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The Anti-Diabetic, Lipidemic, And Pro-Inflammatory Effects of Metformin Versus Glibenclamide on T2DM Obese Patients

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Abstract

Diabetes complications are the primary causes of DM morbidity and mortality. Objectives: comparing the anti-diabetic, lipidemic, and pro-inflammatory effects of metformin and glibenclamide in treating T2DM-obese patients, suggesting a probable novel mode of action and providing a more comprehensive understanding of T2DM pathophysiology for future therapeutic application. The effect of the adipocytokines and pro-inflammatory cytokines in T2DM was also investigated. 150 subjects were allocated into five equal groups (n=30): the normal, the obese control, the obese diabetic control, the obese diabetic treated with 500 mg metformin twice daily, and the obese diabetic glibenclamide-treated, receiving 5 mg glibenclamide once daily. The blood samples were collected after six months of treatment.Metformin improved BMI, FSG, glycosylated hemoglobin, total cholesterol, and triglycerides, but it raised low-density lipoproteins and lowered high-density lipoproteins. The glibenclamide treatment significantly improved fasting insulin and C-peptide serum levels with subsequent better homeostatic model assessment for insulin resistance, but it increased BMI, TC, and TG. Visfatin and Retinol binding protein-4 showed less improvement in the glibenclamide than in the metformin-treated obese diabetic patients. In contrast, resistin decreased with glibenclamide treatment compared to metformin. The IL-1β expression was higher in the obese diabetic control and declined in the glibenclamide-treated obese diabetic patients while, IL-6, TNF-α, and IFN-γ expression decreased in metformin-treated obese diabetic patients than in glibenclamide ones. Our results also demonstrated a high positive correlation between the adipocytokines, visfatin, resistin, and RBP4 and the pro-inflammatory, cytokines IL-1β, IL-6, TNF-α and IFN-γ. From those results, treatment with metformin is recommended in the cases of obesity, inflammation, hypercholesterolemia, and hypertriglyceridemia. In addition, treatment with glibenclamide is recommended in the cases of insulin resistance and dyslipidemia.

Key words: T2DM, obesity class I, Metformin, Glibenclamide, Visfatin, Resistin, RBP-4, lipid profile, insulin resistance.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic progressive disease. The World Diabetes Association's studies predict that by 2030, one in every ten persons will be diabetic if deterrent ways are not offered [1]. DM is a collection of metabolic disorders characterized by vascular resistance to insulin in variable degrees associated with hyperglycemia caused by abnormalities in insulin secretion and/or action [2]. Only a few individuals with genetic insulin resistance (IR) acquire DM, while 25% of non-diabetic individuals exhibit low insulin sensitivity similar to that seen in T2DM [3]. T2DM develops from a complex pathophysiological pattern with several complications related to hyperglycemia, as cardiovascular and renal disease, neuropathy, and others. [4]. C-peptide is a well-established measure for beta cell activity that assesses endogenous insulin production. It is necessary to have comparable and accurate C-peptide measurements to predict how much insulin is produced. [5]. Evidence reveals that adipose tissue is a secretory tissue that produces several biologically active substances known as

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adipocytokines or adipokines with pro- or anti-inflammatory activities. In T2DM, the pro- and anti-inflammatory adipokines balance is disrupted [6]. This occurred due to adipose tissue dysfunction, with unbalanced adipokines secretion, leading to DM metabolic and cardiovascular complications' pathogenesis. [7]. These adipocytokines can also predict cardiac diseases in diabetic patients because they promote vascular dysfunction [8]. Blocking the pro-inflammatory cytokines, e.g., IL-1β, IL-6, IFN-γ, and TNF-α, which cause inflammation, fever, tissue destruction, and even shock and death in humans, has gained attention [9]. Adipokines participate in numerous processes as host defense, inflammation, apoptosis, autoimmune diseases, cell division, and organogenesis [10]. The vital activities of adipocytokines regarding satisfaction, hunger, body fat accumulation and energy management, glucose tolerance, and insulin resistance consolidate their involvement in the initiation and progression of DM and its metabolic disorders [11].

Several adipocytokines, including TNF-α, visfatin, resistin, and retinol-binding protein 4 (RBP4), aroused the curiosity of researchers. Visfatin has h a hypoglycemic effect through activating insulin receptors, increasing insulin sensitivity, and reducing glucose levels [12]. Many researches reveal probable links between visfatin and metabolic diseases. Indeed, visfatin was identified as a prospective biomarker for various metabolic disorders such as DM, IR, and obesity [13]. Resistin inhibits glucose uptake and promotes IR by decreasing fasting serum glucose (FSG) levels. Resistin and visfatin are upregulated in T2DM and increase with obesity. These adipocytokines can also predict cardiac diseases in diabetic patients because they promote vascular dysfunction [11]. Resistin is also linked to metabolic disease and inflammation, as low resistin levels improve insulin sensitivity and glucose homeostasis [14].

