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Beyond the Benefits: High-Dose Metformin and Emerging Metabolic Risks in type 2 diabetic patients

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KEYWORDS

Type 2 diabetes; Dose-dependent effects; Metformin; Metabolic disorder; Oxidative stress.

ABSTRACT:

Introduction: Metformin is well-known for its several beneficial effects on diabetic complications. However, the results regarding the dose-effect relationship remain unclear.

Objectives: This study aimed to evaluate the effects of different doses of metformin on the metabolic profile and oxidative markers in Type 2 diabetes (T2D) patients.

Methods: A total of 285 T2D patients were stratified into three groups based on their metformin dosage: 500–1000 mg/day (Dose 1, n = 58), 1000–1500 mg/day (Dose 2, n = 66), and 1500–3000 mg/day (Dose 3, n = 161). A control group of 90 non-diabetic subjects was included. Metabolic (glucose, lipid profile, renal, and hepatic function), hematological and coagulation parameters, and oxidative stress markers (malondialdehyde (MDA), carbonylated proteins (CP), nitric oxide (NO), superoxide radical (O_2), catalase (CAT), and reduced glutathione (GSH)) were analyzed.

Results: Despite metformin therapy, patients displayed persistent hyperglycemia and elevated HbA1c levels, indicating suboptimal glycemic control. A dose-dependent worsening of lipid parameters was observed, characterized by increased LDL and triglycerides and reduced HDL levels, suggesting an elevated cardiovascular risk. Hematological alterations—including reduced red blood cells and hemoglobin, along with increased bilirubin and ferritin—were more pronounced at higher doses, possibly reflecting hepatic stress. Furthermore, high-dose metformin was associated with elevated inflammatory and oxidative stress markers, potentially compromising its antioxidant and anti-inflammatory properties.

Conclusions: These findings highlight a potential risk associated with higher doses of metformin in T2D patients, emphasizing the need for personalized dosing strategies and regular monitoring of metabolic and oxidative indicators to minimize adverse effects.

1. Introduction

Type 2 diabetes (T2D) is a complex metabolic disorder characterized by chronic hyperglycemia due to insulin resistance and impaired pancreatic beta-cell function [1]. In addition to impaired glucose metabolism, diabetic patients demonstrate other metabolic disturbances, including dyslipidemia, altered fatty acid metabolism and mitochondrial dysfunction, which contribute to cardiovascular risk and other complications [2]. Several lipid/lipoprotein abnormalities have been observed in T2D patients, including elevated cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol and lower high density lipoprotein (HDL) cholesterol levels [3]. Oxidative stress is one of the key contributors to these complications, arising from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses of the organism. High glucose levels and free fatty acids in diabetic patients can exacerbate oxidative stress, leading to further cell and organ damages, especially in insulin-sensitive organs such as the liver, muscles, and pancreas [4]. Previous studies have shown that T2D is associated with oxidative stress, as a result of increased formation of reactive oxidative substances and reduced antioxidant defense mechanisms [5]. It is also associated to an

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inflammatory state that may contribute to the development of lipid metabolism and tissue function alterations and to many diabetes-related co-morbidities [6]. Additionally, several complications have been observed in T2D such as retinopathy, nephropathy, neuropathy, hypertension, hormonal alterations, mineral imbalances and changes in blood cell parameters, platelet function, and coagulation profiles [7].

Metformin, the most widely prescribed drug for T2D, is well-known for its role in reducing hepatic glucose production and improving insulin sensitivity. Additionally, metformin has been shown to have several beneficial effects on hematological parameters [8]. Furthermore, metformin has anti-inflammatory properties that may help to normalize platelet aggregation and improve vascular function and health. However, growing evidence suggests that while metformin effectively treats hyperglycemia, it may also interfere with other metabolic pathways and can exacerbate oxidative stress [9,10]. Previous studies indicate that metformin can affect lipid metabolism, lead to metabolic acidosis, reduce mitochondrial energy production, and impair nutrient absorption leading to additional metabolic challenges in T2D patients [11]. However, despite research on the positive and adverse effects of metformin, the results regarding the dose-effect relationship remain unclear. Indeed, to our knowledge, there is no study on the comparative effects of different metformin doses on both metabolic parameters and redox markers in T2D patients.

