلك بمستشفى سليم زميرلي، باستخدام استمارة استقصاء لجمع المعطيات. تم تحليل البيانات باستخدام اختبار ANOVA ومعامل ارتباط بيرسون.(Pearson)

أظهرت النتائج سيطرة طفيفة للعنصر الرجالي بنسبة 71.9%. كما تم تسجيل فروق ذات دلالة إحصائية في كل من تركيز الغلوكوز، الكرياتينين، فيتامينB12 ، والهيموغلوبين. وأوضحت المعطيات أن 46.9% من المرضى يعانون من نقص في فيتامينB12 ، بمتوسط تركيز بلغ 241.11 بيكوغرام/مل، مع وجود علاقة ارتباط سلبية بين مدة تناول الميتفورمين ومستوى هذا الفيتامين.

تشير هذه النتائج إلى أن الاستعمال المطوّل للميتفورمين قد يزيد من خطر الإصابة بنقص فيتامينB12 ، مما قد يؤدي إلى مضاعفات صحية مثل فقر الدم، والاعتلال العصبي، واضطرابات أخرى مرتبطة بهذا النقص.

**الكلمات المفتاحية:** داء السكري من النوع الثاني، الميتفورمين، نقص، فيتامينB12 ، مؤشرات بيوكيميائية.

**Abstract:**

Metformin is a commonly prescribed medication for the treatment of type 2 diabetes. Several recent studies have shown that long-term metformin therapy in type 2 diabetic patients may alter their vitamin status. The objective of our study is to assess the biochemical and vitamin B12 status in type 2 diabetic (T2D) patients receiving metformin therapy.

We conducted a retrospective cross-sectional analysis study involving 64 patients with type 2 diabetic at Slim Zemirli Hospital. The collected data were analyzed using statistical tests

Significant differences were observed in fasting blood glucose, creatinine, vitamin B12, and hemoglobin levels, particularly in patients receiving metformin for over 10 years. A total of 46.9% of patients were found to have a vitamin B12 deficiency, with an average level of 241.11 pg/mL. Also, a inverse relationship was observed between the duration of metformin use and vitamin B12 levels.

Finally, we conclude that prolonged use of metformin may increase the risk of vitamin B12 deficiency, potentially leading to complications such as anemia, neuropathy, and other related disorders.

**Keywords:** Type 2 diabetes, Metformin, Deficiency, Vitamin B12, Biochemical parameters.

**Résumé** :

La metformine est un médicament couramment prescrit pour le traitement du diabète de type 2. L'objectif d'étudier le statut biochimique et vitaminique chez des patients diabétiques de type 2 (DT2) sous metformine.
On a réalisé une étude rétrospective transversale chez 64 sujets diabétiques de type 2 consultés à l'hôpital Slim Zemirli. Les résultats obtenus ont été analysés à l'aide de tests statistiques
Des différences significatives ont été observées pour la glycémie, la créatinine, la vitamine B12 et l'hémoglobine. 46,9 % des patients présentent une carence en vitamine B12, avec une moyenne de 241,11 pg/mL, notamment chez les patients qui prennent la métformine pour plus de 10 ans. Une relation inversez a été trouvée entre la durée du traitement à la metformine et le taux de vitamine B12. À cet égard, nous pouvons conclure que l'administration prolongée de metformine pourrait induire un risque de carence en vitamine B12, exposant les patients à des complications telles que l'anémie, la neuropathie et d'autres troubles associés.

**Mots clés :** Diabète type 2, Metformine, Carence, Vitamine B12, Paramètres biochimiques.

# List of Abbreviations

**ABCD4** : ATP-Binding Cassette Subfamily D Member 4

**AMPK :** AMP-Activated Protein Kinase

**ADA:** American Diabetes Association

**ATP :** Adenosine Triphosphate

**BMI :** Body Mass Index

**CD320 :** Cluster of Differentiation 320 (Transcobalamin Receptor)

**DM :** Diabetes Mellitus

**DNA :** Deoxyribonucleic Acid

**DPP-4 :** Dipeptidyl Peptidase-4

**FPG :** Fasting Plasma Glucose

**GLP-1 :** Glucagon-Like Peptide-1

**GOD :** Glucose Oxidase

**Hb :** Hemoglobin

**HbA1c :** Glycated Hemoglobin

**HDL :** High-Density Lipoprotein

**IF :** Intrinsic Factor

**IR :** Insulin Resistance

**LDL :** Low-Density Lipoprotein

**LMBD1 :** Lysosomal Membrane Binding Domain 1 Protein

**OCTs :** Organic Cation Transporters

**OGTT :** Oral Glucose Tolerance Test (Two-Hour)

**PG :** Plasma Glucose

**PMAT :** Plasma Membrane Monoamine Transporters

**SGLT1 :** Sodium-Glucose Transporter 1

**SGLT2 :** Sodium-Glucose Co-Transporter 2

**T2D :** Type 2 Diabetes

**TC :** Total Cholesterol

**TC (Vit. B12) :** Transcobalamin

**TG :** Triglycerides

## *List of figures*

## *List of tables*

*Table of contents*

# *Introduction*

# Introduction

Diabetes has become a major public health issue, increasingly affecting developing countries and disadvantaged populations. It refers to a group of metabolic disorders characterized by chronic hyperglycemia. Type 2 diabetes (T2D), in particular, is a multifactorial disease that disrupts the metabolism of carbohydrates, lipids, and proteins. It results from both insulin resistance in peripheral tissues and inadequate insulin secretion **(Meghraoui *et al.*, 2021).**

Among the various therapeutic strategies for diabetes management, including pharmacological (oral antidiabetic drugs and insulin) and non-pharmacological (dietary and lifestyle modifications) approaches, metformin is the most prescribed oral antidiabetic drug **(Ophélie *et al* ., 2022**).

Metformin is widely recognized as the first-line treatment for type 2 diabetes due to its proven efficacy in improving glycemic control, reducing HbA1c levels, and favorably modifying lipid profiles. However, despite its metabolic benefits, prolonged use of metformin has raised concerns regarding potential adverse effects. Among these, a decrease in vitamin B12 levels is frequently observed, particularly in patients receiving high doses or prolonged treatment (**Sayedali *et al*,2023**).

Vitamin B12, also known as cobalamin, is an essential water-soluble vitamin that the body stores large reserves often enough to last several years, which means deficiency symptoms may develop slowly and insidiously over time **(Fernandes *et al.*,2024)** . Deficiency can lead to a wide range of clinical manifestations, affecting hematologic, neurological, and psychological systems **(Miller *et al.*, 2024)**.

Thus, the primary objective of this study is to evaluate the impact of prolonged metformin therapy on vitamin B12 deficiency in patients with type 2 diabetes. The analysis focuses on assessing the correlation between the duration of metformin use and serum vitamin B12 levels, while also exploring associated changes in other biological parameters such as hemoglobin, renal function, glycemic control, and lipid profile.

This manuscript is structured into three main chapters: the first chapter presents a general overview of type 2 diabetes and reviews the pharmacological profile and side effects of metformin, particularly its association with vitamin B12 deficiency. The second chapter describes the study design, methodology, and statistical tools used. The third chapter presents the results and provides a critical discussion based on relevant scientific literature.

# *Bibliographic synthesis*

# I. Type 2 diabetes

## 1 .Definition

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral insulin actions, or both. It is a significant global health concern with increasing prevalence and associated complications **(Goyal *et al.*, 2023).** The most prevalent type of diabetes is type 2 diabetes (T2D), which is characterized by the progressive loss of insulin secretion by pancreatic β-cells combined with insulin resistance (IR) in peripheral tissues **(Abel *et al.*, 2024)**.

### 1.1.Epidemiology

Diabetes is a major global health issue due to its high incidence, disability, and mortality, ranking as the eighth leading cause of death and disability. About 529 million people were living with diabetes in 2021, with a global prevalence of 6.1%, expected to rise to 9.8%, affecting 1.31 billion people.

T2D accounts for over 96% of all cases and is the most rapidly increasing type of diabetes in terms of prevalence **(Figure 1) (Xi Lu *et al.*, 2024).**

T2DM is progressing rapidly in Algeria, with an estimated prevalence of around 14.4% among adults aged 18 to 69 **(Belhadj *et al.*, 2019)**. According to IDF data in 2024, nearly 4.8 million Algerians aged between 20 and 79 are living with the disease. In addition, the Ministry of Health warns of a potential increase in the number of people with diabetes to over 4.2 million by 2025 **(Belhadj *et al.*,2025).**

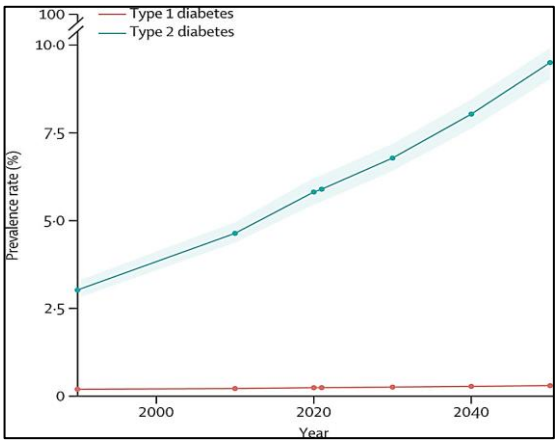



**Figure 1**: Type 1 and 2 diabetes prevalence up to 2050 **(Ong *et al.*, 2023).**

### 1.2. Pathophysiology of T2D

T2D is primarily caused by two key mechanisms: insulin resistance and beta cell dysfunction.

#### 1.2.1 Insulin Resistance (IR)

IR usually begins with skeletal muscles, making them the first dysfunction site. Although muscle insulin resistance may not immediately cause high blood sugar, it contributes to long-term problems by increasing insulin levels, promoting fat buildup in the liver. Over time, this leads to IR **(Andrzej Patyra *et al.*, 2024).**

In the liver, a high-fat diet and chronic high insulin levels lead to increased fat production and accumulation. This disrupts insulin signaling pathways and increases glucose production, causing elevated fasting blood sugar. Key contributors include **(Andrzej Patyra *et al.*, 2024):**

- Diacylglycerol interferes with insulin signaling.
- Ceramides promote glucose production.

#### 1.2.2 Beta Cell Dysfunction

As IR progresses, the pancreas compensates by increasing insulin production. Eventually, beta cells fail to keep up, leading to insufficient insulin. This dysfunction is driven by **(*Khamis* Cureus, 2023)**:

- **Glucotoxicity:** Chronic high glucose depletes insulin stores in beta cells.
- **Lipotoxicity:** Long-term exposure to high fatty acids damages beta cells and impairs their growth.
- **Reduced beta cell mass**: T2D patients often have ~50% fewer beta cells, with increasing loss over time due to apoptosis.
- **Inflammation:** Chronic low-grade inflammation may contribute to beta cell failure

  **( Khamis Cureus,2023)**

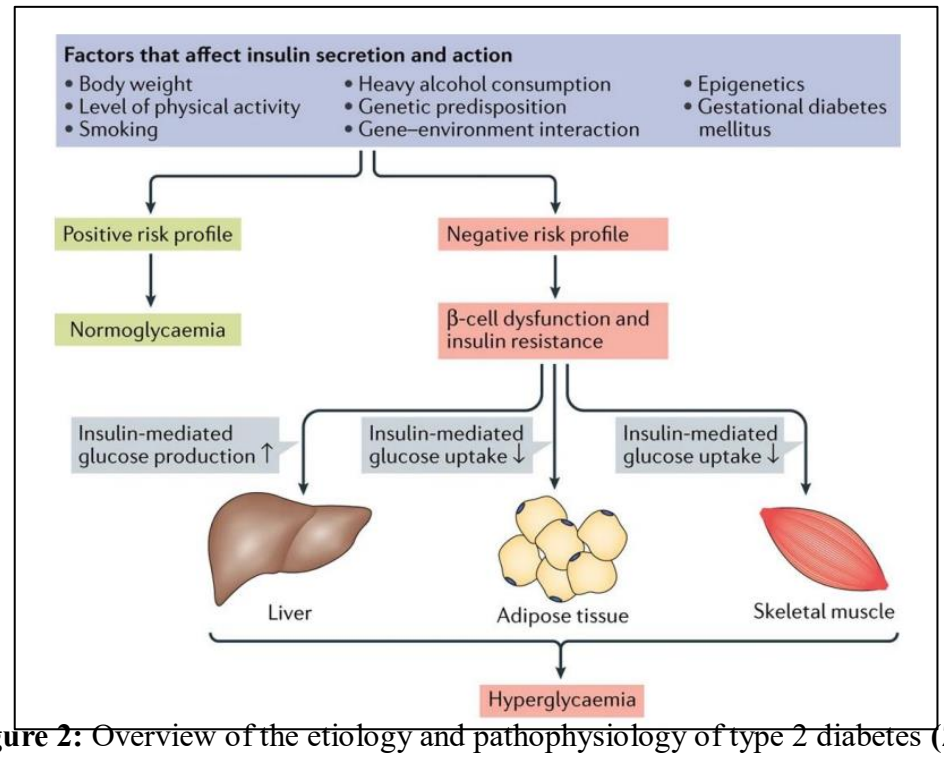



**Figure 2:** Overview of the etiology and pathophysiology of type 2 diabetes **(Zheng *et al.*, 2018).**

### 1.2 Diagnosis

Diabetes can be diagnosed by either the hemoglobin A1C criteria or serum glucose concentration (fasting or 2-hour serum glucose) **(Goyal *et al.*, 2023).**

#### 1.2.1 Fasting Plasma Glucose (FPG)

A blood sample is taken after an 8-hour overnight fast. As per ADA, a fasting plasma glucose (FPG) level of more than 126 mg/dL (7.0 mm/mol) is consistent with the diagnosis. ) **(Goyal *et al.*, 2023).**

#### 1.2.2 Two-Hour Oral Glucose Tolerance Test (OGTT)

In this test, the plasma glucose level is measured before and 2 hours after the ingestion of 75 gm of glucose. DM is diagnosed if the plasma glucose (PG) level in the 2-hour sample is more than 200 mg/dL (11.1 mmol/mol). It is also a standard test but is inconvenient and more costly than FPG and has major variability issues. Patients need to consume a diet with at least 150 g per day of carbohydrates for 3 to 5 days and not take any medications that can impact glucose tolerance, such as steroids and thiazide diuretics. ) **(Goyal *et al.*, 2023).**

#### 1.3.3 Glycated Hemoglobin (Hb) A1C

This test gives an average of blood glucose over the last 2 to 3 months. Patients with an HbA1C greater than 6.5% (48 mmol/mol) are diagnosed as having T2D. HbA1C is a convenient, rapid, standardized test and shows less variation due to pre-analytical variables. It is not much affected by acute illness or stress. ) **(Goyal *et al.,* 2023).**

### 1.3. Management of T2D

The management of T2D involves a non-pharmacological and pharmacological approach:

#### 1.3.1 Non-pharmacological approach

Lifestyle intervention constitutes the only causal, non-pharmacological therapy for Type 2 Diabetes Mellitus (T2DM) and is considered the first-line approach for both its prevention and management. Physical inactivity, poor dietary habits, and excess body weight (overweight and obesity) represent major modifiable risk factors that contribute to the development and progression of the disease **(Rosenfeld *et al.,* 2025).** Furthermore**,** in selected patients who are motivated and have an HbA1c level close to target (e.g., <7.5%), a trial period of lifestyle modification alone for 3 to 6 months may be appropriate before initiating pharmacological therapy, typically with metformin. ***(Dixit et al ., 2022).***

#### 1.3.2Pharmacological approach

If lifestyle modifications alone are insufficient to achieve glycemic targets typically defined as an HbA1c level below 7.5%, though this threshold may vary based on individual patient characteristics, pharmacological intervention becomes necessary. The primary objective of medical therapy is to lower and stabilize blood glucose concentrations as close to normoglycemia as possible **(Oluwafemi *et al* ., 2023).**

Pharmacological treatment options are broadly categorized into oral and injectable agents.