RBP4 is another potential diabesity adipokine that transports plasma retinoids. RBP4 plasma levels have been associated with visual complications in patients with T2DM, implying a role for RBP4 in the etiology of diabetic retinopathy disorders [15]. RBP4 is increased in T2DM associated with obesity and also causes IR. Circulating RBP4 might be used as an early diagnostic indicator for the progression of T2DM [16]. Furthermore, RBP4 was recently proposed as a cardiometabolic risk factor [17]. In most countries, the most widely used oral glucose-lowering medication in the "Biguanides" family is metformin. This is due to its effectiveness, low price, weight neutrality, and good safety record [18]. On the other hand, glibenclamide is an insulin-sensitizing agent with anti-hyperglycemic action that belongs to a group of drugs called "Sulfonylureas" family. It decreases blood glucose levels by raising the pancreatic insulin level [19].

Herein, the study compares the two drugs, metformin and glibenclamide, concerning their effectiveness in changing various adipocytokines and inflammatory mediators' levels after six months of treatment in T2DM obese patients.

2. Results

The demographic data of the studied groups is discussed in **Table 1.**

Table (1): Demographic data of the study groups.

Groups		Normal control	Obese control	Obese Diabetic control	Metformin-treated obese diabetic	Glibenclamide-treated obese diabetic
Number of subjects		30	30	30	30	30
Age (years)		50.11 ± 5.25	45.5 ± 9.7	49.95 ± 7.31	47.52 ± 6.5	48.50 ± 5.43
Duration of DM (years)		-	-	1.7 ± 0.6	1.6 ± 0.9	1.8 ± 1.1
Gender	Male	6 (20%)	8 (26.7%)	8 (26.7%)	6 (20%)	6 (20%)
	Female	24 (80%)	22 (73.3%)	22 (73.3%)	24 (80%)	24 (80%)

- Data are expressed as Mean ± SD (n=30).

Biochemical Investigations

Data showing the effect of metformin and glibenclamide administration on biochemical investigations were represented in (Tables 2 and 3). Metformin caused a statistically significant (P<0.05) higher mean values of FSG, HbA1c, HOMA-IR, BMI, TC, HDL-C, LDL-C, and VLDL-C, with a statistically insignificant (P>0.05) increase in mean values of FI, C-peptide, and TG compared to the obese controls. On the other hand, the mean values of FSG, HbA1c, FI, C-peptide, BMI, and HOMA-IR were statistically significantly (P<0.05) lowered in the metformin-treated than the obese diabetic control patients.

Table (2): Fasting serum glucose, HbA1C, fasting insulin, C-peptide, HOMA-IR, and BMI among the study groups.

Groups	FSG (mg/dl)	HbA1c (%)	FI (µIU/mL)	C-peptide (pg/mL)	HOMA-IR (%)	BMI (kg/m^2)
Normal control	94.70 ± 7.93 ^{a}	4.91 ± 0.26 ^{a}	4.7 ± 1.93 ^{a}	3.12 ± 0.83 ^{a}	1.10 ± 0.46 ^{a}	20.21 ± 3.30 ^{a}
Obese control	96.3 ± 5.61 ^{a}	5.23 ± 0.9 ^{a}	6.2 ± 3.8 ^{b}	4.7 ± 1.93 ^{b}	1.9 ± 0.97 ^{b}	32.5 ± 1.63 ^{c}
Obese diabetic control	186.6 ± 7.87 ^{d}	9.07 ± 0.79 ^{c}	12.9 ± 4.6 ^{d}	10.5 ± 2.4 ^{d}	7.85 ± 2.22 ^{d}	32.63 ± 1.26 ^{c}
Metformin-treated	134.7 ± 20.8 ^{b}	6.97 ± 1.35 ^{b}	7.86 ± 2.33 ^{b}	5.24 ± 1.92 ^{b}	3.35 ± 1.20 ^{c}	27.0 ± 1.8 ^{b}
Glibenclamide-treated	153.5 ± 35.8 ^{c}	8.25 ± 2.22 ^{c}	9.83 ± 2.85 ^{c}	7.10 ± 2.25 ^{c}	2.00 ± 0.81 ^{b}	35.7 ± 3.6 ^{d}
P value	*P*<0.05	*P*<0.05	*P*<0.05	*P*<0.05	*P*<0.05	*P*<0.05

Data are presented as mean ± SD (n = 30).

Means that sharing the same symbol(s) are not significantly different at (*P*<0.05).

In the glibenclamide-treated obese diabetic group, the mean values of FSG, HbA1c, FI, C-peptide, BMI, TC, HDL-C, VLDL-C, and TG showed a statistically significant (P<0.05) increase, with a statistically insignificant (P>0.05) increase in the mean values of HOMA-IR and LDL-C compared to the obese control patients. Moreover, glibenclamide treatment resulted in a statistically significant (P<0.05) decrease in the mean values of FSG, FI, C-peptide, HOMA-IR, HDL-C, LDL-C, VLDL-C, and TG, with a statistically insignificant (P>0.05) decrease in the HbA1c and TC values compared to the obese diabetic control patients.