2. Objectives

This study aimed to evaluate the effects of different doses of metformin on the metabolic profile and oxidative markers in Algerian T2D patients, providing a comprehensive perspective on metformin's impact on metabolic health. These findings will add valuable insights for optimizing metformin treatment in T2D and underscore the importance of monitoring dosage.

3. Methods

Participants

This study was conducted at the Dr. Tidjani Damerdji University Hospital in Tlemcen, Algeria. A total of 285 patients with an established diagnosis of type 2 diabetes were recruited, all of whom were undergoing metformin treatment at varying doses. These patients were categorized into three groups based on their daily metformin dose: T2D-dose 1 (500-1000 mg, n = 58), T2D-dose 2 (1000-1500 mg, n = 66), and T2D-dose 3 (1500-3000 mg, n = 161). Inclusion criteria required diabetic participants aged 45 years or older, with a confirmed diagnosis of type 2 diabetes by physicians, and treated with metformin at doses ranging from 500 to 3000 mg per day. Exclusion criteria included individuals with type 1 diabetes, secondary diabetes, or gestational diabetes. The study gathered comprehensive demographic data, including age, gender, and body mass index (BMI), as well as information on diabetes type, duration of illness, daily metformin dose. In addition, a control group consisting of 90 non-diabetic, healthy individuals were included. The control group was well-matched to the diabetic patients in terms of anthropometric variables (age, BMI) to ensure comparability.

Blood samples

Venous blood samples were drawn from participants following an overnight fast, using the antecubital vein. Standard vacutainers containing EDTA, heparin, and citrate were utilized to collect blood for the analysis of various parameters, including hematological, biochemical, and oxidative stress markers. The investigation of patients and the conditions for blood sampling adhered to a strict ethical code. The protocol was approved by the Tlemcen Hospital Committee for Research on Human Subjects (D01N01UN130120200007), and the study followed the ethical principles outlined in the Helsinki Declaration. All participants were informed of the study's objective and provided their written consent before participating.

Hematological analyses

Laboratory analyses were conducted on various blood components, including erythrocytes, leukocytes (comprising lymphocytes, monocytes, and neutrophils), hemoglobin, hematocrit, and platelets (thrombocytes) were performed using the Mindray BC-30s instrument. Prothrombin time (quick), International normalized ratio (INR), Kaolin-cephalin clotting time (KCCT), Prothrombin time activity (PT-act), Fibrinogen measurements were performed using the TromboTimer 4 Channel instrument. The Erythrocyte Sedimentation Rate (ESR) was determined using the Westergren

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method with citrate tubes. Blood samples were mixed with sodium citrate as an anticoagulant to prevent clotting, and the rate at which red blood cells settled in a vertical tube was measured.

Biochemical parameters

Biochemical parameters were evaluated using the Mindray BS-240 clinical chemistry analyzer, which employed specific test kits provided by Spinreact. This encompassed an array of measurements, including glycemic factors (fasting glucose, glycated hemoglobin (HbA1c)), lipid profile (triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)), renal profile (blood urea, blood creatinine, blood uric acid), hepatic profile, enzymatic activities of the transaminases were determined (aspartate transaminase (TGO), alanine transaminase (TGP), direct bilirubin, total bilirubin, indirect bilirubin), and C-reactive protein (CRP) as an inflammatory marker. The JOKOH apparatus, employed to measure various ions and electrolytes levels in the blood, operates based on electrochemical methods. This approach involves specialized electrodes designed for each ion (calcium, sodium, potassium, chloride) that engage with the ions within the biological sample. During this interaction, electrochemical reactions take place, resulting in the generation of measurable electric currents. The magnitude and characteristics of these currents are directly correlated with the concentrations of ions present in the sample. Ferritin levels were measured using the Maglumi 800.