**Oral antidiabetic drugs include:**

- Biguanides (e.g., **metformin**),
- Sulfonylureas (e.g., gliclazide),
- Dipeptidyl peptidase-4 (DPP-4) inhibitors (e.g., alogliptin),
- Sodium-glucose co-transporter 2 (SGLT2) inhibitors (e.g., canagliflozin).

**Injectable therapies encompass :**

- Insulins, which vary by onset and duration of action, including rapid-acting (e.g., insulin aspart), intermediate-acting (e.g., isophane insulin), and long-acting (e.g., zinc-crystalline insulin),

- Incretin-based therapies, particularly glucagon-like peptide-1 (GLP-1) receptor agonists (e.g., exenatide), which enhance glucose-dependent insulin secretion.

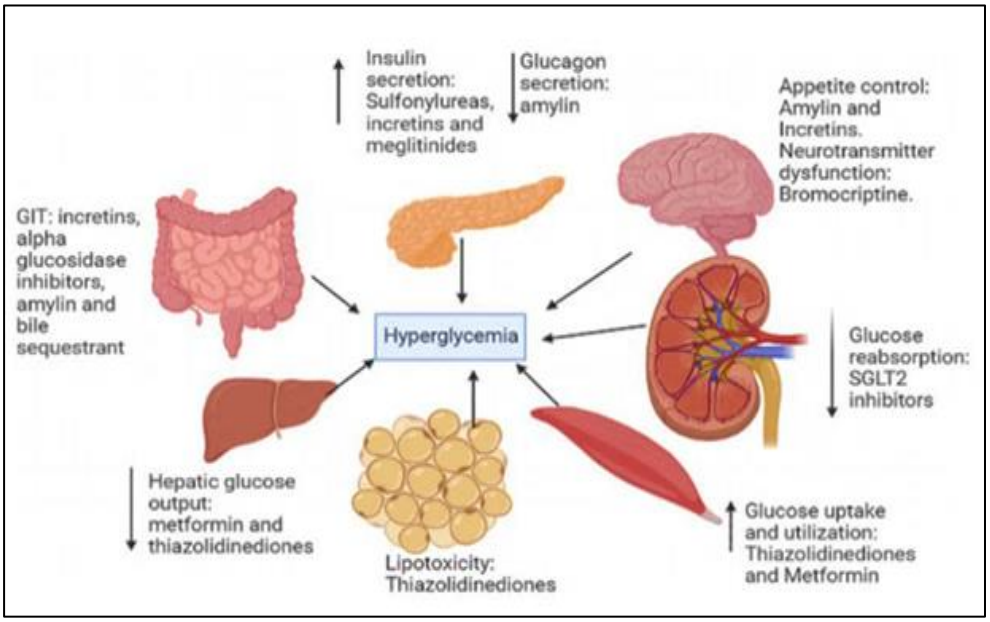



**Figure 3:** Sites of action of the pharmacological agents **(Oluwafemi *et al* ., 2023).**

## 2 .Metformin: a potential T2D manager

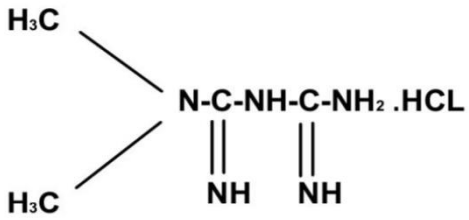
Metformin (1,1-dimethylbiguanide hydrochloride) is an oral anti-diabetic drug of the biguanide class and one of the most prescribed drugs worldwide. It is a widely used first-line oral antihyperglycemic medication for the management of T2D **(Safar Kheder *et al.*, 2018).**

### I.2.1 Metformin structure and origin

Metformin, chemically known as 1,1-dimethylbiguanide hydrochloride (molecule formula $C_4H_{12}ClN_5$, molecular weight approximately 165.62 g/mol) is a synthetic biguanide. Its structure features a biguanide core, which consists of two linked guanidine groups with two methyl substituents at the 1-position, making it highly hydrophilic and positively charged at physiological pH. This unique chemical structure underpins metformin's biological activity, including its ability to improve glycemic control by targeting liver and muscle tissues ***(*Safar Kheder *et al* ., *2018 ).***

In fact, metformin originates from a traditional herbal remedy *Galega officinalis* (French lilac) **(figure 4)**, used traditionally for diabetes-like symptoms. Active guanidine derivatives found in this plant inspired early research into glucose-lowering agents. First synthesized in 1922, metformin was introduced clinically in the 1950s by Jean Sterne and has since become the cornerstone treatment for T2D due to its effectiveness and safety **(Campbell, 2023).**

**(A)**




**(B)**

**Figure 4**: **(A)** *Galega officinalis* ; **(B)** metformin chemical formula**(Campbell, 2023).**

### 2.2 Metformin's Pharmacokinetics

Metformin's pharmacokinetics are characterized by oral absorption via transporter proteins, primarily organic cation transporters (OCTs) and plasma membrane monoamine transporters (PMAT), which facilitate its uptake in the small intestine and liver. Metformin exhibits low protein binding and limited distribution into most tissues. Minimal metabolism leads to a predictable duration of action. Renal excretion is the primary elimination pathway, making kidney function critical in metformin therapy. Variations in pharmacokinetic parameters can occur due to age, renal impairment, and transporter gene polymorphisms (***Lewis et al.,2024).***

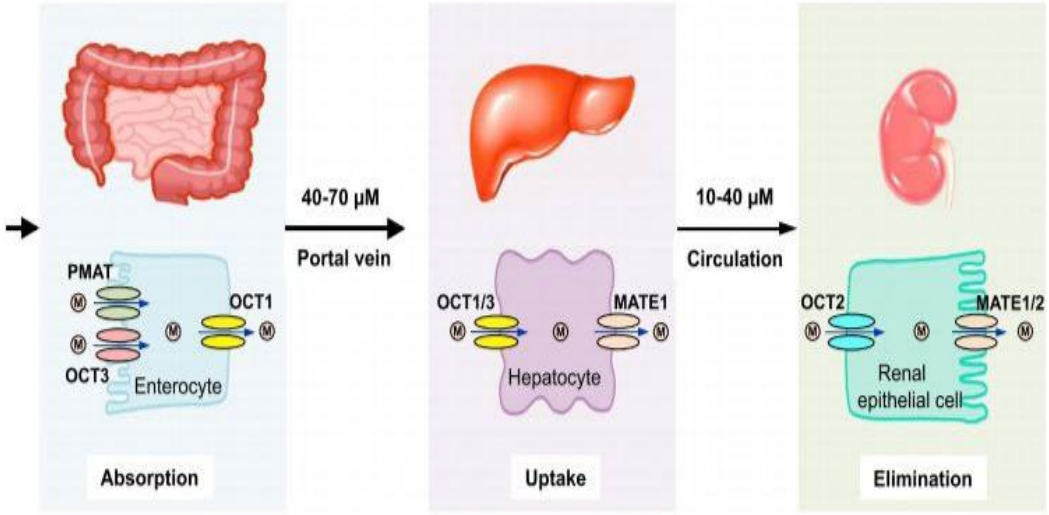



**Figure 5:** Pharmacokinetics of metformin (***Lewis et al.,2024).***

## 2.3 Pharmacodynamic

Metformin is effective in lowering fasting and postprandial blood glucose, as well as HbA1c levels. It acts on multiple organs, including the liver, muscle, fat, pancreas, and intestine. Its main hypoglycemic effects come from reducing hepatic glucose production and intestinal glucose absorption, while enhancing insulin sensitivity and β-cell function **(Ayyanna, 2021)**.

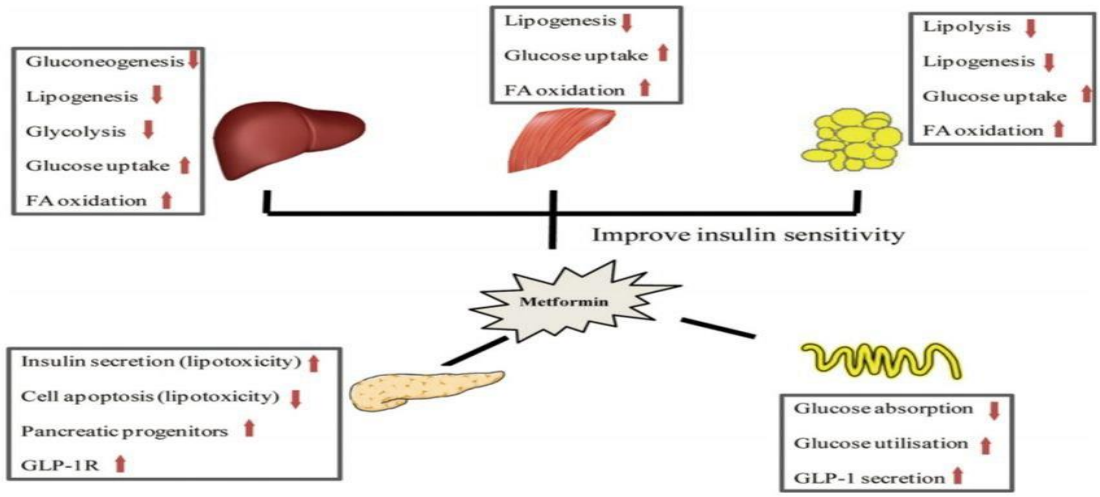



**Figure 6:** Action of metformin on target organs **(Ayyanna, 2021)**.

Metformin's pharmacodynamics primarily involve inhibition of hepatic gluconeogenesis, which lowers both fasting and postprandial blood glucose levels. It achieves this mainly by inhibiting mitochondrial complex I in liver cells, reducing ATP production and increasing the AMP:ATP ratio, which activates AMP-activated protein kinase (AMPK). AMPK activation leads to decreased glucose production, enhanced insulin sensitivity, and increased peripheral glucose uptake **(Ayyanna, 2021)**.

Besides the liver, metformin also acts in the intestines by reducing glucose absorption through downregulation of sodium-glucose transporter 1 (SGLT1) and promoting glucose uptake into enterocytes, which can increase glucose excretion into the gut lumen. It also stimulates glucagon-like peptide-1 (GLP-1) secretion, enhancing insulin release and inhibiting glucagon. ***(Tingting Zhou,et al.,2018)***

### 2.4 Metformin side effects

Generally, metformin is well tolerated; however, digestive issues (in 5–20% of patients) may occur, such as abdominal pain, cramps, diarrhea, nausea, vomiting, bloating, flatulence, loss of appetite, and a metallic taste. These usually appear at the beginning of treatment and are reversible. They can be reduced by starting with a low dose and taking the medication at the end of meals (**Tingting *et al.,* 2018)**.

Other less common side effects include vitamin B12 deficiency with long-term use. Since B12 is essential for DNA synthesis, fatty acid metabolism, and nervous system function, deficiency may cause anemia, fatigue, or mood disorders **(Hussain *et al.,* 2025)**.

## 3. Vitamin B12

Vitamin B12 (Cobalamin) is a water-soluble vitamin containing cobalt and functions as a crucial cofactor for various metabolic enzymes. The term vitamin B12 encompasses all biologically active cobalamin forms in humans, including cyanocobalamin, hydroxocobalamin, methylcobalamin, and adenosylcobalamin. While the first three are available in pharmaceutical preparations, the two physiologically active forms, adenosylcobalamin and methylcobalamin, are synthesized within cells from these precursors ***(Sayedali et al.,2023***).

Since the human body does not synthesize vitamin B12, dietary intake is essential. It is primarily found in animal-based foods such as liver, meat, poultry, egg yolk, fish, and shellfish.

The recommended dietary intake of vitamin B12 is approximately 2 micrograms per day for adults. However, requirements may vary depending on the stage of life ***(Rose Issam Ghemrawi et al., 2013)***

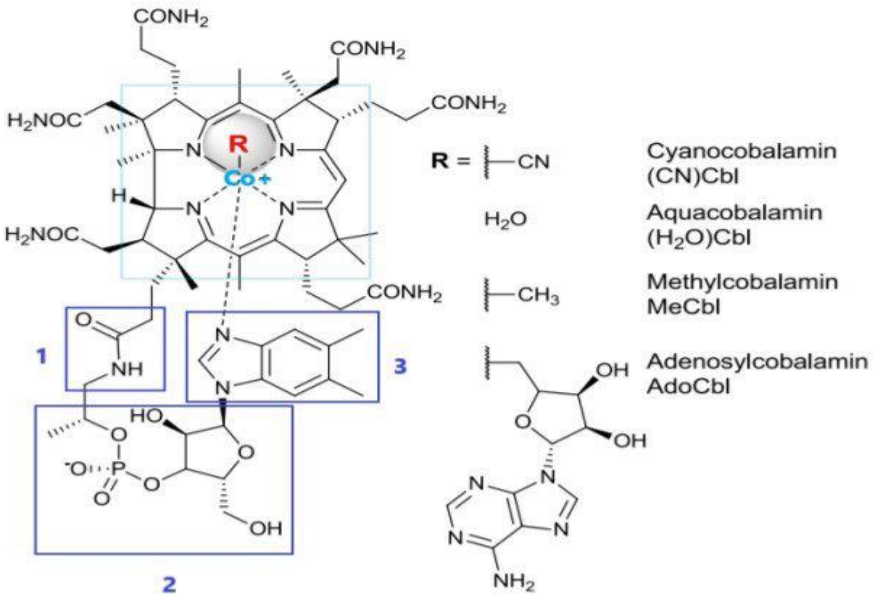



**Figure 7:** Structure of Cobalamin and its four derivatives ***(Varutti et al., 2024***).

### 3.1 Vitamin B12 absorption

Vitamin B12 undergoes a complex, multi-step absorption and transport process in the human body. Initially, it is released from food proteins in the stomach through the action of gastric acid and proteases and binds to the salivary protein haptocorrin. In the small intestine, this complex is degraded, allowing vitamin B12 to bind with intrinsic factor (IF) in the ileum. This IF-B12 complex is then absorbed via specific receptors and released into the bloodstream, where it binds to transcobalamin (TC) for distribution. Cellular uptake occurs through the CD320 receptor, followed by lysosomal release into the cytosol, mediated by ABCD4 and LMBD1 proteins. Intracellular processing involves conversion to methylcobalamin in the cytoplasm and adenosylcobalamin in mitochondria.