Table (3): Lipid profile among the study groups.

Groups	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	TG (mg/dL)
Normal control	158.5 ± 16 [a]	93.6 ± 18 [d]	43.5 ± 18.8 [a]	21.5 ± 3.8 [a]	107.2 ± 19 [a]
Obese control	198.4 ± 30.5 [b]	57.38 ± 8.4 [a]	117.7 ± 24.4 [b]	22.3 ± 2.5 [a]	138 ± 20.5 [b]
Obese diabetic control	347 ± 48 [d]	75.3 ± 29 [b]	233.5 ± 58 [d]	28.3 ± 7.8 [c]	205.5 ± 23 [d]
Metformin-treated	296.4 ± 28.4 [c]	80.4 ± 11.5 [c]	138.4 ± 18.5 [c]	25.6 ± 5.6 [b]	141.3 ± 38.8 [b]
Glibenclamide-treated	340.9 ± 74.9 [d]	83.5 ± 13.7 [c]	119.6 ± 42 [b]	23.6 ± 10.4 [b]	190.3 ± 57.2 [c]
P value	*P*<0.05	*P*<0.05	*P*<0.05	*P*<0.05	*P*<0.05

Data are presented as mean ± SD (n = 30).
Means that sharing the same symbol(s) are not significantly different at (*P*<0.05).

Nevertheless, compared to the glibenclamide, metformin caused statistically significant (P<0.05) lower results in FSG, HbA1c, FI, C-peptide, BMI, TC, and TG except for HOMA-IR and LDL-C that were statistically significantly (P<0.05) lowered with glibenclamide treatment. Also, a statistically insignificant (P>0.05) difference in the mean values of HDL-C and VLDL-C could be noticed between metformin- and glibenclamide-treated obese diabetic patients.

Adipocytokines

Comparison between serum levels of the adipokines visfatin, resistin, and RBP4 levels among the studied groups is represented in Figure (1).

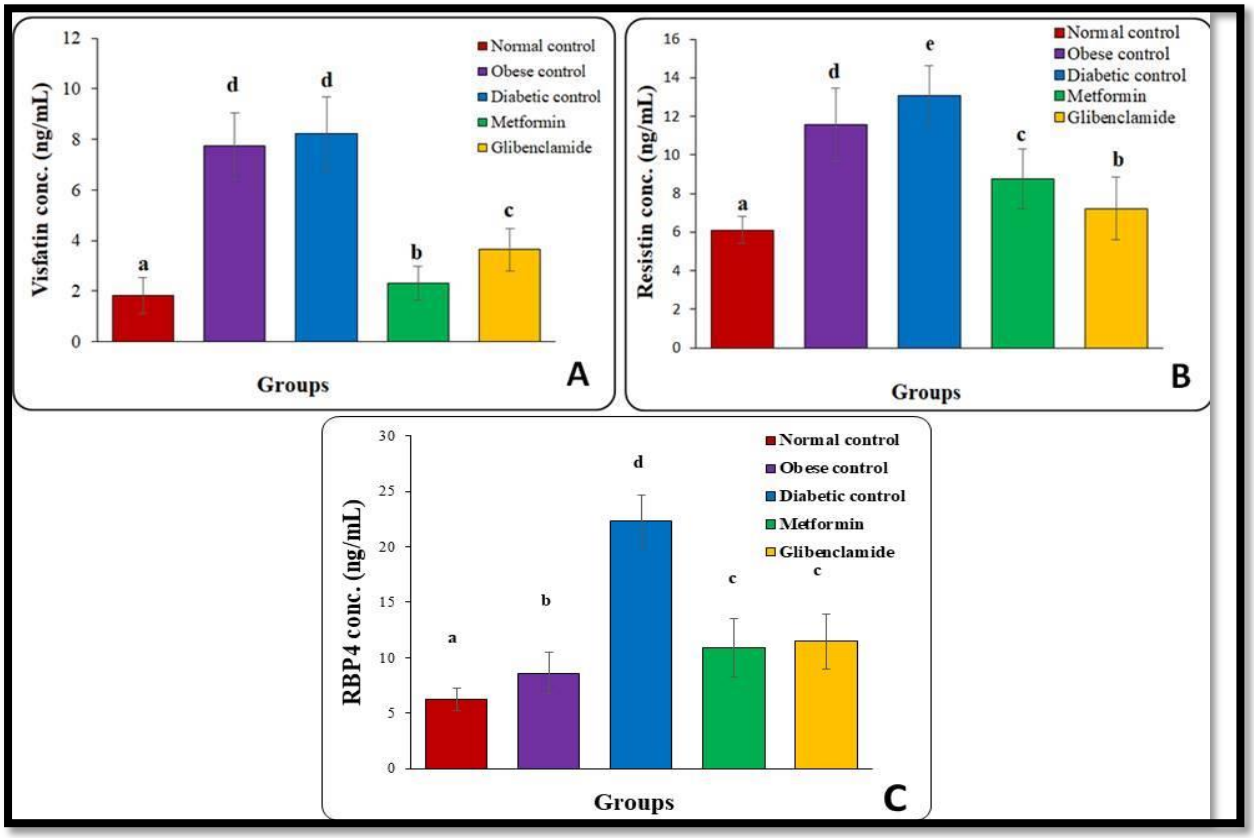

Figure (1): Serum levels of (A) visfatin, (B) resistin, and (C) RBP4 among the study groups.
Data are presented as mean ± SD (n = 30).
Means that sharing the same symbol(s) are not significantly different at (*P*<0.05).