Oxidative stress parameters

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) using the Sigma-Aldrich Kit (St. Louis, MO, USA), while protein oxidation was measured through carbonylated proteins (CP) using the Sigma-Aldrich Kit (St. Louis, MO, USA. Additionally, oxidative stress markers such as nitric oxide (NO) and superoxide radical (O_2) were measured using Sigma-Aldrich kit (St. Louis, MO, USA). The concentration of reduced glutathione (GSH) was determined using a colorimetric assay, where 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) reacts with GSH to produce 2-nitro-5-thiobenzoic acid, following the protocol from Sigma-Aldrich (St. Louis, MO, USA). To measure the activity of erythrocyte catalase (CAT, EC 1.11.1.6), the Cayman Chemical Catalase Assay Kit (Cayman Chemical Company, Ann Arbor, MI) was used, with the enzyme reacting with methanol in the presence of H_2O_2.

Statistical analysis

Statistical analysis was conducted using SPSS software version 26.0.0.0. Normally distributed continuous variables are expressed as mean ± standard deviation. To compare normally distributed variables, an analysis of variance (ANOVA) was employed, followed by the Tukey's post hoc test for subgroup comparisons. A p-value <0.01 was considered statistically significant.

4. Results

Diabetic patients were categorized into three groups based on their daily metformin dose: T2D-dose 1 (500–1000 mg, n = 58), T2D-dose 2 (1000–1500 mg, n = 66), and T2D-dose 3 (1500–3000 mg, n = 161). A control group of 90 non-diabetic individuals was also included. Key demographic and physical characteristics, including age, weight, BMI, and metformin dose, are summarized in Table 1.

Table 1. Characteristics of the investigated population.

	Controls	T2D-Dose 1	T2D-Dose 2	T2D-Dose 3	p Value
N	90	58	66	161	
Age (years)	57 ± 3	57 ± 3	58 ± 4	59 ± 3	0.084
Gender, male/female (%)	52.20/47.80	41.40/58.60	43.90/56.10	49.10/50.90	0.541
Weight (kg)	66 ± 5	69 ± 4	71 ± 6	68 ± 4	0.134
Height (m)	1.68 ± 0.03	1.67 ± 0.02	1.69 ± 0.05	1.67 ± 0.03	0.169
BMI (kg/m²)	23.80±2.26	24.91± 1.33	24.82± 1.48	24.64± 1.96	0.126
Diabetes duration, (years)	/	6 ± 2	7 ± 3	7 ± 2	0.333

Values are means ± SD. BMI: Body mass index (weight/height^2); Controls: non-diabetics; T2D-dose 1: diabetics treated with 500–1000 mg of metformin; T2D-dose 2: diabetics treated with 1000–1500 mg of metformin; T2D-dose 3: diabetics treated with 1500–3000 mg of metformin. Statistical comparisons between the groups were performed by one-way ANOVA test followed by Tukey post hoc test.

Hematological Parameters

Compared to the control group, diabetic patients showed significant differences in most hematological parameters. Red blood cell count (RBC), hemoglobin, and hematocrit levels were significantly lower in all diabetic patients whatever the dose of metformin used ($p < 0.001$). Platelet count, monocytes, neutrophils, and erythrocyte sedimentation rate (ESR) at both 1 and 2

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hours were significantly higher while lymphocytes were significantly lower in all diabetic patients compared to controls ($p < 0.001$) (Table 2).

Within diabetic groups, hematological differences were dose-dependent. Diabetic patients in the highest dose group (T2D-dose 3) had significantly lower lymphocyte counts and hemoglobin levels compared to the other groups ($p < 0.001$), while platelet count and ESR increased with higher metformin doses. Hematocrit was also lower in the T2D-dose 3 group compared to T2D-dose 2 (Table 2).

In comparison to the control group, diabetic patients exhibited significant alterations in coagulation parameters ($p < 0.001$). Prothrombin time (PT) was significantly prolonged in diabetic patients, with the highest values in T2D-dose 3 (Table 2). Similarly, PT activity (%) decreased significantly in all diabetic groups, with the T2D-dose 3 group showing the most reduction ($p = 0.005$). Fibrinogen levels were significantly elevated in all diabetic groups, with the highest fibrinogen levels seen in the T2D-dose 3 group ($p < 0.001$). Both the International Normalized Ratio (INR) and the KCCT (Kaolin Cephalin Clotting Time) were significantly increased in all diabetic groups compared to controls, with no significant differences between the metformin doses (Table 2).