These active forms are essential cofactors in critical biochemical reactions, including DNA synthesis and energy metabolism (**Mucha *et al.*, 2024**).

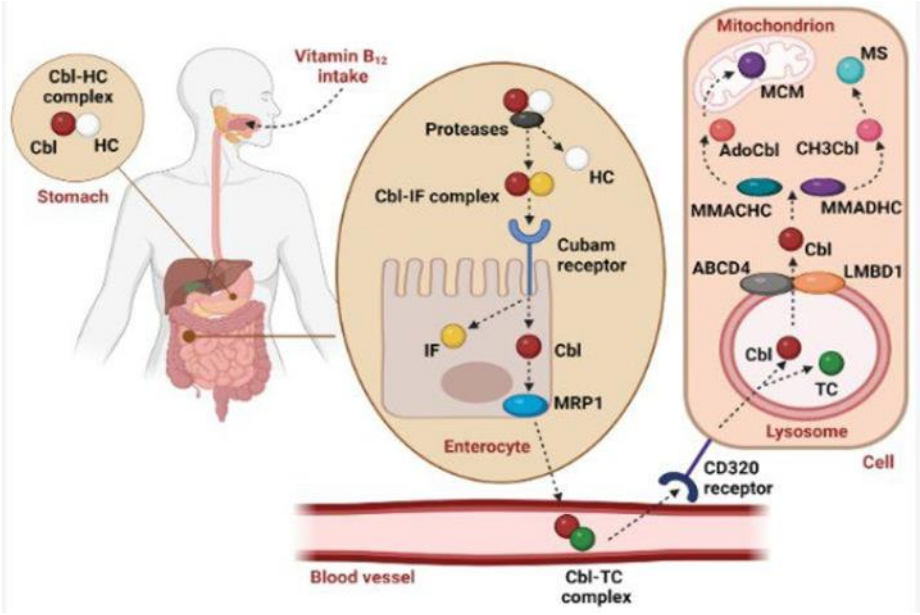



**Figure 8**: Adsorption, blood transport, and intracellular metabolism of vitamin B12 (**Mucha *et al.,* 2024).**

### 3.2 Clinical outcomes of vitamin B12 deficiency

Disruptions of vitamin B12 absorption pathways can lead to elevated homocysteine and methylmalonic acid levels contributing to neurological, hematological, and cardiovascular complications:

- **Neuropathy:**

Vitamin B12 deficiency contributes to peripheral neuropathy, characterized by numbness, pain, and weakness. It may also exacerbate cardiac autonomic neuropathy, increasing the risk of cardiovascular complications such as arrhythmias and sudden cardiac death ***(*Sayedali *et al.*,2023*).***

- **Anemia**

Vitamin B12 plays a crucial role in red blood cell maturation. Although evidence remains inconclusive, several studies and case reports suggest a potential association between vitamin B12 deficiency and megaloblastic anemia or reduced hemoglobin levels ***(*Sayedali *et al.*,2023*).***

# *Practical section*

# *Chapter II:*
# *Patients and Methods*

## II.1. Type and study setting

Our descriptive, analytic, retrospective, and cross-sectional study was conducted over a period from March to June 2025 in the internal medicine department of Salim Zemirli Hospital.

It aims to evaluate the effect of prolonged Metformin intake on certain biological parameters, especially vitamin B12, in patients with type 2 diabetes. The study included both clinical and laboratory data from a diverse group of patients.

## II.2. Study population

The study included patients diagnosed with T2D who were attending regular follow-up visits and were under metformin treatment for varying durations. A total of 64 patients were enrolled in the study, including 27 women and 37 men, with ages ranging from 21 and 94 years.

To ensure the relevance and reliability of the study population, specific inclusion and exclusion criteria were applied as follows:

### II.2.1Inclusion criteria

-Confirmed diagnosis with Type 2 diabetes.

-Continuous treatment with Metformin for a minimum duration of 6 months.

-Informed consent was obtained from all patients.

### II.2.2 Exclusion criteria

-Patients diagnosed with Type 1 diabetes.

-Patients with gestational diabetes.

-Patients receiving hypoglycemic treatment other than Metformin.

-Patients taking vitamin B12 supplementation or multivitamins

-History of pernicious anemia or gastrointestinal surgery affecting absorption

## II.3. Data Collection Methods

Data for this study were meticulously gathered using an information sheet **(appendix)** through a combination of clinical and biological measurements, as well as relevant patient records. The collection process involved two primary methods: the review of medical records and direct clinical evaluations. The selection period for medical records spanned from 2018 to 2024, ensuring the inclusion of comprehensive and relevant clinical data for analysis.

### II.3.1. Sociodemographic and clinical data

Data were obtained retrospectively from medical records and included variables such as age, sex, doses, and duration of metformin intake treatment. These variables were recorded at the time of the patients' most recent clinical evaluation. The population was further categorized into three groups based on the duration of metformin use:

- Less than 5 years
- Between 5 and 10 years
- More than 10 years

### II.3.2. Blood Pressure

Blood pressure was measured using an automatic blood pressure cuff on the right arm, after the patient had rested for at least five minutes in a supine position. This measurement was crucial for evaluating the cardiovascular health of participants, as hypertension is a common comorbidity in diabetic patients and may influence the study's outcomes.

### II.3.3. Biochemical parameters analysis

Biochemical parameters were assessed to evaluate the metabolic and nutritional status of the patients, with particular focus on markers potentially influenced by prolonged metformin use. The primary outcome of interest was serum vitamin B12 level, given its known association with metformin therapy. Additional biochemical data included fasting blood glucose, glycated hemoglobin (HbA1c), hemoglobin concentration, serum creatinine, urea, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. These values were obtained from the patients' most recent laboratory tests recorded in their medical files.

### II.3.3.1. Blood Sampling

Blood samples were collected between 8:00 and 9:00 a.m. following a 12-hour fasting period. Venous blood was drawn using a tourniquet and collected in either heparinized or dry tubes. The samples were then promptly centrifuged to separate the plasma for analysis.

### II.3.3.2. Measuring techniques

- **Blood Glucose (fasting glycemia)**

Plasma glucose is measured using an enzymatic and colorimetric method involving glucose oxidase (GOD). In this reaction, glucose is oxidized to gluconic acid and hydrogen peroxide. In the presence of peroxidase and phenol, the hydrogen peroxide oxidizes a colorless chromogen (4-aminoantipyrine) into a red-colored quinoneimine compound. The intensity of the resulting color is directly proportional to the glucose concentration in the sample and is measured photometrically at a wavelength of 505 nm.

- **HbA1c**

The HbA1c level was determined using an immunoturbidimetric assay. Blood samples were collected in EDTA tubes to prevent coagulation. The principle of the method is based on the agglutination reaction between HbA1c in the sample and latex particles coated with anti-HbA1c antibodies. The formation of immune complexes causes turbidity, which is measured photometrically. The intensity of turbidity is directly proportional to the concentration of HbA1c in the sample.

Results were expressed as a percentage (%) of total hemoglobin, with the analysis performed using an automated biochemistry analyzer.

- **Triglyceride assay**

This is based on the hydrolysis of triglycerides into glycerol and fatty acids under the action of a lipoprotein lipase. The glycerol formed is then converted to glycerol-3-phosphate, then oxidized to dihydroxyacetone-phosphate with the formation of hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed reacts in a TRINDER reaction with 4-aminophenazone and 4-chlorophenol to form a red-colored derivative.

The intensity of the red color developed is directly proportional to the triglyceride concentration and is measured photometrically. Normal values 0.40 to 1.5 g/l.

- **Total Cholesterol**

Cholesterol esterase hydrolyzes cholesterol esters to form free cholesterol and fatty acids. In a subsequent reaction catalyzed by cholesterol oxidase, cholesterol is converted, in the presence of oxygen, to cholestene-4 one-3 with the formation of hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed reacts with 4-aminophenazone (4-AAP) and phenol to form a red-colored derivative. The intensity of the coloration developed is directly proportional to the cholesterol concentration. It is determined by the increase in absorbance at 512 nm. Normal values : 1.10 to 2.00g/1.

- **HDL (High-Density Lipoprotein)**

HDL cholesterol is quantified using a direct enzymatic colorimetric method. Following blood collection after a 12-hour fast, serum is separated by centrifugation. Non-HDL lipoproteins are precipitated or blocked enzymatically, allowing specific measurement of HDL. The enzymatic reaction yields a colored product, the intensity of which is proportional to the HDL concentration. The absorbance is measured photometrically, and results are expressed in mg/dL.

- **LDL (Low-Density Lipoprotein)**

LDL cholesterol can be measured either directly using an enzymatic method or estimated indirectly using the Friedewald equation, provided that triglyceride levels are below 400 mg/dL. The equation is as follows:
LDL = Total Cholesterol – HDL – (Triglycerides / 5)
In the direct method, LDL particles are selectively measured after removal of other lipoprotein fractions. The analysis is typically performed using automated biochemistry analyzers, and results are also expressed in mg/dL.

- **Total hemoglobin (Hb)**

Total hemoglobin was determined by colorimetric methods using Drabkin's reagent, or by automated methods on hematology analyzers. Hemoglobin reacts to form a stable complex measured spectrophotometrically at a specific wavelength. This assay is used to assess blood status and detect anemia.

Normal values vary according to sex:

In women: 12 - 16 g/dL

Men: 13 - 17 g/dl

- **Vitamin B 12**

Vitamin B12 is assayed by an immunological method using chemiluminescence. The principle is based on a competitive reaction between serum vitamin B12 and a labelled form, in the presence of a specific antibody. The intensity of the light emitted is inversely proportional to the concentration of B12. Normal values range from 200 to 900 pg/mL.

- **Urea**

Urea was determined by an enzymatic method using urease. This enzyme hydrolyzes urea into ammonia and carbon dioxide. Ammonia is then measured by a colorimetric reaction. Normal values range from 0.15 to 0.45 g/L.

- **Creatinine**

Creatinine was measured by the Jaffé method, where it reacts with picric acid in an alkaline medium to form a colored complex measured at 520 nm. Normal values are 6 to 11 mg/L in women and 7 to 13 mg/L in men.

**II.4. Statistical analysis**

Data were collected and analyzed using XLStat 2019 (Addinsoft Inc., New York, USA). Descriptive statistics were used to summarize demographic and clinical characteristics.

After verification of Normality using Shapiro Wilk test, ANOVA test was used to compare biochemical parameters across groups categorized by the duration of metformin treatment. Tukey Post hoc test was applied when significant differences were found. Also, correlation analysis using Pearson test was performed to asses relationship between metformin duration and biochemical variables. Data was expressed as the Mean ± SD, and statistical significance was set at $p<0,05$

# *Chapter III :*

# *Results and discussion*

### 3.1. Data and population descriptions

#### 3.1.1.Gender distribution

The analysis reveals a predominance of male participants in the studied sample, with 59.38% males compared to 40.63% females, yielding a sex ratio of 1.46 in favor of males. This finding may reflect a higher prevalence of type 2 diabetes among men in the target population or could be attributed to differences in healthcare-seeking behavior and health awareness between sexes.

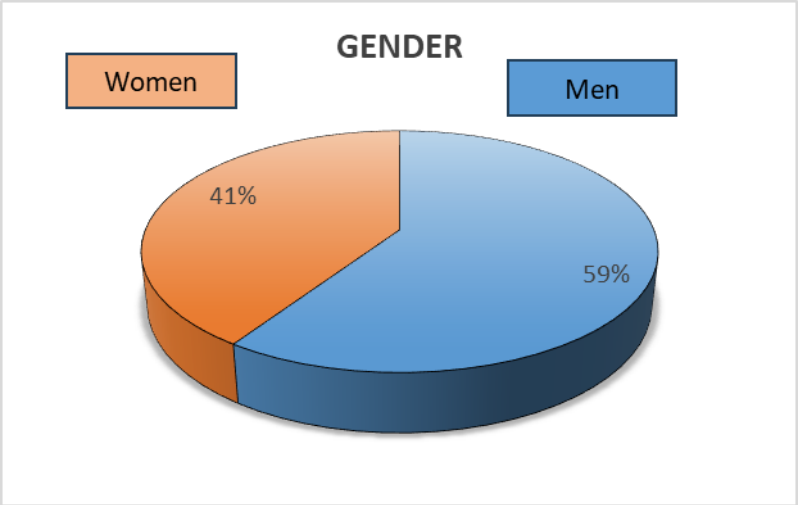



**Figure 09**: The distribution of the study population according to sex.

#### 3.1.2. Age distribution of subjects

The analysis indicates that most participants were aged 61 to 75 years (37.50%), followed by those aged 46 to 60 years (18.75%), and those over 75 years (17.19%).
Lower proportions were observed among younger age groups, with 15.63% in the 18–30 years category and 10.94% in the 31–45 years group.