The mean values of the serum levels of visfatin and resistin in the metformin- and glibenclamide-treated obese patients were statistically significantly (P<0.05) lower than the obese control and the obese diabetic control patients, with statistically significantly (P<0.05) better metformin results than glibenclamide.
However, the mean values of the serum levels of RBP4 with metformin and glibenclamide treatments were statistically significantly (P<0.05) higher than the obese control patients and statistically significant (P<0.05) lower than the obese diabetic control patients without any statistically significant (P>0.05) difference between metformin and glibenclamide treatments.

The pro-inflammatory cytokines

In Figure (2), the expression of the pro-inflammatory cytokines IL-1β, IL-6, TNF-α and IFN-γ exhibited a statistically significant (P<0.05) decrease with both the metformin and glibenclamide treatments compared to the obese control and obese diabetic control patients, except for IL-1β in the metformin-treated group that was statistically insignificant (P>0.05) decreased compared to the obese control group.
Moreover, metformin treatment statistically significantly (P<0.05) decreased pro-inflammatory cytokines IL-6 and TNF-α expression compared to glibenclamide. On the other hand, glibenclamide treatment statistically significantly (P<0.05) lowered the pro-inflammatory cytokine IL-1β expression compared to metformin without any statistically significant (P>0.05) difference between metformin and glibenclamide concerning the expression of IFN-γ.