Biochemical Parameters

Diabetic patients, whatever the dose of metformin used, exhibited significantly elevated glucose and glycated hemoglobin (HbA1c) levels compared to controls (Table 3). The lipid profile revealed an increase in low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) in all diabetic groups, accompanied by a marked reduction in high-density lipoprotein cholesterol (HDL-C) levels (Table 3). Kidney function markers, specifically urea and creatinine, were significantly elevated in all diabetic groups. Liver enzymes, namely serum glutamate oxaloacetate transaminase (TGO) and serum glutamate pyruvate transaminase (TGP), were also significantly higher in all diabetic groups, whatever the dose of metformin used. Additionally, the bilirubin profile (indirect, direct, and total bilirubin) was elevated in all diabetics (Table 3).

Table 2. Hematological parameters

	Controls	T2D-Dose 1	T2D-Dose 2	T2D-Dose 3	p Value
WBC (10^3/mm^3)	7.03 ± 0.75	7.08 ± 0.66	7.28 ± 0.34	7.47 ± 0.54	0.519
RBC (10^6/mm^3)	4.89 ± 0.52^{a}	4.68 ± 0.59^{b}	4.58 ± 0.72^{b}	4.26 ± 0.74^{b}	<0.001
Monocytes (10^3/mm^3)	0.20 ± 0.02^{c}	0.30 ± 0.02^{b}	0.30±0.01^{b}	0.40 ± 0.02^{a}	<0.001
Neutrophils (10^3/mm^3)	4.70 ± 0.20^{a}	5.20 ± 0.10^{b}	5.40 ± 0.10^{b}	6.20 ± 0.10^{a}	<0.001
Lymphocytes (10^3/mm^3)	2.10 ± 0.30^{a}	1.90± 0.20^{a,b}	1.92±0.10^{a,b}	1.60± 0.11^{c}	<0.001
Hemoglobin (g/dL)	12.95± 0.72^{a}	11.51±0.91^{b}	11.34±0.38^{b}	10.57±0.98^{b}	<0.001
Hematocrit (%)	41.28±2.20^{a}	38.51±2.29^{b}	37.38±2.10^{b,c}	34.75±2.05^{c}	<0.001
Platelets (10^3/mm^3)	301.78±29.49^{d}	363.60±28.53^{c}	389.26±36.38^{b}	402.17±23.69^{a}	<0.001
ESR (1hour),mm	8.76 ± 2.52^{c}	19.61 ± 2.62^{b}	18.19 ± 2.46^{a}	19.82 ± 2.33^{a}	<0.001
ESR (2hour),mm	21.64 ± 5.78^{c}	43.67 ± 6.92^{b}	43.79 ± 4.23^{b}	45.46 ± 6.91^{a}	<0.001
Prothrombin time (sec)	13.69 ± 0.45^{c}	14.95 ± 0.60^{b}	15.44± 0.17^{a,b}	15.72 ± 0.43^{a}	0.005
PT-act (%)	84.98 ± 4.25^{a}	75.53 ± 5.49^{b}	71.80 ± 7.15^{c}	71.51 ± 5.06^{c}	0.005
INR	1.21 ± 0.03^{b}	1.34 ± 0.02^{a}	1.46 ± 0.07^{a}	1.47 ± 0.04^{a}	0.005

Values are means ± SD. Controls: non-diabetics; T2D-dose 1: diabetics treated with 500–1000 mg of metformin; T2D-dose 2: diabetics treated with 1000–1500 mg of metformin; T2D-dose 3: diabetics treated with 1500–3000 mg of metformin; WBC: White Blood Cell; RBC: Red Blood Cell; INR: International normalized ratio; KCCT: Kaolin-cephalin clotting time; PT-act: Prothrombin time activity; ESR: Erythrocyte Sedimentation Rate. Statistical comparisons between the groups were performed by one-way ANOVA test followed by Tukey post hoc test. Values for each parameter with different superscripts (a,b,c,d) are significantly different for $P < 0.01$.