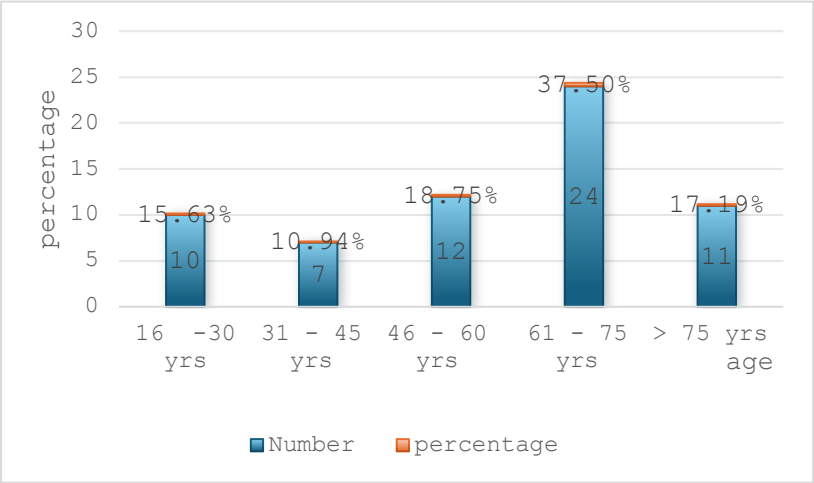



**Figure 10**: The distribution of study participants according to age.

### 3. 1.3. Metformin intake duration

The data reveal that more than half of the participants (51.56%) had been taking metformin for a duration between 5 and 10 years, indicating a widespread long-term reliance on this therapy in the management of T2D. Meanwhile, 31.25% of the patients had been on metformin for over 10 years, whereas only 17.19% had used it for less than 5 years.

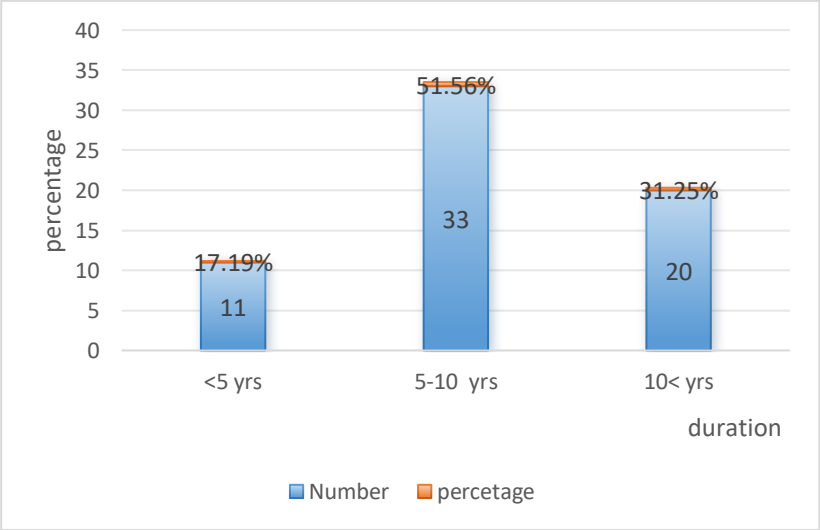



**Figure 11:** The distribution of patients based on the duration of metformin intake.

### 3.1.4. Metformin dose

The results indicate that the vast majority of patients (85.94%) were prescribed a daily dose of 850 mg, reflecting the preference for this intermediate dose in the management of T2D. Only 9.38% of patients were on a lower dose of 500 mg, while 4.69% received a higher dose of 1000 mg.

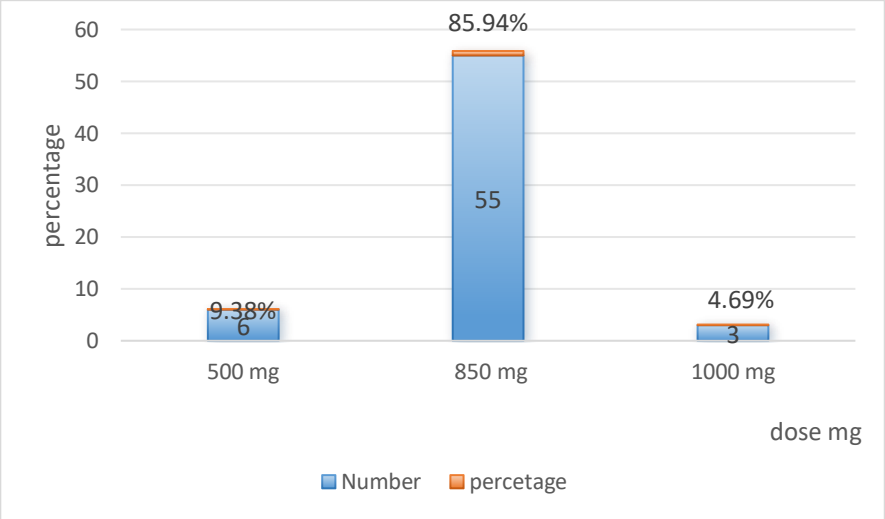



**Figure 12:** The distribution of patients according to their daily metformin dose.

### 3.1.5. Biochemical parameters in the study population

- **Distribution of fasting glycemia**

The analysis reveals that a significant majority (71.88%) had fasting glucose levels exceeding 1.26 g/L, indicating poor glycemic control among these patients. In comparison, 17.19% had glucose levels between 1.10 and 1.26 g/L, while only 10.94% had levels below 1.10 g/L. These findings highlight a high prevalence of fasting hyperglycemia in the studied sample, reflecting the chronic nature of T2D.

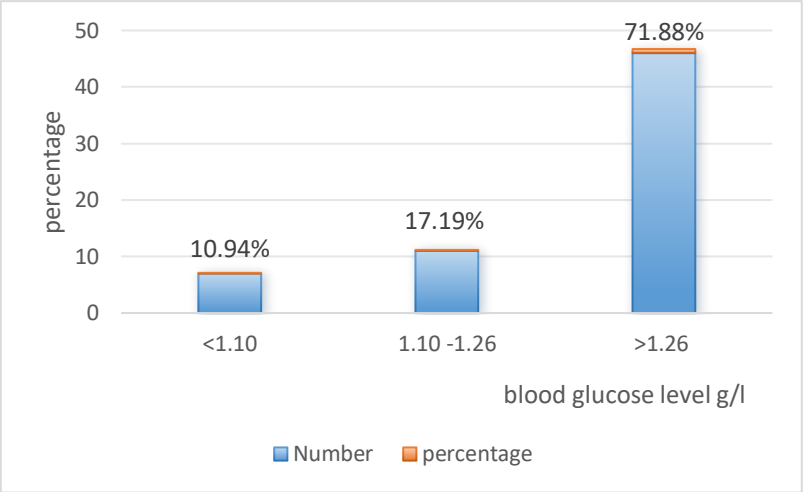



**Figure 13** : The distribution of participants according to fasting glycemia levels.

- **Distribution according to vitamin B12 levels**

The analysis shows that more than half of the participants (54.69%) had a confirmed vitamin B12 deficiency (levels <200 pg/mL), highlighting a high prevalence of deficiency among type 2 diabetic patients treated with metformin.

An additional 10.94% had borderline levels (200–300 pg/mL), while only 34.38% of the subjects had normal B12 concentrations (>300 pg/mL).

These findings underscore the necessity for routine screening of vitamin B12 levels in patients on metformin therapy, to prevent potential hematological and neurological complications associated with its deficiency.

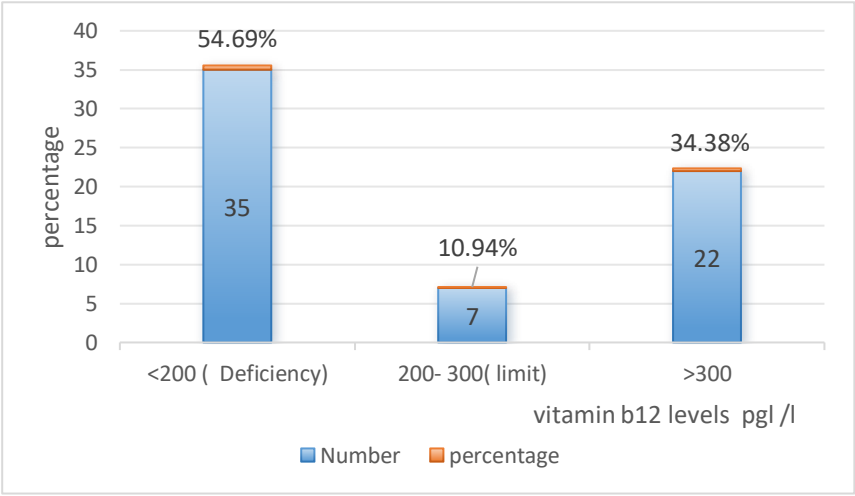



**Figure 14:** The distribution of participants according to serum vitamin B12 levels.

- **Hemoglobin**

Among female patients, 53.85% had hemoglobin levels within the normal range (12–15 g/dL), while 38.46% exhibited hemoglobin levels below 12 g/dL, indicating a substantial prevalence of anemia. Elevated levels (>15 g/dL) were observed in only 7.69% of women, suggesting that such occurrences are relatively uncommon in this group.

In male patients, 50% had hemoglobin values within the reference range (13–17 g/dL). However, 44.74% showed levels below 13 g/dL. Elevated hemoglobin levels (>17 g/dL) were noted in 5.26% of cases.

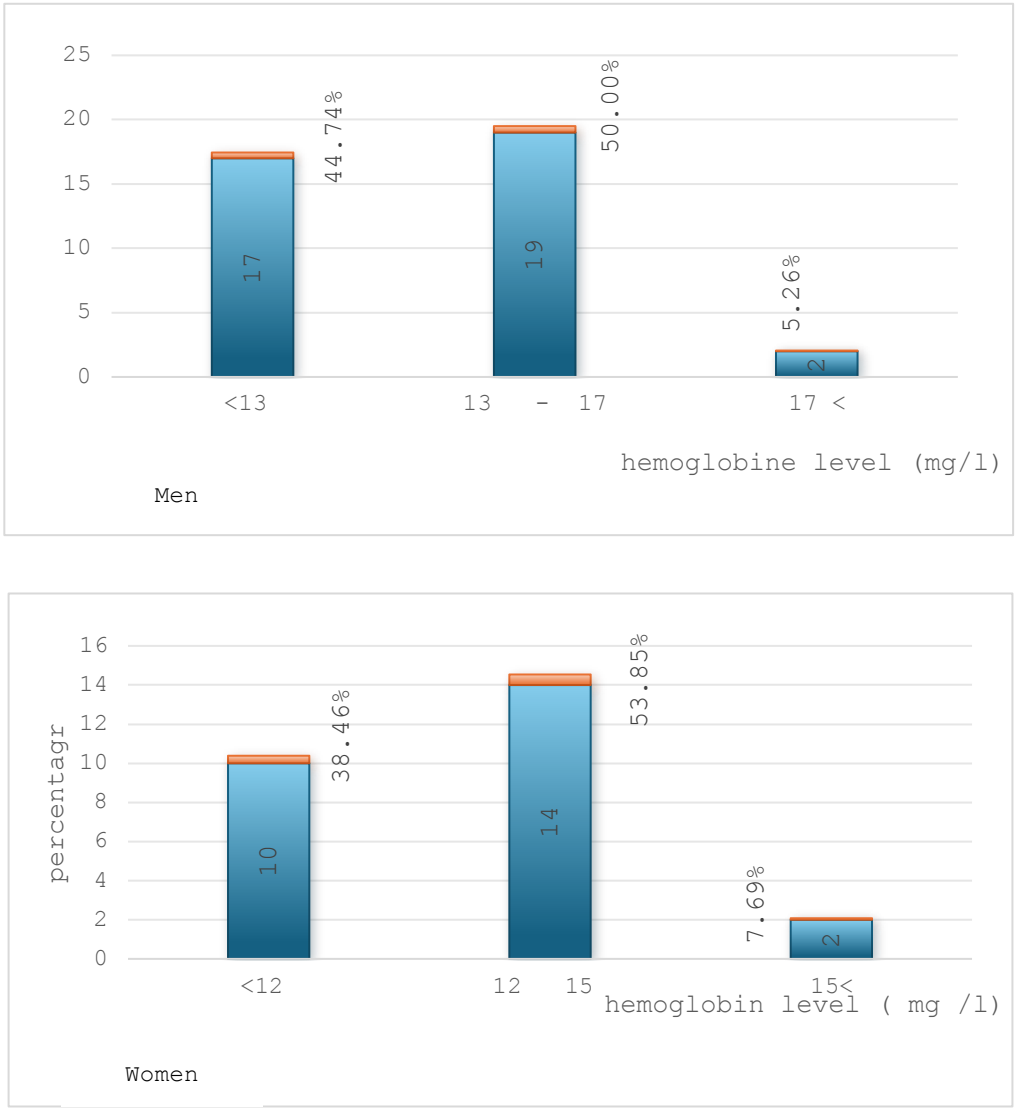



**Figure 15**: The distribution of hemoglobin values among participants, categorized by sex.

- **Distribution of diabetics according to HbA1c**

The analysis reveals that the vast majority of participants (79.69%) had HbA1c levels above 6.5%, which is consistent with poor glycemic control and confirms the diagnosis of type 2 diabetes in this group. 17.19% of individuals had HbA1c levels ranging between 5.7% and 6.4%, corresponding to a prediabetic state. Only 3.13% of participants showed normal HbA1c values (<5.7%).

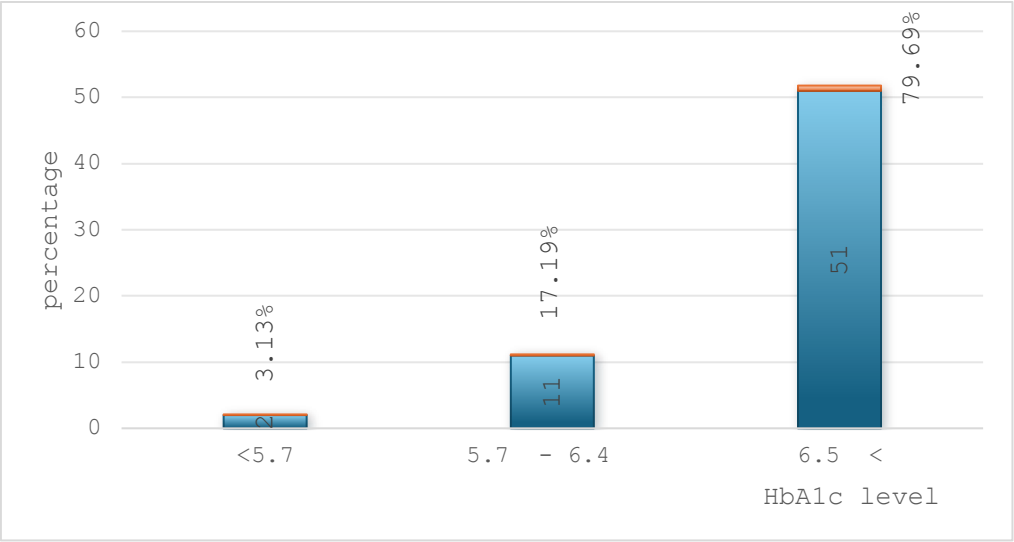



**Figure 16:** The distribution of patients according to their HbA1c levels.

- **Distribution according to urea and creatinine values**

The analysis revealed that the majority of participants (64.62%) had creatinine levels within the normal reference range (0.6–1.3 mg/dL), suggesting relatively stable kidney function in most cases. However, elevated creatinine levels were observed in 33.85% of the patients, which may reflect some degree of renal impairment. Only 1.54% of patients had creatinine levels below the normal range.