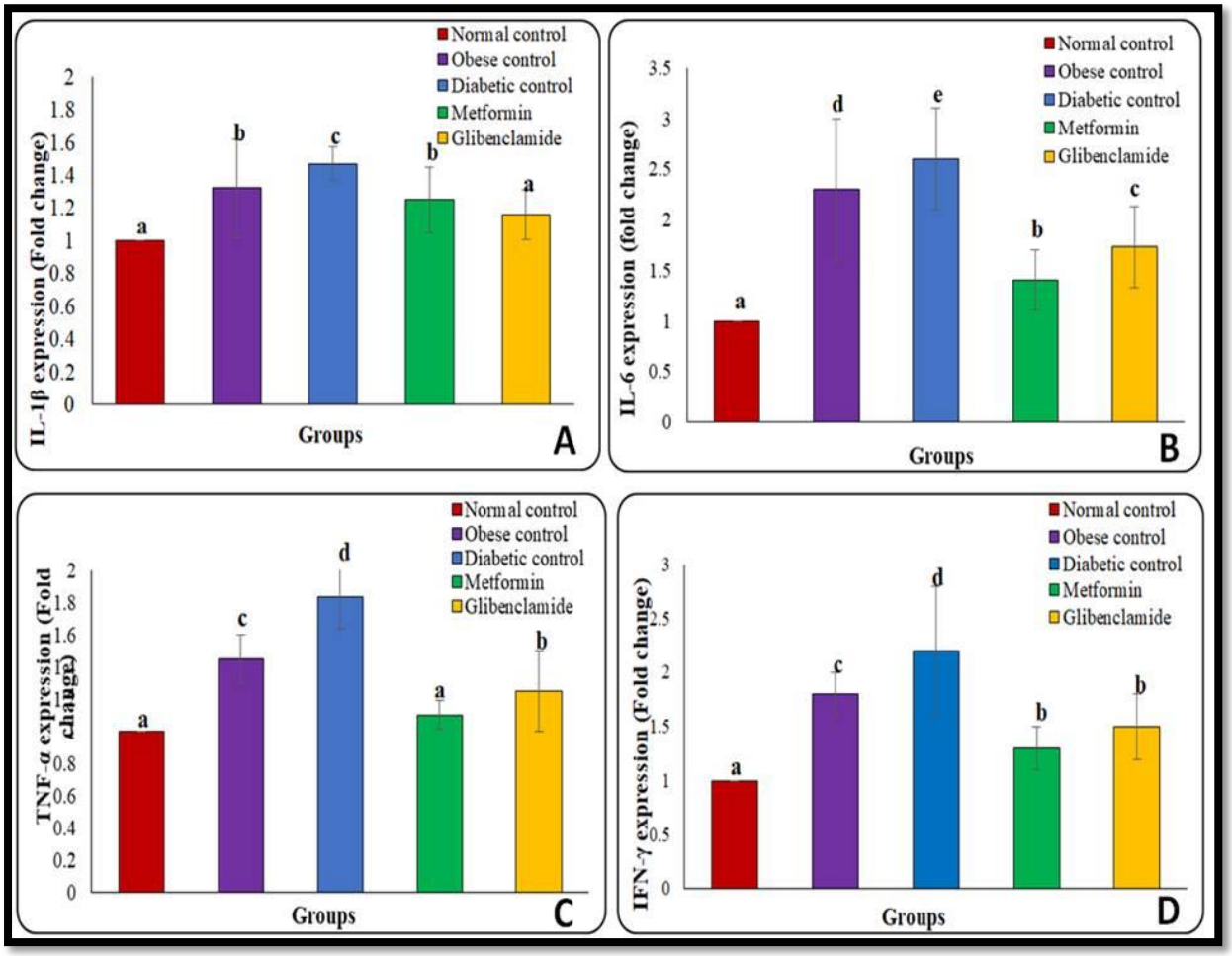

Figure (2): mRNA expression of (A): IL-1β, (B): IL-6, (C): TNF-α, and (D): IFN-γ among the study groups.
Data are presented as mean ± SD (n = 30).
Means that sharing the same symbol(s) are not significantly different at (*P*<0.05).
The cross correlation between the study cytokines expression values showed a strong positive correlation ranging from 0.535 and 0.967, with a significant statistical difference at P-value < 0.01 (Table 4).

Table (4): Correlation matrix between adipokines and pro-inflammatory cytokines among the study participants.

	Visfatin	Resistin	RBP4	IL-1β	IL-6	TNF-α	IFN-γ
Visfatin	1						
Resistin	0.910**	1					
RBP4	0.535**	0.653**	1				
IL-1β	0.737**	0.883**	0.684**	1			
IL-6	0.967**	0.919**	0.642**	0.833**	1		
TNF-α	0.903**	0.872**	0.723**	0.778**	0.935**	1	
IFN-γ	0.927**	0.888**	0.691**	0.713**	0.906**	0.906**	1

** High positive correlation at the 0.01 level (2-tailed).

3. Discussion

T2DM is a public health issue that has reached epidemic proportions due to its rapidly increasing rates worldwide, especially in developing nations [31], which is caused by aberrant glucose uptake linked to improper lipid metabolism [32]. Metformin, a biguanide derivative, is an insulin sensitizer used primarily as the first-line oral therapy to treat T2DM alone or with other hypoglycemic therapies [33]. Glibenclamide is the most widely prescribed sulfonylureas therapy for treating T2DM [34]. While the effect of metformin and glibenclamide in treating T2DM has been well assessed, little evidence exists about its activity on adipocytokines and inflammatory mediators in T2DM. Understanding the new mechanisms of action of metformin and glibenclamide is essential for advancing treatment strategies, minimizing side effects, developing combination therapies, overcoming drug resistance, fostering drug development, and ultimately improving public health.

The study results revealed significantly lower FSG, HbA1c, FI, HOMA-IR, C-peptide, and BMI levels compared to the obese diabetic control patients after six months of metformin, with more effectively lowered FSG and HbA1c than glibenclamide in T2DM obese patients. However, glibenclamide treatment decreased FI and HOMA-IR better than metformin. These results were compatible with previous studies that found metformin monotherapy consistently lowers FSG levels and HbA1c. Metformin action involves multiple pathways that help lower blood glucose levels, including reduced hepatic gluconeogenesis, enhanced insulin sensitivity, mainly in skeletal muscle cells, and inhibition of intestinal glucose absorption, which lowers blood glucose levels after meals. Metformin can also help in weight management, as it reduces appetite and promotes weight loss that further increases insulin sensitivity, reducing hepatic glucose output, preserving pancreatic β-cells, and indirectly affecting insulin secretion. These actions contribute to better glycemic control and overall management of T2DM [35].

Because of the tight relationship between managing glucose levels and lipid profiles, it is critical to consider both elements to minimize micro- and macro-vascular problems linked to DM and obesity [36]. Lin et al. discovered that diabetic patients had higher levels of lipids (LDL-C, TC, TG) and lipid ratios (TG/HDL, LDL/HDL) than normal subjects, as well as a strong positive correlation between lipid profile and HbA1c levels [37]. This suggests a probable link between glycemic

management along with dyslipidemia in diabetic individuals [38], with a rise in LDL-C and a decline in HDL-C in diabetics with poor glycemic control [39]. Thus, controlling lipid profiles and the glycemic index is important in preventing cardiovascular complications [40].

In the same context, our results revealed an increase in TC and TG in obese diabetic patients treated with glibenclamide, which works primarily by stimulating pancreatic β-cells to release insulin for blood glucose regulation. Its effects on lipid profile are secondary and less significant. It can mildly reduce TC and LDL-C while having a neutral or slightly positive impact on HDL-C. This occurs due to better glycemic control, affecting protein glycosylation in lipid metabolism. In T2DM patients, HDL-C levels may be affected by various factors such as IR, inflammation, and lifestyle factors. Although glibenclamide can improve glycemic control in T2DM patients, its direct impact on HDL-C levels is relatively minimal [41].