Electrolyte levels demonstrated no significant differences in sodium, potassium and chloride levels between diabetic patients and controls. However, calcium levels were elevated in all diabetic groups. Furthermore, inflammatory markers such as C-reactive protein (CRP) and ferritin were significantly elevated in all diabetic groups (Table 3).

Within the diabetic groups, dose-dependent changes were noted across various parameters. T2D-Dose 3 showed significantly higher glucose and glycated hemoglobin (HbA1c) levels compared to T2D-Dose 1

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and Dose 2. Additionally, T2D-Dose 3 exhibited significantly higher TG levels with a marked reduction in HDL-C levels compared to both T2D-Dose 1 and Dose 2. Creatinine and urea levels also increased with higher doses, particularly in T2D-Dose 3. Bilirubin levels progressively increased with metformin doses. CRP and ferritin were highest in the group receiving the highest metformin dose (Table 3).

Table 3. Biochemical Parameters

	Controls	T2D-Dose 1	T2D-Dose 2	T2D-Dose 3	p Value
Glucose (mmol/L)	0.89 ± 0.02 ^{c}	1.57 ± 0.03 ^{b}	1.63 ± 0.02^{a}	1.62 ± 0.03 ^{a}	0.001
HbA1c (%)	4.97 ± 0.19 ^{c}	7.53 ± 0.13 ^{b}	8.20 ± 0.30 ^{a}	8.14 ± 0.48 ^{a}	<0.001
Total Cholesterol (mmol/L)	1.64 ± 0.15	1.65 ± 0.26	1.54 ± 0.32	1.53 ± 0.32	0.128
HDL-C (mmol/L)	0.45 ± 0.02 ^{a}	0.29 ± 0.03 ^{b}	0.31 ± 0.04 ^{b}	0.31 ± 0.03 ^{b}	0.005
LDL-C (mmol/L)	1.07 ± 0.14 ^{c}	1.36 ± 0.04 ^{a}	1.22 ± 0.05 ^{b}	1.21 ± 0.03 ^{b}	0.001
Triglycerides (mmol/L)	0.93 ± 0.08 ^{c}	1.43 ± 0.03 ^{b}	1.49 ± 0.04 ^{b}	1.56 ± 0.05 ^{a}	<0.001
Uric acid (µmol/L)	0.22 ± 0.04 ^{b}	0.31 ± 0.01 ^{a}	0.32 ± 0.03 ^{a}	0.33 ± 0.02 ^{a}	0.005
Urea (g/L)	0.24 ± 0.09 ^{c}	0.38 ± 0.08 ^{b}	0.41 ± 0.07 ^{a}	0.46 ± 0.04 ^{a}	<0.001
Creatinine (mg/L)	8.91 ± 0.36 ^{c}	10.91±0.24 ^{b}	11.29± 0.28 ^{a}	11.69± 0.32 ^{a}	0.001
TGO (UI/L)	17.93± 1.66 ^{c}	37.72±1.02 ^{b}	39.21± 2.64 ^{b}	41.70±1.98^{a}	<0.001
TGP (UI/L)	27.37± 2.81 ^{d}	36.09±1.97 ^{c}	39.85± 2.31 ^{b}	43.31±2.99 ^{a}	<0.001
Indirect bilirubin (mg/L)	4.28 ± 0.14 ^{d}	5.30 ± 0.12^{c}	5.72 ± 0.09 ^{b}	6.43 ± 1.10 ^{a}	0.001
Direct bilirubin (mg/L)	1.34 ± 0.08 ^{d}	2.37 ± 0.04 ^{c}	2.68 ± 0.07 ^{b}	3.21 ± 0.02 ^{a}	0.001
Total bilirubin (mg/L)	5.62 ± 0.19 a	7.67 ± 0.12 b	8.40 ± 0.16 c	9.64 ± 1.20 d	0.001
Calcium (mg/L)	94.72±4.57^{b}	104.26±4.14^{a,b}	111.93±4.11 ^{a}	116.50±3.79 ^{a}	0.005

Values are means ± SD. Controls: non-diabetics; T2D-dose 1: diabetics treated with 500–1000 mg of metformin; T2D-dose 2: diabetics treated with 1000–1500 mg of metformin; T2D-dose 3: diabetics treated with 1500–3000 mg of metformin; HbA1c: Glycated hemoglobin; HDL-C: High-density Lipoprotein Cholesterol; LDL-C: Low-density Lipoprotein Cholesterol; TGO: Aspartate Transaminase; TGP: Alanine Transaminase; CRP: C-reactive protein.