Regarding urea levels, the results showed that over half of the participants (56.25%) had values within the accepted reference range (0.15–0.45 g/L). Elevated urea levels were found in 39.06% of cases, potentially indicating impaired renal clearance or an indirect effect on kidney function. Conversely, low urea levels were reported in just 4.69% of patients.

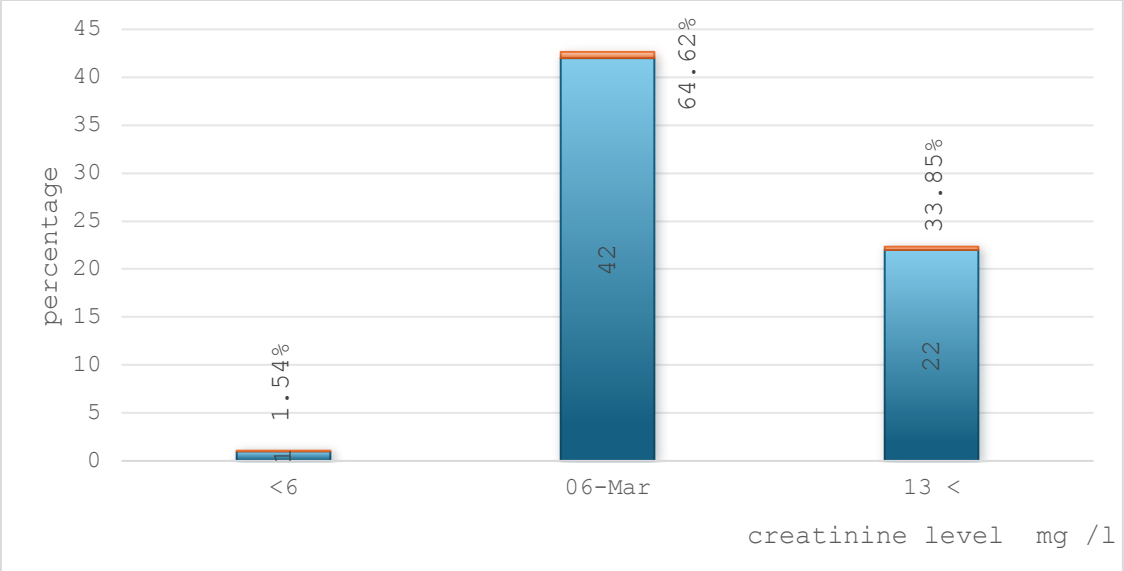



**Figure 17:** The distribution of serum creatinine levels.

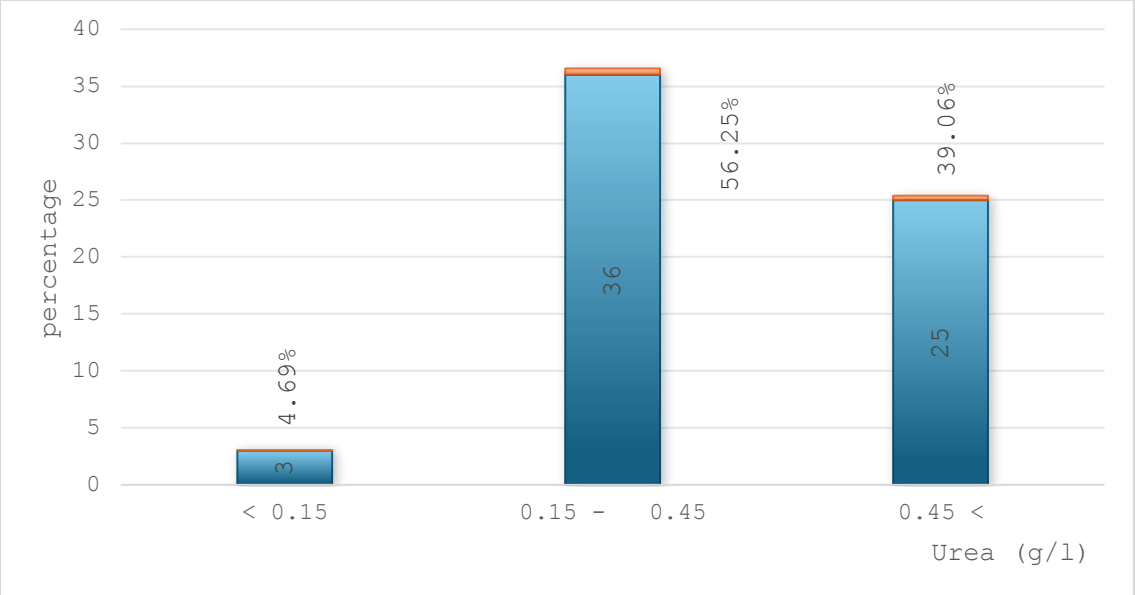



**Figure 18:** The distribution of urea levels.

- **Distribution of diabetics according to lipid parameters**

The analysis of triglyceride levels indicates that 46.88% of the participants had values below 1.5 g/L, reflecting normal levels, while 29.69% were in the borderline range (1.5–2 g/L), and 23.44% exceeded 2 g/L, suggesting moderate hypertriglyceridemia.
Regarding total cholesterol, 68.75% of the patients had values under 2 g/L, 21.88% were between 2 and 2.4 g/L, and 9.38% had elevated levels above 2.4 g/L, indicating varying degrees of cholesterol control within the sample.
For LDL cholesterol, 33.78% of subjects showed optimal levels below 1 g/L, 36.49% fell in the intermediate range (1–1.3 g/L), and 29.73% had elevated values.
In terms of HDL cholesterol, 68.75% of patients had levels above 0.4 g/L, considered protective, while 31.25% had values below this threshold.

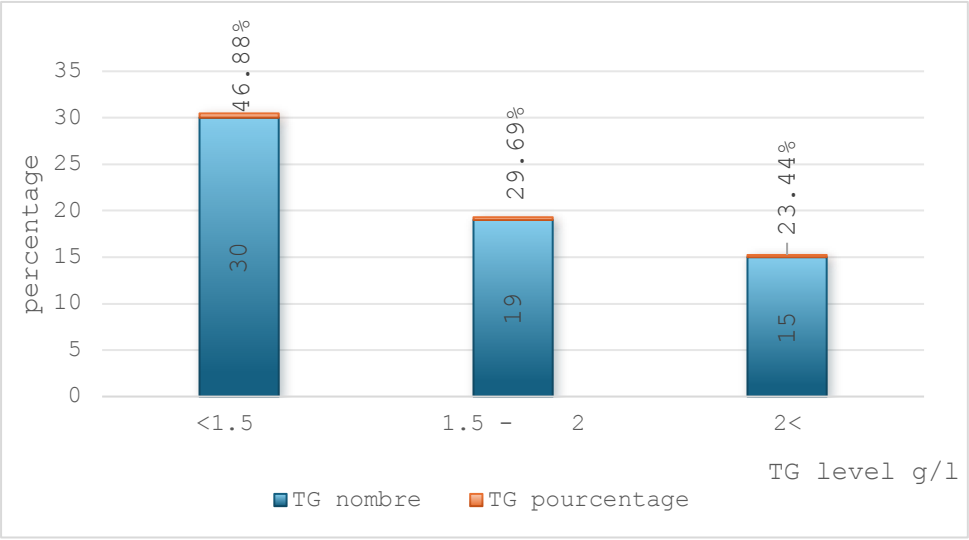



**Figure 19:** The distribution of triglyceride levels.

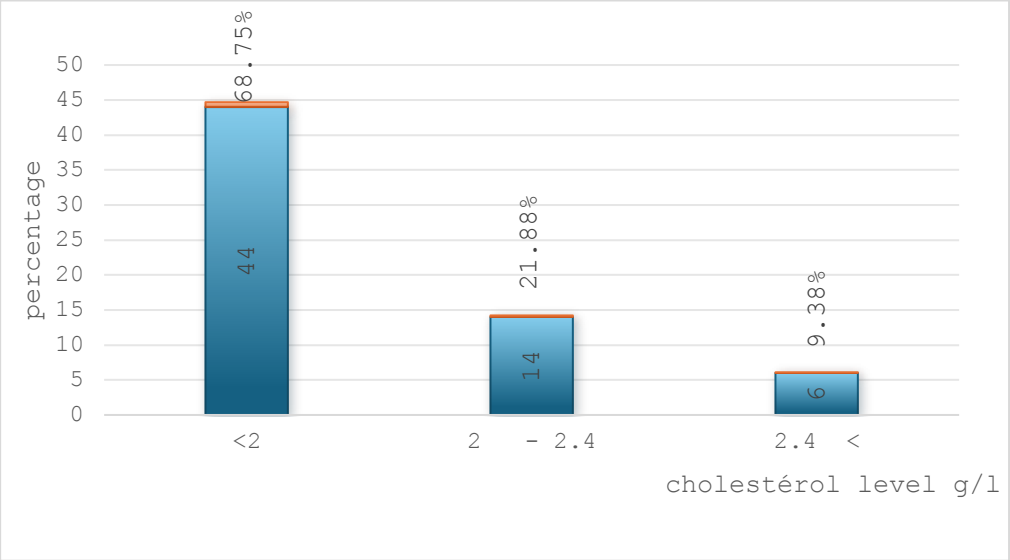



**Figure 20:** The distribution of total cholesterol levels

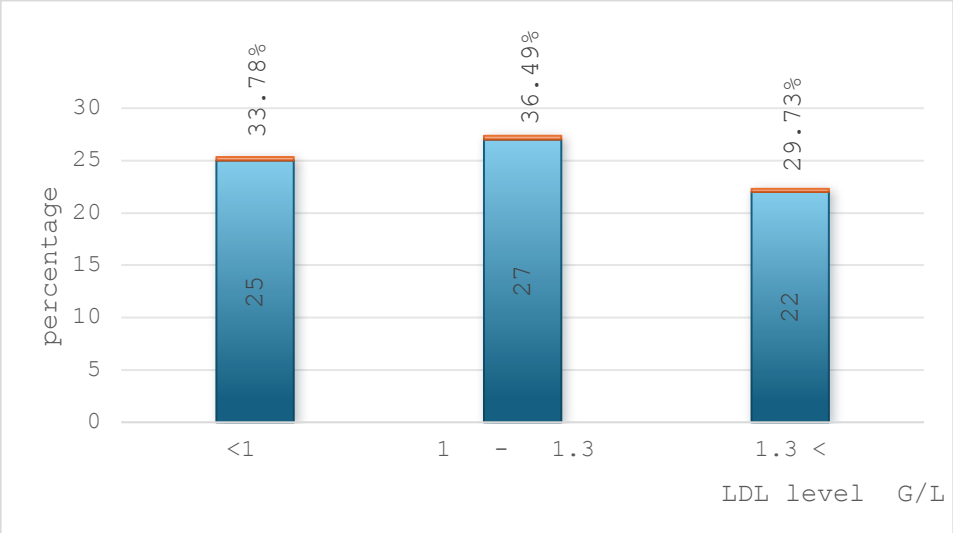



**Figure 22:** The distribution of LDL levels.

### 3.2. Comparison of biochemical parameters by metformin intake duration

#### 3.2.1.Vitamin B12 levels

The statistical analysis revealed a highly significant variation among the groups (p = 0.001). According to the Tukey post-hoc comparison, no significant difference was observed between the first two groups, with mean values of 492.574 ± 83.300 and 409.718 ± 37.240, respectively.

Conversely, the group with over 10 years of metformin exposure showed a markedly lower mean (219.685 ± 39.256), suggesting a notable decline in vitamin B12 levels with prolonged therapy.

**Table I.** Comparison of mean serum vitamin B12 levels according to duration of metformin use in T2D patients.

| Groups | Means ± SD | P value |
|---|---|---|
| <5 years | 492.574 ± 83.300[a] | **0.001** |
| 5 – 10 years | 409.718 ± 37.240[a] | |
| >10 years | 219.685 ± 39256[b] | |

Groups with different superscripts (a, b) are significantly different at $p < 0.05$ (post hoc test)

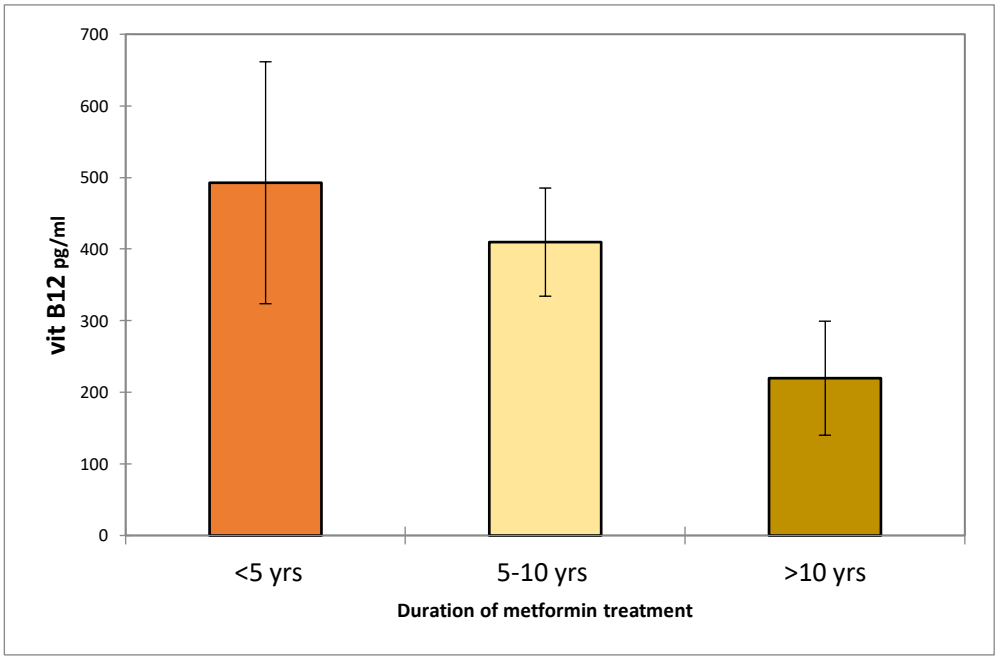



**Figure 23:** Mean serum vitamin B12 levels according to duration of metformin treatment.

### 3.2.2. Hemoglobin levels

The results revealed a statistically significant difference between the groups (p = 0.025). According to the post-hoc Tukey test, no significant difference was observed between the <5 years and 5–10 years groups (12.545 ± 0.603 and 12.126 ± 0.354, respectively). However, the group with metformin use for more than 10 years showed a significantly lower mean hemoglobin level (10.659 ± 0.485) compared to the other two groups.