In addition, an elevation of LDL-C and a decline in HDL-C takes place in metformin-treated obese diabetic patients. Metformin reduces hepatic gluconeogenesis, increasing insulin sensitivity, modulating lipid metabolism, and inhibiting mitochondrial respiratory chain complex 1, which in turn impacts glucose and lipid levels [42]. So, the treatment of obese diabetics with either metformin or glibenclamide had a bad influence on lipid profile in T2DM obese patients.

In this study, we discussed visfatin, resistin, and RBP4 as examples of adipocytokines, and IL-1β, IL-6, TNF-α, and IFN-γ as examples of inflammatory mediators that are involved in T2DM severity, IR, obesity and inflammation.

Several studies have identified a link between circulating visfatin and several forms of DM. In T2DM patients, serum visfatin levels are appropriately related to dietary carbohydrates and unsaturated fatty acids [43]. Some research implies that visfatin levels are higher in T2DM [44], while others show that visfatin levels are lower in insulin resistance [45]. Several investigations have found a link between visfatin and fasting glucose levels [46], insulin sensitivity, and BMI [13], while others didn't discover any significant links between them [47]. Visfatin was shown to stimulate the production of many inflammatory mediators [48]. There is increasing evidence of the association between IR and subclinical inflammation involving adipocytokines [49].

Our findings indicated that visfatin is positively associated with T2DM, obesity, and dyslipidemia. Metformin treatment was found to have a better effect on lower visfatin levels in type 2 diabetic obese patients than glibenclamide. Visfatin causes a rapid decline in serum insulin by lowering blood glucose and enhancing insulin sensitivity. Obesity-related increases in visfatin levels may constitute an attempt to maintain a stable blood glucose level. Excess weight seems to be linked to an elevation of inflammation as that could lead to the progression of IR, T2DM, and its related consequences that involve cardiac and kidney diseases [50].

Resistin appears to be a linking factor between visceral obesity and DM [51]. Higher levels of resistin identified in diabetic patients are linked with diabetes-related and CVD complications. A positive association exists between serum resistin, BMI, and HOMA-IR [52]. Treatment of T2DM obese patients with glibenclamide was more effective in diminishing resistin levels than metformin. Resistin contributes to the onset and worsening of T2DM through various mechanisms. It yields IR by disturbing insulin signaling pathways in target tissues. Resistin also promotes inflammation by activating various immune cells and improving pro-inflammatory cytokines synthesis, affects β-cell function, and alters adipokine secretion, all contributing to T2DM development and worsening the condition [53].

RBP4 is a potential cardiometabolic risk factor produced by liver cells and fat tissue. RBP4 was demonstrated to cause IR, and plasma RBP4 levels are elevated in T2DM, obesity, metabolic syndrome, as well as CVDs. Furthermore, it has been discovered that RBP4 is reduced with pharmacological therapies, leading to metabolic layout improvement, which includes exercise, diet, and hypolipidemic and oral hypoglycemic medications [54]. So, RBP4 may serve as an early predictor for T2DM progression. A possible cause in conditions of IR is the down-regulated GLUT4 expression in visceral adipose tissue with increased RBP4 expression [55]. While, there is a positive association between RBP4 mRNA and GLUT4 mRNA in subcutaneous adipose tissue [56], there is an inverse link between RBP4 mRNA and GLUT4 mRNA expression in visceral adipose tissue [15] or no association between them [57].

Our results stated that the obese diabetic control patients showed higher RBP4 levels than the obese control. In addition, metformin-treated obese diabetics were more effective in reducing RBP4 levels than glibenclamide. Metformin's mechanism of action involves reducing glucose synthesis in the liver, decreasing glucose absorption in the gut, and improving insulin sensitivity by increasing peripheral glucose absorption and consumption. Additionally, metformin stimulates adenosine monophosphate-activated protein kinase (AMPK) in the liver, suppressing fatty acid production and gluconeogenesis. [58].

Cytokines have emerged as a significant front in medicine, serving as medical, predictive, and treatment tools for human diseases. [59]. The pro-inflammatory cytokine IL-1β is rapidly produced in most tissues due to many factors, including infection or injury [60]. Multiple investigations have revealed the relevance of IL-1β in adipose tissue, particularly in IR and obesity-related inflammation. IL-1β may be used as a therapy to reverse the negative metabolic effects of obesity [61].

Our results estimated that IL-1β increased in newly obese diabetic patients and IR. Also, obese diabetic treatment with glibenclamide was more effective in reducing IL-1β levels than metformin. That may be because hyperglycemia increases IL-1β levels in the pancreas, increasing receptor responsiveness in islets and β-cells, potentially affecting insulin secretion and β-

cell function [62]. Glibenclamide effects on IL-1β in T2DM can be indirect and secondary to its primary action. By improving glycemic control and insulin sensitivity, glibenclamide may help reduce inflammation and the generation of IL-1β. This occurs through several potential mechanisms: reduced glucose levels, improved insulin sensitivity, and antioxidant effects. Some studies suggest that glibenclamide may have antioxidant properties, which could help reduce oxidative stress and inflammation in DM [63].