Oxidative Stress Biomarkers

Diabetic patients had significantly higher malondialdehyde (MDA) and carbonyl proteins (CP) levels compared to controls, whatever the dose of metformin. Nitric oxide (NO) and superoxide anion (O2) levels were also elevated, while reduced glutathione (GSH) and catalase (CAT) levels were lower in all diabetic groups (Figure 1).

Among diabetics, a dose-dependent increase was observed in MDA and CP, with T2D-Dose 3 showing the highest levels compared to T2D-Dose 1 and Dose 2. NO and O2 levels were similarly elevated in T2D-Dose 3, while GSH and CAT levels were lowest in this group (Figure 1).

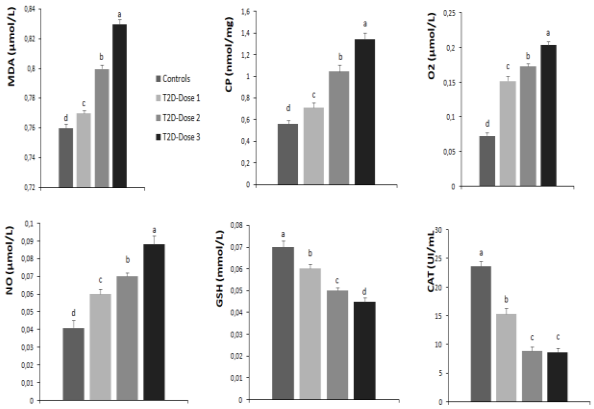

Figure 1. Oxidative stress biomarkers

Values are means ± SD. Controls: non-diabetics; T2D-dose 1: diabetics treated with 500–1000 mg of metformin; T2D-dose 2: diabetics treated with 1000–1500 mg of metformin; T2D-dose 3: diabetics treated with 1500–3000 mg of metformin; MDA: Malondialdehyde; CP: Carbonylated Proteins; O2: Superoxide Anion; NO: Nitric Oxide; GSH: Reduced Glutathione; CAT: Catalase. Statistical comparisons between the groups were performed by one-way ANOVA test followed by Tukey post hoc test. Values for each parameter with different superscripts (a,b,c,d) are significantly different for P <0.01.

5. Discussion

The aim of the present study was to evaluate the effects of different doses of metformin on the metabolic profile and oxidative markers in Algerian T2D patients. Our findings highlight that, despite metformin treatment, type 2 diabetic patients exhibit hematological and metabolic alterations, inflammation and oxidative stress, which worsen with increasing dosage.

Despite metformin administration, the observation of elevated HbA1c and fasting blood glucose levels suggests that optimal glycemic control remains a challenge for T2D patients studied. This highlights that simply managing blood glucose with medication is not sufficient; the severity of hyperglycemia, lifestyle factors, and individual responses to treatment must be considered. Previous research emphasizes that dietary interventions and nutritional support can reduce diabetes-related complications [12]. A diet high in carbohydrates can counteract the effects of metformin,

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leading to elevated blood glucose levels despite the treatment. It has been demonstrated that carbohydrate restriction in conjunction with metformin is an effective treatment option for T2D patients [13]. Indeed, several mechanisms related to insulin resistance, such as chronic inflammation or lipid abnormalities, may limit the effectiveness of metformin. It has been shown that in T2D patients treated with metformin, adding other medications can lead to a significant improvement in both beta cell function, insulin resistance and in markers of inflammation [14].

The lipid profile observed in our study, marked by decreased HDL- and increased LDL-cholesterol and triglycerides levels, indicates a potential risk for atherosclerosis. These results are in agreement with the findings of previous studies that also identified lipid imbalances in diabetic populations, raising concerns about vascular complications [3,7,15]. However, in our study, lipid alterations significantly increased in T2D patients treated with metformin, with a significant increase at higher doses. It is worth mentioning that some studies suggest protective effects of metformin against vascular disease with lipid profile improvement in individuals with T2D [16]. While metformin is recognized for its cardiovascular protective effects, its impact on lipid profiles and atherosclerosis prevention has shown variability, with some studies reporting inconsistent outcomes depending on individual patient characteristics [17]. Previous study revealed that therapeutic Effects of Metformin are different in T2D patients with altered lipidomic profiles [18].