**Table II.** Comparison of mean hemoglobin levels according to duration of metformin use in T2D patients.

| Groups | Means ± SD | P value |
|---|---|---|
| <5 yrs | 12.545 ± 0.603 [a] | **0.025** |
| 5- 10 yrs | 12.126 ± 0.354[a] | |
| >10 yrs | 10.659 ± 0.485 [b] | |

Groups with different superscripts (a, b) are significantly different at $p < 0.05$ (post hoc test)

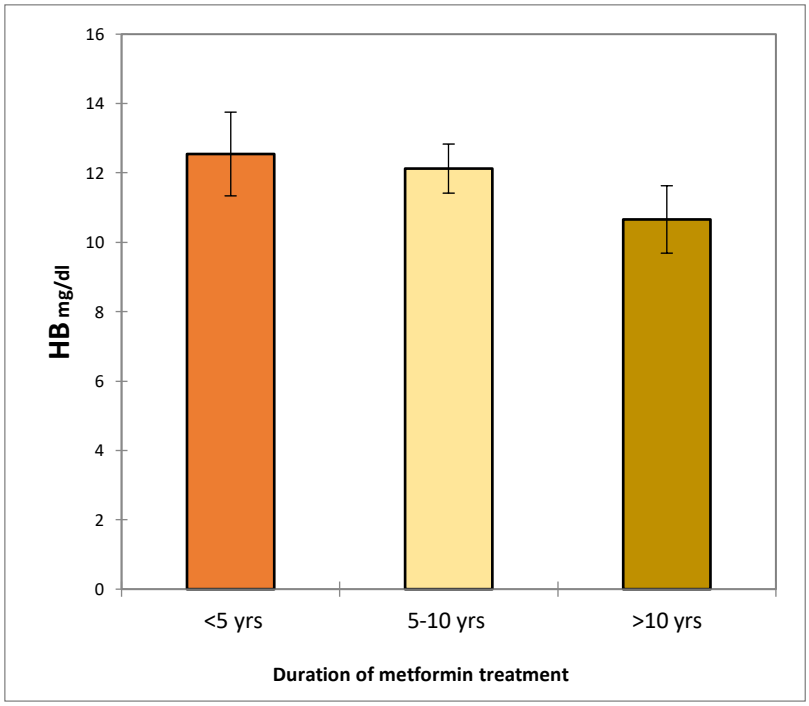



**Figure 24:** Mean hemoglobin levels according to duration of metformin treatment.

### 3.2.3. Fasting glycemia

Patients using metformin for less than 5 years showed the highest mean level (3.554 ± 0.256), while lower levels were observed in the 5–10 years and >10 years groups (Table III).

**Table III.** Comparison of mean blood glucose levels according to duration of metformin use in T2D patients.

| Groups | Means ± SD | P value |
|---|---|---|
| < 5 yrs | 3.554 ± 0.256[a] | **<0.0001** |
| 5 – 10 yrs | 1.650 ± 0.148[b] | |
| >10 yrs | 1.842 ± 0.195[b] | |

Groups with different superscripts (a, b) are significantly different at $p < 0.05$ (post hoc test)

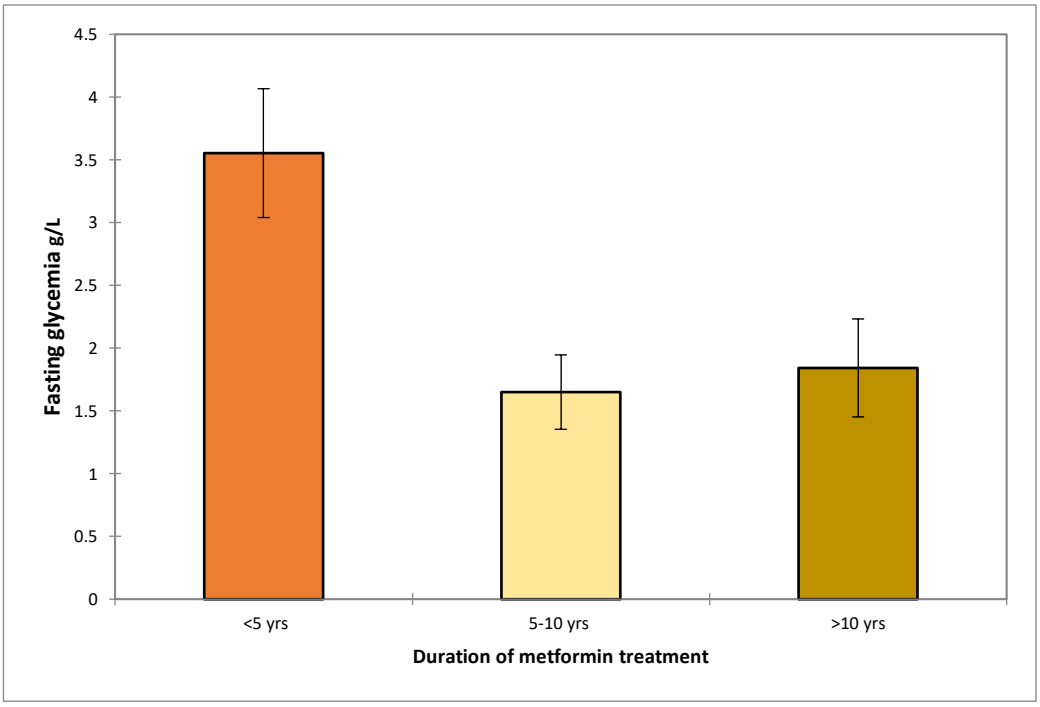



**Figure 25:** Mean fasting glycemia levels according to duration of metformin treatment.

### 3.2.4. HbA1c levels

ANOVA analysis showed a statistically significant difference in mean HbA1c levels across treatment duration groups (p = 0.030).

Patients treated for less than 5 years exhibited the highest average (10.597 ± 0.713), with progressively lower values in the 5–10 years and >10 years groups (Table IV; Figure 24).

**Table IV.** Comparison of HbA1c levels according to duration of metformin use in T2D patients.

| **Groups** | **Means ± Sd** | **P value** |
|---|---|---|
| <5 yrs | 10.597±0.713[a] | **0.030** |
| 5-10 yrs | 8.647 ± 0.590[ab] | |
| >10yrs | 8.342 ±0.441[b] | |

Groups with different superscripts (a, b) are significantly different at $p < 0.05$ (post hoc test)

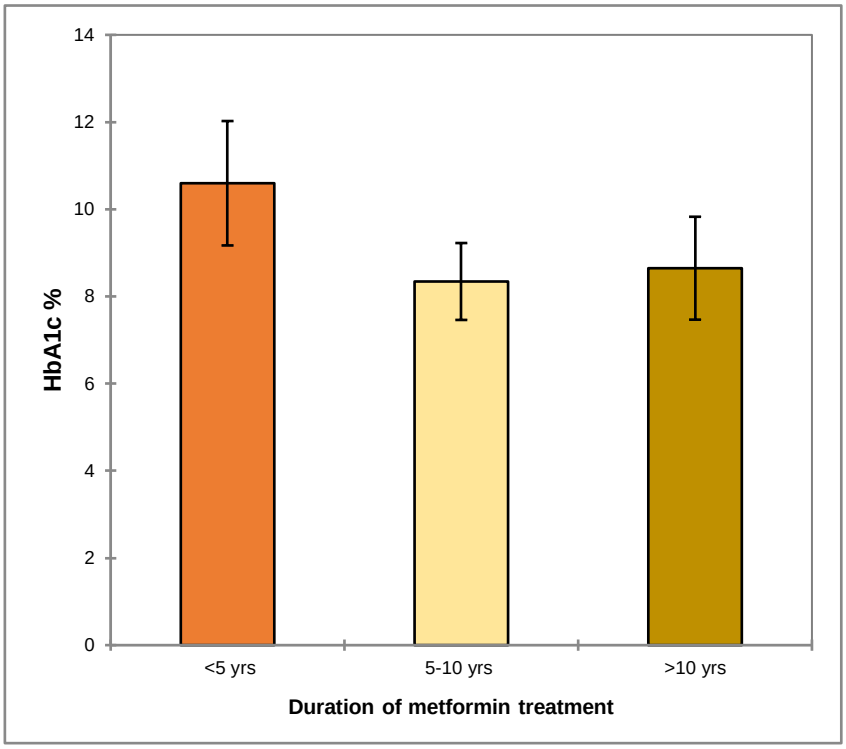



**Figure 26:** Mean HbA1c levels according to duration of metformin treatment.

### 3.2.5. Renal function parameters

The ANOVA analysis showed no statistically significant differences in mean urea levels among the three groups based on the duration of metformin use (p = 0.350).

Also, the comparison of serum creatinine levels across the three groups based on metformin treatment duration showed no statistically significant difference (p = 0.075).

**Table V.** Comparison of mean urea and creatinine levels according to duration of metformin use in T2D patients.

| Renal parameters | Groups | Means ± SE | P value |
|---|---|---|---|
| **Urea mg/dL** | 10yrs < | 0.495 ± 0.054[a] | **0.350** |
| | 5- 10 yrs | 0.453 ± 0.039[a] | |
| | < 5 yrs | 0.361 ± 0.074[a] | |
| **Creatinin mg/L** | < 5 yrs | 9.935 ± 0.912[a] | **0.075** |
| | 5- 10 yrs | 10.314 ± 0.563[a] | |
| | >10 yrs | 12.276 ± 0.754[a] | |

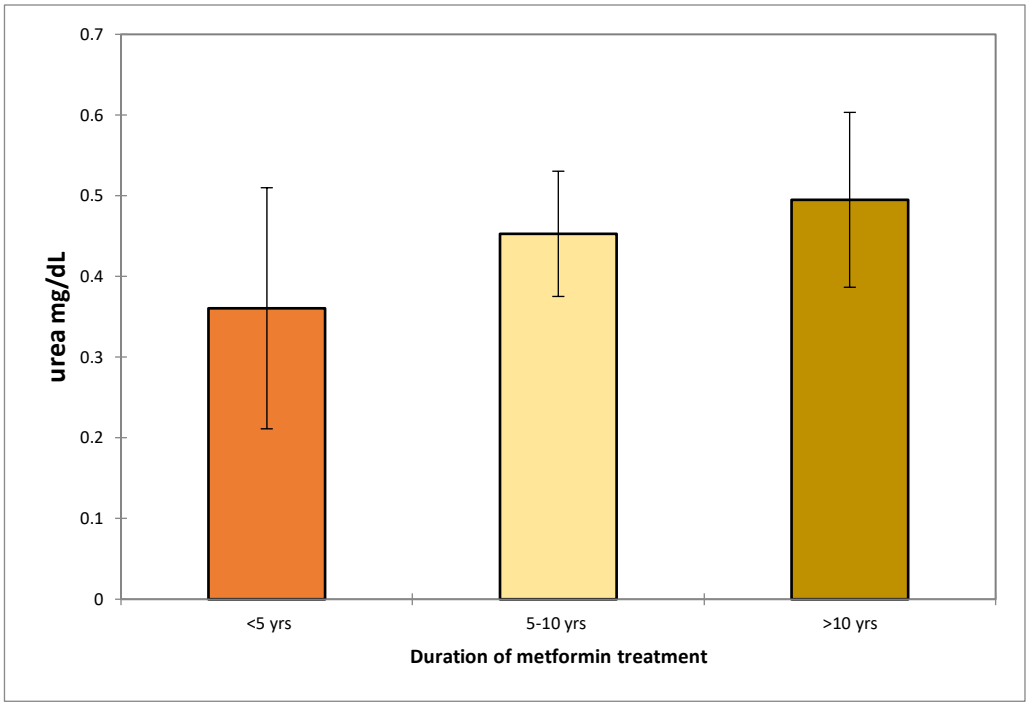



**Figure 27:** Mean urea levels according to duration of metformin treatment.

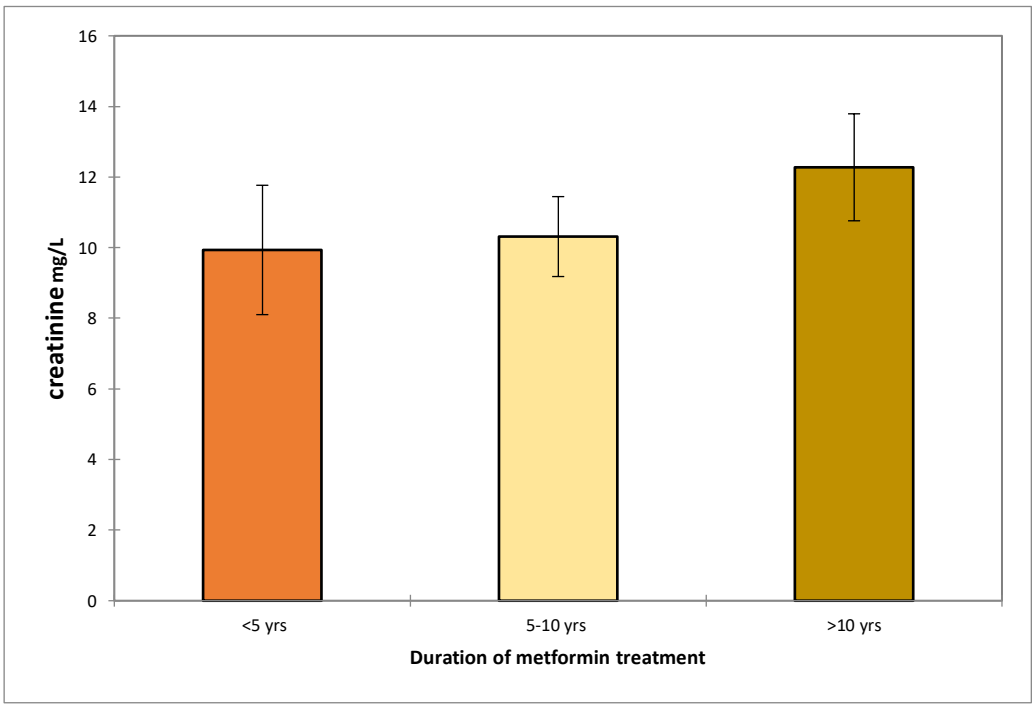



**Figure 28:** Mean creatinine levels according to duration of metformin treatment.

### 3.2.6. Lipid profile parameters

The statistical analysis of triglycerides (TG) levels across the three groups based on metformin treatment duration revealed no statistically significant differences ($p = 0.461$). Although slight variations in mean values were observed (Table VI; Figure 27)

The comparative analysis of total cholesterol levels across the three metformin treatment duration groups revealed no statistically significant differences ($p = 0.233$). Although the group with less than 5 years of metformin use had a slightly lower mean cholesterol level (1.527 g/l ± 0.129)

The evaluation of HDL cholesterol levels across the different metformin exposure groups did not reveal any statistically significant differences ($p = 0.128$).
Although participants with less than 5 years of treatment displayed a slightly reduced average HDL value (0.371 g/l ± 0.038), this decrease was not statistically meaningful.