IL-6 levels were higher in patients with lipid issues and IR [64]. IL-6 is regarded as a probable cause of obesity-related chronic inflammation and IR. IL-6 appears to produce cellular IR in both primary hepatocytes and HepG2 cells in vitro [65]. Previous studies discovered a substantial variance in IL-6 levels between diabetic patients and healthy subjects [66]. T2DM increases circulating IL-6 levels in humans that can be considered as an independent predictor of T2DM. Obesity is associated with higher IL-6 concentrations. The main sources of increased IL-6 in T2DM obese patients are adipocytes and macrophages located in adipose tissue [67].

TNF-α is a pleiotropic cytokine with numerous biological functions, the most important of which is to promote inflammation [68]. It has been linked to IR development. TNF-α production dysregulation has been linked to many human illnesses, including T2DM [69]. TNF-α levels in plasma correlate with visceral fat in obese T2DM patients and do not immediately influence poorly controlled diabetic patients by high glucose level reduction [70]. The substantial connection of TNF-α with HOMA and insulin may imply that β-cells are overworking to compensate for the IR caused by TNF-α in the peripheral tissues. TNF-α levels in T2DM patients correlate with higher HbA1c values, suggesting they potentially predict glycemic control [71].

IFN-γ is a prominent player in phagocytosis and opsonization, perhaps protecting against local infection in diabetic foot infection. As T2DM worsens, both T-natural killer cells and IFN-γ levels decrease. IFN-γ downregulation plays a more sensitive and particular effect on amplifying acute foot ulcer infection [72].

Our results estimated that IL-6, TNF-α, and IFN-γ increased in obese and newly obese diabetic patients. Metformin was more effective in reducing IL-6, TNF-α, and IFN-γ levels than glibenclamide. In summary, the mechanism of metformin on these cytokines likely involves multiple pathways, including adipokine regulation, AMPK activation, mitochondrial function, gut microbiota, inhibition of hepatic gluconeogenesis, and reduction of oxidative stress [56]. These actions contribute to the overall amelioration of insulin sensitivity and glucose homeostasis in obese.

4. Experimental

The study's target population is T2DM obese patients treated with metformin or glibenclamide. A total of 150 individuals were included in this study, 34 males and 116 females with age range between 45.5 – 57.26 years. The samples were collected after six months of treatment from five equal groups (n=30):

The normal control group: included healthy individuals.

The obese control group: included non-diabetic obese individuals.

The obese diabetic control group: newly diagnosed and previously untreated T2DM obese patients.

The metformin-treated obese diabetic group: diabetic obese patients receiving 500 mg metformin twice daily treatment.

The glibenclamide-treated obese diabetic group: diabetic obese patients receiving 5 mg glibenclamide once-daily treatment.

The sample size was calculated considering an effect size of 30%, alpha error of 0.05, power of the study of 80%, and number of groups of five. The results revealed a total sample size of 140 participants, and for more accuracy, the sample size was enlarged to 150 participants [20].

Before enrollment, subjects underwent thorough and detailed laboratory tests to exclude any conditions that could affect glucose tolerance.

Inclusion Criteria

Subjects with fasting serum glucose (FSG) level of 70-110 mg/dL without any hypoglycemic medications and BMI from 18.5-25 or 30-34.9 kg/m2 represented the normal control (n=30) and the obese control (n=30) groups, respectively. Patients with FSG from 140-250 mg/dL, HbA1c above 6.5%, and BMI from 30-34.9 kg/m2 represented the obese diabetics (n=90). From the latter group, the newly diagnosed diabetic patients on two separate tests without any previous diabetic treatment represented the obese diabetic control (n=30) and those treated with metformin and glibenclamide represented the metformin-treated (n=30) and glibenclamide-treated (n=30) obese diabetic groups, respectively. All groups received an exercise regimen of 40 ± 10 min daily walking [21].

Exclusion Criteria

Diabetic patients with FSG above 250 mg/dL underweighted and preobese patients, or those with class III obesity (BMI out of the study range) were excluded. Patients suffering from autoimmune disorders or recently hospitalized for infection were excluded. Neither the patients nor the normal subjects had a history of clinical or standard laboratory results diagnostic for parasitic, viral, or other infections, impaired hepatic or renal function, or the chronic use of anti-inflammatory drugs. We excluded also pregnant or lactating women, patients with different medical conditions other than T2DM, and smokers were

also excluded. Patients who didn't receive the treatment properly (dosage and timing) or missed two consecutive follow-up visits were excluded.

Methods

Body weight and Body mass index (BMI)

Subjects' weight and height were recorded at the study beginning and after six months of treatment. An automatic electronic balance (GRANZIA SRLS Instrument, via Sant anna 1, 16035 Rapallo, Genova, Italy) was utilized to measure body weight changes. BMI was calculated in Kg/m² [22]. BMI from 30-34.9 kg/m2, represented as class I obesity [23]. The medical history, anti-diabetic therapy, and daily meals were recorded.