The significant dose-dependent increases in urea, creatinine, and uric acid observed in all patients suggest the presence of renal complications. This finding aligns with the fact that metformin is primarily eliminated through the kidneys. When clearance is reduced, metformin accumulation can lead to toxicity [19]. It has been demonstrated that metformin may have an adverse effect on renal function in patients with type 2 DM and moderate chronic kidney disease [20]. These results emphasize the importance of monitoring inflammatory markers, kidney function, and urinary health in patients undergoing metformin therapy. In our study, both TGO and TGP were significantly higher in all T2D groups, especially in T2D-Dose 3. Metformin, particularly at higher doses, may affect liver function, leading to elevated liver enzymes in T2D patients [21].

The significant differences in hematological parameters among diabetic patients treated with metformin reveal a dose-dependent decrease in red blood cells (RBC), hemoglobin, and hematocrit levels, accompanied by elevated bilirubin and ferritin levels. While this finding may suggest a potential link to liver function, it is important to consider other contributing factors such as hemolytic anemia, which could also elevate bilirubin levels. Furthermore, the multifactorial nature of metabolic diseases like T2D, along with metformin's potential interference with vitamin B12 absorption, may exacerbate conditions such as hemolytic anemia. This interplay warrants further investigation into the relationship between long-term metformin therapy, liver function, vitamin B12 deficiency, and associated hematological complications [22,23]. Although many studies have demonstrated metformin's beneficial impact on hemostasis [24], our findings suggest that its effectiveness is dose-dependent.

The significant rise in inflammatory markers, such as ferritin and CRP, with increasing metformin doses suggests a dose-dependent inflammatory effect, particularly in patients with complications. Additionally, the increase in erythrocyte sedimentation rate (ESR) across all diabetic groups further points to underlying inflammation. This contrasts with findings from recent studies that highlight metformin's anti-inflammatory properties in various conditions [25]. Metformin, beyond its hypoglycemic effects, has been recognized for its anti-inflammatory, anti-aging, and anti-cancer benefits. Preventing inflammation is critical, as chronic inflammation contributes to many public health concerns. However, the precise anti-inflammatory mechanisms of metformin remain inconsistent across studies, indicating that its effects may vary depending on dosage, context, and patient characteristics. Therefore, further clinical trials are required to confirm the metformin effect on inflammatory markers in T2DM patients [26].

Previous studies have demonstrated that metformin generally reduces oxidative stress in patients with newly diagnosed type 2 diabetes [27], but our findings suggest that these antioxidant benefits may diminish at higher doses. In fact, in our study, pro-oxidants are increased and antioxidants are decreased in T2D patients particularly with higher dose of metformin. This underscores the importance of carefully controlling

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metformin dosage, as higher doses in our study were associated with increased oxidative stress. These differences highlight the need for dose adjustments to optimize the balance between metformin's therapeutic effects and its impact on oxidative stress.

The dose-dependent adverse effects observed in our study suggest that lower doses of metformin may provide therapeutic benefits with minimal adverse effects. However, in patients with advanced diabetes or complications, dose adjustments become crucial to avoid adverse outcomes.

It is clear then that metformin can negatively impact metabolic and redox parameters in T2D patients, particularly those treated with higher doses. However, our study has some limitations, particularly in characterizing the recruited patients. Some information must be included such as adherence to medication, dietary interaction, metformin intolerance by analyzing gut microbiota and lifestyle factors affecting metabolic control.

Conclusion

The parameters analyzed in this study emphasize the importance of individualized treatment approaches and careful monitoring in patients with type 2 diabetes receiving metformin therapy. Monitoring glycemic control, lipid profiles, and inflammatory markers, liver and kidney function in patients on metformin is crucial to prevent the adverse effects of high doses of this anti-diabetic drug.

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Conflict of interest

The authors declare no conflicts of interest.

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