The comparison of LDL cholesterol levels between the three treatment duration groups did not yield statistically significant differences ($p = 0.655$).
The group treated with metformin for less than 5 years exhibited a lower mean LDL value (0.895 g/l ± 0.289), while slightly higher averages were noted in the 5–10 years (1.204 g/l ± 0.179) and over 10 years (1.163g/l ± 0.239) groups.

**Table VI .** Comparison of lipid profile parameters according to duration of metformin use in T2D patients.

| **Groups** | **Triglyceride g/L** | **P value** |
|---|---|---|
| < 5 yrs | 1.548 ± 0.191 [a] | **0.461** |
| 5-10 yrs | 1.307±0.118 [a] | |
| >10 yrs | 1.495 ± 0.158 [a] | |

| **Groups** | **Total cholesterol g/L** | **P value** |
|---|---|---|
| < 5 yrs | 1.527 ± 0.129[a] | **0.233** |
| 5-10 yrs | 1.710 ± 0.075[a] | |
| >10 yrs | 1.808 ± 0.098[a] | |

| Groups | HDL g/L | P value |
|---|---|---|
| <5yrs | 0.371±0.038 [a] | **0.128** |
| 5-10 yrs | 0.456 ±0.023 [a] | |
| 10yrs | 0.461 ± 0.031 [a] | |

| Groups | LDL g/L | P value |
|---|---|---|
| < 5 yrs | 0.895± 0.289 [a] | **0.655** |
| 5-10 yrs | 1.204 ± 0.179 [a] | |
| >10 yrs | 1.163 ±0.239 [a] | |

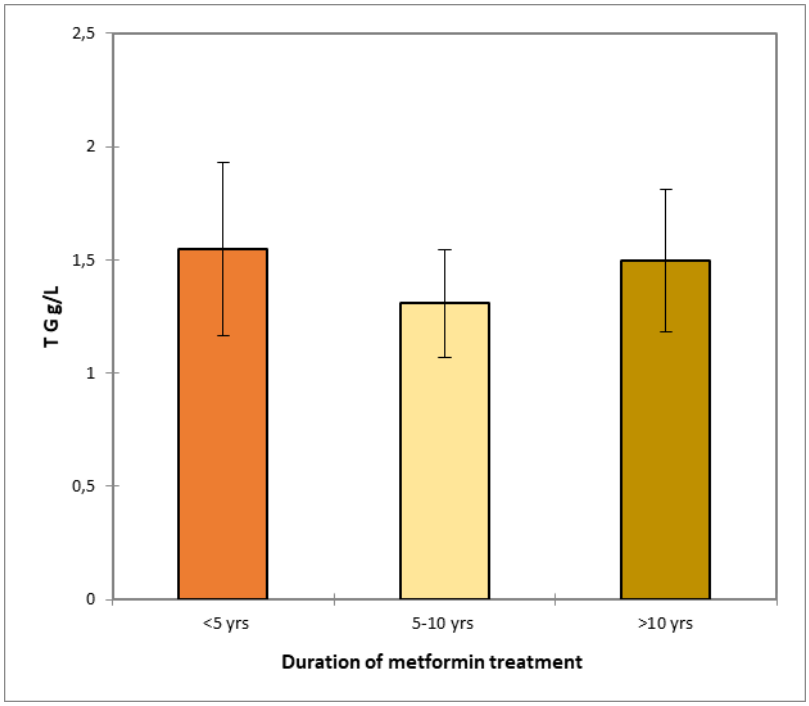



**Figure 29:** Mean triglyceride levels according to duration of metformin treatment.

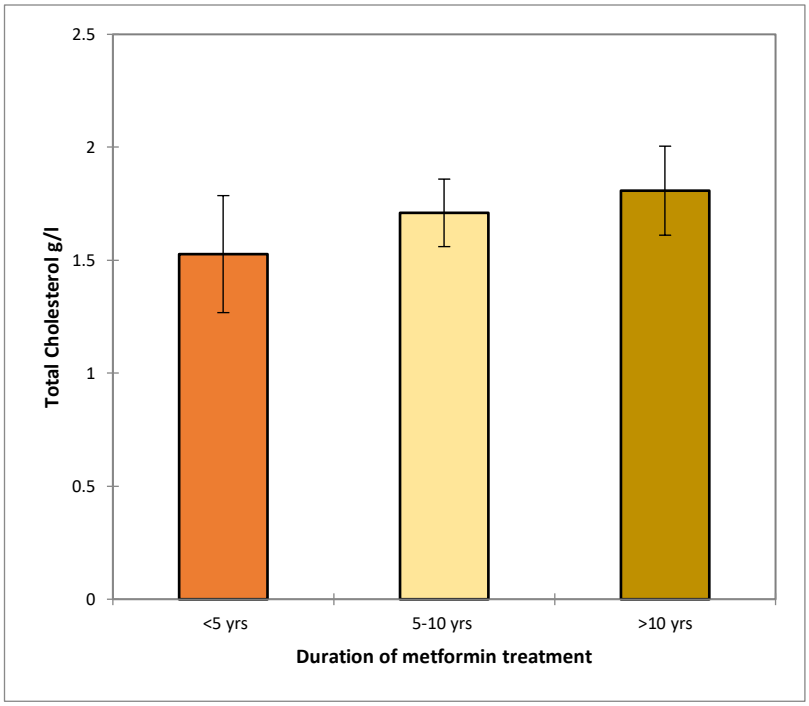



**Figure 30:** Mean total cholesterol levels according to duration of metformin treatment.

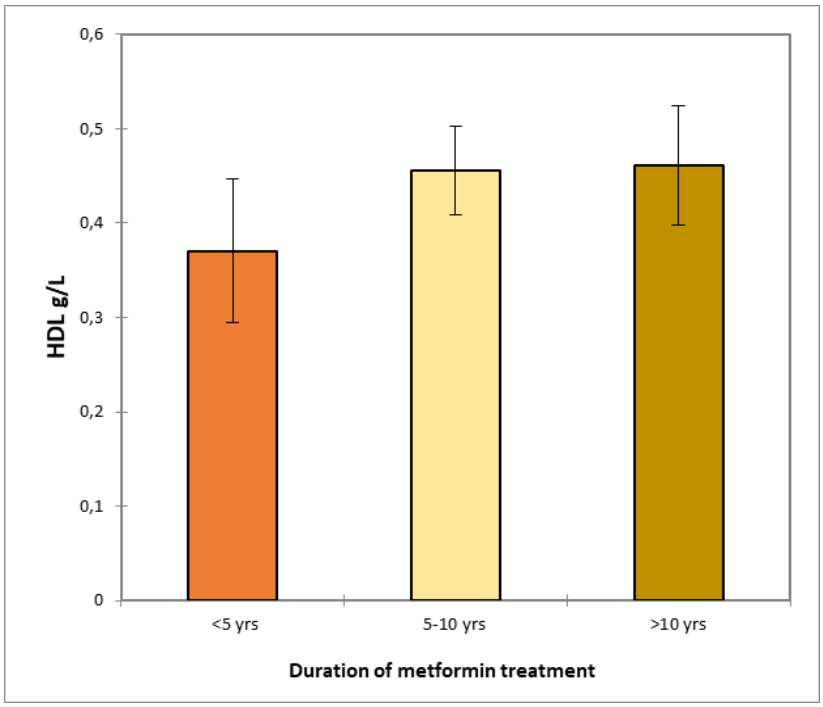



**Figure 31:** Mean HDL levels according to duration of metformin treatment.

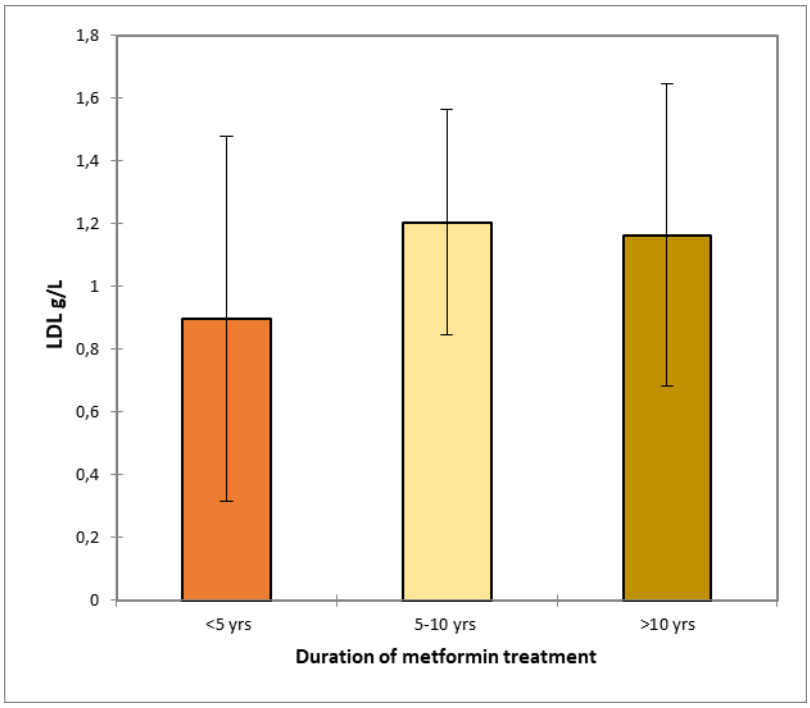



**Figure 32:** Mean LDL levels according to duration of metformin treatment.

### 3.3. Correlation between metformin duration and biochemical parameters

The correlation analysis conducted between the duration of metformin use and various biochemical parameters revealed several notable associations:

In fact, a moderate positive correlation was found between metformin use duration and serum creatinine levels ( $p = 0.037$), suggesting a slight increase in creatinine with prolonged treatment, which may reflect mild changes in renal function.

Also, a moderate inverse correlation was observed between treatment duration and hemoglobin levels ( $p = 0.006$), implying that longer metformin exposure may be associated with a reduction in hemoglobin.

A strong negative correlation was found between the duration of metformin use and vitamin B12 levels ( $p < 0.0001$), supporting the results found with the comparison test above.

A moderate negative correlation was identified between the duration of metformin therapy and fasting blood glucose levels ( $p = 0.002$), demonstrating a statistically significant association with improved glycemic control over time.

Regarding the rest of the biochemical parameters studied, no significant correlation ($p>0,05$) was observed.

## 4. Discussion

In this study, data from 64 patients with type 2 diabetes mellitus (T2DM) were analyzed. All participants were undergoing metformin therapy for varying durations. The primary aim was to assess the impact of long-term metformin use on key biological markers such as vitamin B12 levels, hemoglobin, blood glucose, renal function, and lipid profile, as well as to explore any potential complications associated with the treatment. The findings reveal a relationship between the duration of metformin use and biological changes in patients, offering deeper insight into the long-term effects of this therapy on patient health.

Our results indicate an inverse relationship between the duration of metformin use and serum vitamin B12 levels, suggesting that prolonged treatment with metformin may be associated with reduced vitamin B12 concentrations. These results are in line with recent studies that consistently demonstrate that long-term metformin therapy is associated with a significant reduction in serum vitamin B12 levels. The risk of deficiency increases with both the dose and duration of metformin use. For example, a large prospective study found that patients with a higher metformin usage index (a product of daily dose and duration) had a markedly increased risk of vitamin B12 deficiency, with the highest risk observed in those with the longest and highest exposure **(Infante *et al.,* 2021)**. Another study confirmed that prolonged and high-dose metformin treatment is inversely related to vitamin B12 levels, with reductions ranging from 14% to 30% in serum concentrations **(Bhal *et al.,* 2023)**. These findings have led to recommendations for periodic monitoring of vitamin B12, especially in patients with risk factors or symptoms suggestive of deficiency **(Hussein *et al.,* 2025).**

Researchers have indicated that metformin may contribute to vitamin B12 deficiency by reducing the absorption of the intrinsic factor–vitamin B12 complex via the cubilin receptor in the terminal ileum. This can result in complications such as peripheral neuropathy, autonomic cardiac neuropathy, neuropsychiatric disorders, or hematological abnormalities **(Hussein *et al.,* 2025).**

The results of our study revealed a moderate, statistically significant negative correlation between metformin use and hemoglobin levels, indicating that prolonged use of metformin is associated with a decrease in hemoglobin concentrations. Several studies, including two randomized clinical trials and a real-world observational study, have demonstrated that metformin use is associated with an increased risk of early-onset anemia in patients with type 2 diabetes. Although the precise mechanism underlying this early decline in hemoglobin levels remains unclear, the timing suggests that vitamin B12 deficiency alone does not fully explain the phenomenon, highlighting the need for further research to elucidate the potential underlying mechanisms **(Yang *et al.,* 2019; Donelly *et al.,*2020; Albai *et al.,* 2020).**

As shown by our findings, a weak but statistically significant positive correlation was observed between the duration of metformin use and serum creatinine levels, suggesting a potential mild impact of prolonged therapy on this renal parameter. In contrast, no statistically significant relationship was found between the duration of metformin use and blood urea levels, indicating an absence of a measurable effect of the drug on this indicator. Overall, these results suggest a limited effect of metformin duration on renal function among the studied sample of patients with type 2 diabetes.

Metformin is primarily eliminated by the kidneys, and its clearance is closely related to renal function, as measured by creatinine clearance. Studies indicate that as renal function declines (reflected by increased creatinine levels), metformin clearance decreases, leading to higher plasma concentrations of the drug **(Lipska *et al.,* 2011; Tseng, 2024).** This relationship underscores the importance of monitoring renal function in patients on long-term metformin therapy, as impaired clearance can increase the risk of adverse effects, including lactic acidosis.