Blood Sampling

After getting a brief history from all participants, 10 mL morning venous fasting blood samples were collected after 8 hours of overnight fasting. The collected blood was subdivided into 5 mL in plain tube allowed to coagulate, then centrifugate at 4000 r.p.m. for 15 minutes to obtain serum for insulin, C-peptide, and adipokines determination using enzyme-linked immune-sorbent assay (ELISA) and biochemical measuring of FSG concentration. Additionally, 3 mL of the collected blood was gathered in EDTA tubes, thoroughly mixed, and then centrifugated to obtain plasma for molecular detection of pro-inflammatory cytokines using the real-time polymerase chain reaction (RT-PCR) technique. Finally, 2 mL of the collected blood were collected in EDTA tubes for biochemical measuring of HbA1c. Then, all subjects were asked to complete 12 hours of fasting, and another 5 mL of venous samples were collected in plain tubes for lipid profile investigation.

Biochemical Investigations

FSG was measured using glucose oxidase and 4-amino antipyrine by a reagent kit from BioSystems, Maadi, Cairo, Egypt, according to manufacturer instructions.

Serum fasting insulin (FI) was obtained using the quantitative ELISA method (Catalog Number 10801, using a BIOS Human INS, enzyme-linked immunoassay (ELISA) kit purchased from Chemux Bio Science, Inc., South San Francisco, USA)). Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated based on FI and FSG levels, where HOMA-IR = (FI * FSG) / 405 [24].

Assessment of C-peptide was performed using BIOS Human ELISA kit (Catalog Number is 10802) purchased from Chemux BioScience, Inc, South San Francisco, USA, as instructed by the manufacturer. Quantitative determination of HbA1c was performed utilizing a reagent kit bought from Egypt's BioMed according to manufacturer instructions.

Lipid profile

Total cholesterol (TC) and triglycerides (TG) levels were measured by TC and TG kits purchased from DiaSys Diagnostic Systems, GmbH, Germany. High-density lipoprotein cholesterol (HDL-C) level was measured by HDL-C kit was purchased from Spinreact, Girona, Spain [31]. LDL-C and VLDL-C were calculated as follows:

VLDL-C = TG/5 [25]

LDL-C = TC – HDL-C – (TG/5) [26].

RNA Extraction

The total RNA was isolated from white blood cells (WBCs) using a Trizol/tri kit purchased from Qiagen, Inc., South San Francisco, USA, [27]. A reagent kit from Applied Biosystems, California, USA, synthesized cloned DNA (cDNA). The total RNA concentration was evaluated using a Nanodrop 2000 spectrophotometer. The ratio of the extracted RNA's integrity (A260/280) was approximately 1.8, and the total RNA used for cDNA synthesis was about ten μL.

Pro-inflammatory mediators Expression

The mRNA expression of the pro-inflammatory cytokines IL-1β, TNF-α [28], IL-6 [29], and IFN-γ [30] were detected using the RT-PCR technique (Table 5). RNA purification kit was purchased from Qiagen Inc., Germantown, USA (Catalog Number is 52906), and primers were purchased from Bioresearch Technologies, 2199 South McDowell Blvd, Petaluma, CA, USA. Using the following primers:

Table (5): Primers pairs used for RT-PCR

Gene	Sequence (5′-3′)
IL-1β	F: 5′-AAA CAG ATG AAG TGC TCC TTC CAG G-3′ R: 5′-CTC CTT AAT GTC ACG CAC GAT TTC-3′
IL-6	F: 5′-ACTCACCTCTTCAGAACGAATTG-3′ R: 5′-CCATCTTTGGAAGGTTCAGGTTG-3′
TNF-α	F: 5′-GGG AAG AGT TCC CCA G-3′ R: 5′-GGT CTG GTA GGA GAC G-3′
IFN-γ	F: 5′-ATG GAT GCT ATG GAA GGA A-3′ R: 5′-ACT TAT GTT GTT GCT GAT GG-3′

Expression of adipocytokines by ELISA

The quantitative determination of the adipocytokines visfatin, resistin, and RBP4 was determined using ELISA kits from RayBio, Parkway Lane, Norcross, GA (Catalog Number EIA-VIS and EIA-RES and EIA-RBP respectively) according to the manufacturer instructions.

Statistical Analysis

The IBM Statistical Program for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA) was applied to analyze the study results using one-way ANOVA and Duncan's test post hoc test. Mean ± standard deviation (SD) was used to represent the results, and P values ≤ 0.05 were considered statistically significant.

5. Conclusions

The study found elevated levels of adipocytokines, visfatin, resistin, and RBP4 in obese and obese-diabetic patients. Metformin treatment improved these adipocytokines but increased LDL-C and decreased HDL-C. Glibenclamide treatment improved IR but increased TC and TG levels. IL-1β, IL-6, TNF-α, and IFN-γ expressions increased in obese patients, while metformin-treated patients showed a decline. Metformin is recommended for obesity, inflammation, hypercholesterolemia, and hypertriglyceridemia, while glibenclamide is recommended for insulin resistance and dyslipidemia.

6. Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

There are no funding sources to declare.

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