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Concomitant Treatment of Type 2 Diabetics with Dipeptidyl Peptidase-4 Inhibitor and Metformin Increases Insulin Sensitivity

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Abstract

Objective: Vildagliptin is one of the DiPeptidylPeptidase-4 (DPP-4) inhibitors that act to delay endogenous degradation of the incretin hormones. This work was designed to evaluate the clinical effectiveness of vildagliptin in controlling hyperglycemia and lowering insulin resistance in Type 2 Diabetes Mellitus (T2DM) as monotherapy, and as combined therapy with metformin.

Patients and methods: Forty-five obese T2DM patients were enrolled in the study in addition to 10 healthy volunteers, who served as control. The patients were divided into 3 groups: Vildagliptin group received 50 mg vildagliptin twice daily. Metformin group received 500 mg metformin 3 times daily. Co-treatment group received 50 mg vildagliptin once and 500 mg metformin twice per day. Fasting blood samples were taken at the start of study and after 2 months of treatment.

Results: Fasting Blood Glucose (FBG) was decreased by 48.54%, 21.87%, and 32.27% in metformin, vildagliptin, and co-treatment groups, respectively. The decrease of FBG was associated with an improvement of lipid profile and a parallel decrease in fasting insulin, HOMA-IR, leptin, and free fatty acids. Vildagliptin produced a significant increase in glucagon-like peptide-1 by 30.15%, as monotherapy, and 45.38% as combined therapy with metformin.

Conclusion: The use of vildagliptin as add-on therapy to metformin in subjects with inadequate glycemic ling. The strategy of the use of DPP-4 inhibitors is expected to be of increasing value in the future treatment control produced a better achievement in decreasing insulin resistance and improving glucose hand of T2DM.

Keywords: DPP-4 inhibitors; T2DM; Insulin resistance; GLP-1; Leptin; Free fatty acids

Introduction

Type 2 diabetes mellitus is a dual disease, characterized by islet beta- and alpha-cell dysfunction in the setting of insulin resistance [1]. The prevalence of diabetes in Egypt is high and growing; in 2003 Report by the International Diabetes Federation, it was estimated that 9.8 % of the adult Egyptians have diabetes and 5% had Impaired Glucose Tolerance (IGT). In 2007 the prevalence increased to 11% and is predicted to grow to 13.3% by the year 2025 [2].

The need to address the underlying islet cell deficit has led to the rediscovery of the incretin hormones and their role in glucose homeostasis [3]. Incretins are gastrointestinal hormones released during nutrient absorption to increase insulin secretion. The two gut peptides accounting for most of the incretin effect are GLP-1 (Glucagon-Like Peptide 1), which synthesized in L-cells primarily found in the distal small bowel and colon, and GIP (Glucose-dependent Insulinotropic Peptide), which is secreted by duodenal and proximal jejunal K cells [4].

Both GIP and GLP-1 receptors are expressed in various tissues. GLP-1 receptors are found in pancreatic islets, vagal nerves, stomach, lung, kidney, and the brain, whereas GIP receptors are expressed in pancreatic islets, the brain and adipose tissue [5]. Within some minutes of release from their intestinal sites, GIP and GLP-1 undergo rapid metabolism (proteolytic cleavage) to inactive metabolites by the enzyme DiPeptidylPeptidase-4 (DPP-4). The cleavage is rapid accounting for the short half-life of the native GLP-1 (less than 2 min) [6]. The small amounts of active hormones that reach the pancreas act on receptor sites residing on beta-cells to stimulate insulin secretion in a glucose-dependent manner; furthermore, GLP-1 acts on alpha cells and inhibits the secretion of glucagon [7].

Analogs of GLP-l have been developed to mimic its insulinotropic effect such as liraglutide and exenatide, which are resistant to degradation by the DPP-4 enzyme [8]. Another group of incretin enhancers acts as selective inhibitors of DPP-4. Vildagliptin is a potent and selective inhibitor of DPP-4 that increases the levels of active incretins and enhances pancreatic islet α- and β-cell responsiveness to glucose, thus improving insulin secretion and reducing inappropriate glucagon production, improving insulin sensitivity, improving postprandial lipid and lipoprotein metabolism, and reducing fasting and postprandial glucose and HbA1c [9]. Vildagliptin does not appear to increase the risk for hypoglycemia and the oral dosage form gives a distinct advantage over GLP-l agonists, which must be given parenterally [10].

Since DM is a progressive disease, the majority of patients will eventually require the addition of a second drug to achieve acceptable glycemic control. So, the present work was conducted to evaluate the

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clinical effectiveness of vildagliptin in controlling hyperglycemia and lowering insulin resistance in T2DM patients as monotherapy, and as combined therapy with metformin.

Patients and Methods

A total of 45 obese insulin-resistant T2DM patients were enrolled in the study. The patients were selected from the Diabetes Clinic, Internal Medicine Department, Tanta University Hospitals, between October 2010 and March 2011, under supervision of Dr. Mamdouh Gabre. Ten healthy volunteers with matched age and sex served as control group. Body weight and height were measured and Body Mass Index (BMI) was calculated: BMI=weight (kg)/height (m^2).