Despite these risks, metformin remains highly effective in improving glycemic control, lowering HbA1c, and favorably modifying lipid parameters over time. Long-term studies have shown that metformin therapy is associated with sustained reductions in blood glucose and HbA1c levels, as well as beneficial effects on body weight and insulin requirements **(Olga *et al.,* 2019)**. Some evidence also suggests a reduction in macrovascular complications with prolonged use **(Kooy *et al.,* 2009)**. These findings confirm the efficacy of metformin as a cornerstone in the management of type 2 diabetes.

Moreover, other studies have indicated that initiating metformin therapy early, within three Months of a diabetes diagnosis consistently lead to improvements in fasting blood glucose And HbA1c levels during the first 24 months following diagnosis **(Zheng *et al.*, 2024).**

Furthermore, other researchers have confirmed that in newly diagnosed type 2 diabetic patients, metformin therapy led to a significant decrease in LDL and triglyceride (TG) levels, as well as an increase in HDL, without the use of lipid-lowering medications **( Szu *et al.*, 2018; Flores *et al.*, 2025 ).**

# *Conclusion*

In this study, we conducted a retrospective cross-sectional analysis on a sample of 64 patients with type 2 diabetes undergoing metformin therapy. The results revealed a negative correlation between the duration of metformin use and vitamin B12 concentrations, suggesting a potential impact of prolonged treatment on this biological parameter. A moderate decrease in hemoglobin levels was also observed, while no significant effect was found on lipid parameters, fasting glycemia, HbA1c levels, or urea levels.
However, a weak but statistically significant correlation was noted with serum creatinine.

Prolonged metformin intake is effective for glycemic and metabolic control but is associated with a time- and dose-dependent decrease in vitamin B12 and hemoglobin levels and may impact renal function over time. Therefore, it is essential to consider regular monitoring and individualized patient management to maximize benefits and minimize risks

Finally, this study highlights the need to:

- Expand the sample size and the geographic scope of the study
- Conduct longitudinal studies to assess the long-term clinical consequences of vitamin B12 deficiency in diabetic patients treated with metformin;
- Evaluate the effectiveness of targeted preventive supplementation.
- Explore the underlying biological mechanisms in order to develop more precise prevention and management strategies.

# *References*

- Abel, E. D., Gloyn, A. L., Evans Molina, C., Joseph, J. J., Misra, S., Pajvani, U. B., Simcox, J., Susztak, K., & Drucker, D. J. (2024). *Diabetes mellitus—Progress and opportunities in the evolving epidemic*. *Cell, 187*(15), 3789–3820.

- Akalu, Y., & Birhan, A. (2020). *Peripheral arterial disease and its associated factors among type 2 diabetes mellitus patients at Debre Tabor general hospital, Northwest Ethiopia*. *Journal of Diabetes Research, 2020*, 9419413.

- Albai, O., Timar, B., Paun, D. L., Sima, A., Roman, D., & Timar, R. (2020). *Metformin treatment: a potential cause of megaloblastic anemia in patients with type 2 diabetes mellitus*. *Diabetes, Metabolic Syndrome and Obesity*, 13, 3873–3878.

- Ayyanna, M. C. (2021). *Pharmacokinetic and Pharmacodynamic Interaction between Metformin and Fenofibrate in Animal Models*. *Asian Journal of Pharmaceutics (AJP), 15*(1).

- Bahl, G., Hussain, M. S., Saraswat, N., & Agrawal, M. (2023). *Beyond Diabetes Management: Unraveling Metformin's Long-Term Effects on Vitamin B12*. *Clinical Diabetology*, *12*(5), 279–282.

- Belhadj, M., Arbouche, Z., Brouri, M., Malek, R., Semrouni, M., Zekri, S., ... & Abrouk, S. (2019). *BAROMÈTRE Algérie: enquête nationale sur la prise en charge des personnes diabétiques. Médecine des maladies Métaboliques, 13*(2), 188–194.

- Belhadj, M., Malek, R., Baghous, H., Boukheloua, M., Arbouche, Z., Nouri, N., ... & Haireche, M. A. (2025). *Perspectives of type 2 diabetes mellitus management in Algeria: a comprehensive expert review. Frontiers in Clinical Diabetes and Healthcare, 6*, 1495849.

- Campbell, J. M. (2023). *Metformin*. In *Anti-Aging Pharmacology* (pp. 71–88). Academic Press.

- Dixit, J. V., Badgujar, S. Y., & Giri, P. A. (2022). *Reduction in HbA1c through lifestyle modification in newly diagnosed type 2 diabetes mellitus patient: A great feat*. *Journal of Family Medicine and Primary Care, 11*(6), 3312–3317.

- Donnelly, L. A., Dennis, J. M., Coleman, R. L., Sattar, N., Hattersley, A. T., Holman, R. R., & Pearson, E. R. (2020). *Risk of anemia with metformin use in type 2 diabetes: a MASTERMIND study*. *Diabetes Care, 43*(10), 2493–2499.

- Flores-Estrada, J. J., Pinto-García, L. J., Nájera-García, N., Marché-Fernández, O. A., & Cáceres-Carranza, F. J. (2025). *Metformin in the management of non-alcoholic fatty liver disease: current evidence and future perspectives*. *Cardiovascular and Metabolic Science, 36*(1), 51–57.

- Ghemrawi, R. I. (2013). *Vitamin B12 deficiency induces endoplasmic reticulum stress due to decreased SIRT1 deacetylase and increased HSF1 acetylation* (Doctoral dissertation, University of Lorraine).

- Gillani, S. W., et al. (2021). *Effet de la metformine sur les profils lipidiques du diabète de type 2 : méta-analyse d'essais contrôlés randomisés*. *Journal of Pharmacy and Bioallied Sciences, 13*(1), 76–82.

- Goyal, R., Singhal, M., & Jialal, I. (2023). *Type 2 diabetes*. *StatPearls [Internet]*.

- Hsu, W.-H., et al. (2017). *Effet de la metformine sur la fonction rénale chez les patients atteints de diabète de type 2 et d'insuffisance rénale chronique modérée*. *Oncotarget, 9*(4), 5416.

- Hussain, M. S., Srivastava, N., Singh, G., & Kumar, R. (2025). *Long-term use of metformin and vitamin B12 Deficiency in Diabetes*. *Current Drug Safety, 20*(3), 258–270.

- Infante, M., Leoni, M., Caprio, M., & Fabbri, A. (2021). *Long-term metformin therapy and vitamin B12 deficiency: an association to bear in mind*. *World Journal of Diabetes, 12*(7), 916.

- Kefi, M. (2021). *Contribution to the study of vitamin B12 deficiency and homocysteine metabolism in patients treated with metformin* (Doctoral dissertation, Université Côte d'Azur, Nice, France).

- Khamis, A. M. (2023). *Physiopathologie, critères diagnostiques et approches de la rémission du diabète de type 2*. *Cureus, 15*(1), e33908.

- Kheder, S. (2018). *The investigation of the effects of Metformin in Thyroid Cancer* (Doctoral dissertation, The University of Sheffield).

- Kooy, A., De Jager, J., Lehert, P., Bets, D., Wulffelé, M. G., Donker, A. J., & Stehouwer, C. D. (2009). *Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus*. *Archives of Internal Medicine, 169*(6), 616–625.

- Lewis, A., Williams, K., & Oroszi, T. (2024). *Metformine : parcours pharmacocinétique et pharmacodynamique à travers l'organisme*. *Pharmacology & Pharmacy, 15*(12), 466–477.

- Lipska, K. J., Bailey, C. J., & Inzucchi, S. E. (2011). *Use of metformin in the setting of mild-to-moderate renal insufficiency*. *Diabetes Care, 34*(6), 1431–1437.

- Mucha, P., et al. (2024). *Métabolisme de la vitamine B12 : un réseau de processus multiprotéiques*. *Revue Internationale des Sciences Moléculaires, 25*(15), 8021.

- Ojo, O. A., et al. (2023). *Diabète sucré : du mécanisme moléculaire à la physiopathologie et à la pharmacologie*. *Medicine in Novel Technology and Devices, 19*, 100247.

- Ong, K. L., Stafford, L. K., McLaughlin, S. A., Boyko, E. J., Vollset, S. E., Smith, A. E., ... & GBD 2021 Diabetes Collaborators. (2023). *Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: A systematic analysis for the Global Burden of Disease Study 2021*. *The Lancet, 402*(10397), 203–234.

- Patyra, A. (2024). *Effets et mécanisme sous-jacent des métabolites secondaires des plantes sur la régulation de la sécrétion d'insuline* (Doctoral dissertation, Université de Montpellier ; Université de médecine de Varsovie).

- Rosenfeld, R. M., Grega, M. L., & Gulati, M. (2025). *Lifestyle interventions for treatment and remission of type 2 diabetes and prediabetes in adults: implications for clinicians*. *American Journal of Lifestyle Medicine*. https://doi.org/10.1177/15598276251325802

- Sayedali, E., Yalin, A. E., & Yalin, S. (2023). *Association entre la metformine et la carence en vitamine B12 chez les patients atteints de diabète de type 2*. *World Journal of Diabetes, 14*(5), 585.

- Tseng, C. H. (2024). *Metformin-associated lactic acidosis and mortality in type 2 diabetes patients hospitalized with heart failure and/or acute coronary syndrome: A neglected clinical scenario and the potential role of insulin*. *International Journal of Cardiology, 414*.

- Varutti, L. (2024). *Vitamin B12: Biochemical, nutritional, pathological data and diagnostic and therapeutic uses in animals of veterinary interest* (Doctoral dissertation, Université Paris-Est Créteil).

- Wójcik-Kulig, M., Ulatowska, J., & Plech, T. (2022). *Effects of metformin–current perspectives in treatment of selected diseases*. *Biomedicine & Pharmacotherapy, 149*, 112832.

- Xie, C., et al. (2024). *Impact du moment d'administration de la metformine sur les réponses glycémiques et du glucagon-like peptide-1 à la perfusion intraduodénale de glucose dans le diabète de type 2 : une étude croisée, randomisée, en double aveugle et contrôlée par placebo*. *Diabetologia, 67*(7), 1260–1270.

- Yang, W., Cai, X., Wu, H., & Ji, L. (2019). *Associations between metformin use and vitamin B12 levels, anemia, and neuropathy in patients with diabetes: a meta-analysis*. *Journal of Diabetes, 11*(9), 729–743.

- Zheng, M., et al. (2024). *Comparaison de l'effet d'un traitement précoce ou tardif par metformine sur les paramètres glycémiques chez les adultes australiens atteints de diabète : données probantes issues d'une base de données nationale de médecine générale*. *Clinical Therapeutics, 46*(5), 396–403.

- Zhou, T., et al. (2018). *Aperçu préclinique de la metformine pour le traitement du diabète de type 2*. *Biomedicine & Pharmacotherapy, 106*, 1227–1235.

# *Annex*

## Fiche d'exploitation

<table>
<tr><td rowspan="4">Informations générales</td><td>Numéro du Dossier :</td><td>Sexe : Femme ☐ Homme ☐</td></tr>
<tr><td>Nom et Prénom :</td><td>Numéro Téléphone :</td></tr>
<tr><td>Age :</td><td>Adresse :</td></tr>
<tr><td>Date de 1 ère consultation :</td><td>Fréquence des consultations :</td></tr>
<tr><td rowspan="5">Examen Clinique</td><td>Poids : Kg / Taille : Cm</td><td>Dose de Metformine :</td></tr>
<tr><td>IMC : ☐ entre 25 et 29 Kg/m² ☐≥ 30 Kg/m²</td><td>Rythme de prise de Metformine :</td></tr>
<tr><td>Tour de Taille (cm) :</td><td>Maladie de Crohn : ☐ Oui ☐ Non</td></tr>
<tr><td>Début du diabète :</td><td>Maladie de Biermer : ☐ Oui ☐ Non</td></tr>
<tr><td>Durée du diabète :</td><td></td></tr>
<tr><td rowspan="15">Examen biologique</td><td colspan="2">Bilan glycémique</td></tr>
<tr><td>Glycémie à jeun : ☐ ≥ 1.1 g/l</td><td>HOMA-IR : ☐ > 2.6 ☐ non</td></tr>
<tr><td>HbA1c : ☐ > 6 % ☐ non</td><td>Insulino-résistance : ☐ oui ☐ non</td></tr>
<tr><td colspan="2">Bilan lipidique</td></tr>
<tr><td>Cholestérol Total : ☐ ≥ 2 mg/l ☐ non</td><td>Triglycerides: ☐ ≥ 1.5 g/l ☐ non</td></tr>
<tr><td>HDL cholestérol : ☐ ≤ 0.40 g/l ☐ non</td><td>Dyslipidémie : ☐ Oui ☐ Non</td></tr>
<tr><td colspan="2">LDL cholestérol : ☐ ≥ 1 g/l ☐ non</td></tr>
<tr><td colspan="2">Syndrome carentiel</td></tr>
<tr><td>Vitamine B 12 : pg/mL</td><td>Hémoglobine : g/dL</td></tr>
<tr><td>Vitamine B 9 : ng/mL</td><td>Ferritine : ng/mL</td></tr>
<tr><td>Vitamine D : ng/mL</td><td>Homocystéine : mmol/l</td></tr>
<tr><td colspan="2">Bilan immunologique</td></tr>
<tr><td>Anticorps anti-Cellules pariétales : UI/ml</td><td>Anticorps anti-Facteur Intrinsèque : UI/ml</td></tr>
<tr><td>Neuropathie périphérique :</td><td>Fibroscopie œsogastrique :</td></tr>
</table>

People's Democratic Republic of Algeria

The Ministry of Higher Education and Scientific Research

Saad Dahlab Blida1 University

Faculty of Natural and Life Sciences
Department of Biology

**Final Dissertation**

To obtain a master's degree in Natural and Life Sciences

**Branch:** Biological Sciences

**Option:** Pharmacotoxicology

**Theme**

# Impact of prolonged metformin intake on vitamin B12 levels in type 2 diabetic patients



Examinatrice
Dr. Djairene N

Presented by :

**Mrs. Gaci Meriem and Mrs. Mazouz Mounia**

**In front of a jury composed of:**

| | | | |
|---|---|---|---|
| **Dr Douaouri N.H** | MCB | Chairman | USDB1 |
| **Dr Djairene N.** | Docteur | Examiner | USDB1 |
| **Dr Settar A.** | MAB | Supervisor | USDB1 |
| **Pr Zitouni L.** | Professor | Co-supervisor | ESH Salim Zemirli |

**Academic year :2024-2025**