The diagnosis was confirmed by FBG >126 mg/dL and fasting insulin >25 µIU/mL (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus Report, 2003) [11]. The patients were excluded if they had T1DM or associated hepatic or renal disorders. All subjects were informed about the study and gave an informed consent. The study was carried out according to the ethical guidelines approved by the Ethical Committee at Faculty of Pharmacy, Tanta University.

The epidemiological data of the patients and controls are shown in table 1. Within metformin group two patients were hypertensive, one was cardiac, and four were newly diagnosed as diabetic (naïve). Within vildagliptin group two patients were hypertensive and two were naïve. Within co-treatment group two patients were hypertensive and three were naïve. The diabetic patients were on conventional anti-hyperglycemic therapy.

The patients were divided into 3 groups; 15 patients each. First group received 500 mg metformin (Cidophage, CID CO, Egypt) three times daily. Second group received 50 mg vildagliptin (Galvus, Novartis Pharma Stein AG, Switzerland) twice daily. Third group received both metformin (500 mg twice daily) and vildagliptin (50 mg once daily). Fasting blood samples were collected in the morning between 8.00 am and 11.00 am at the start of the study and after 2 months of treatment. Each blood sample was divided into four portions; the first portion was allowed to clot, centrifuged, and the serum was used for immediate measurement of glucose. The second portion was added to Ethylene Diamine Tetraacetic Acid disodium salt (EDTA) (BDH, England) and used for immediate measurement of glycated hemoglobin. The third portion was added to EDTA and aprotinin (Phoenix Pharmaceuticals, INC), centrifuged at 1600 rpm (Chilspin 2 MSE, England) at 4°C for 20 min, and the obtained plasma was kept frozen at -70°C for up to one month for analysis of GLP-1. The fourth portion of blood was heparinized, centrifuged at 1600 rpm (4°C) for 20 min, and the plasma was stored at -2°C till analysis of insulin, leptin, lipids, and free fatty acids.

Serum glucose [12], Plasma triglycerides [13], total cholesterol [14], and High-Density Lipoprotein-Cholesterol (HDL-C) were measured colorimetrically using commercial kits (Elitech Diagnostics Company, France). Low-Density Lipoprotein-Cholesterol (LDL-C) was calculated [15]. Glycohemoglobin was measured spectrophotometrically [16] using kits obtained from Stanbio Laboratories, Texas, USA. Enzyme-Linked Immunosorbent Assay (ELISA) was followed for estimation of insulin [17] using kits from BioSource Europe S.A, leptin [18] using kits from Diagnostic Biochem Canada Inc., free fatty acids using kits from Cusabio Biotech Co., Ltd., China, and GLP-1 [19] using kits from Phoenix Pharmaceuticals, INC. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated: HOMA-IR=Insulin× Glucose/405 [20].

Data were analyzed by Minitab 6 system and presented as Mean ± SEM. For intergroup comparisons, measured variables were analyzed by paired *t*-test and difference between two groups was analyzed by unpaired *t*-test. Linear regression test and *Pearson`s* correlation were utilized to study correlation between measured parameters. The level of significance was set at $P<0.05$.

Results

Treatment of diabetic patients with each of metformin (Table 2), vildagliptin (Table 3) and both drugs (Table 4) for two months resulted in a significant decrease in FBG, but insignificant decrease in HbA1c, compared to pretreatment level. However, FBG remained higher than the control value. The diabetic patients also exhibited a significant decrease in both insulin and HOMA-IR *versus* their pretreatment values in all treated groups.

Plasma leptin and FFA were significantly higher in diabetic patients before treatment *versus* controls and were significantly decreased following treatment in all examined groups (Tables 2-4). Whereas plasma insulin in all treated patients returned to near its value in the control group, leptin and FFA remained significantly higher than the control level. Plasma GLP-1 was significantly decreased in diabetic patients before treatment *versus* the control group (Tables 2-4). Both vildagliptin and co-treatment groups showed a significant increase in GLP-1 after treatment *versus* pretreatment level. Treatment with metformin produced insignificant change in GLP-1.

The mean values of pretreatment levels of total cholesterol, triglycerides and LDL-C were significantly higher in all studied groups than in controls (Tables 2-4). Treatment for two months resulted in

Group		Control group	Metformin group	Vildagliptin group	Co-treatment group
Number		10	15	15	15
Sex (M/F)		3/7	3/12	2/13	4/11
Age (years)	Range	33-60	33-60	34-60	34-60
	Mean ± SEM	45.80 ± 2.67	47.40 ± 1.81	50.40 ± 1.85	49.73 ± 2.20
BMI (kg/m^2)	Range	28-33	27-40	27– 37.3	26.7-40
	Mean ± SEM	30.62 ± 0.53	32.56 ± 1.25	31.02 ± 0.87	31.45 ± 1.14
FBG (mg/dL)	Range	70-118	160-390	150-292	140-270
	Mean ± SEM	100.47 ± 3.74	229.9 ± 15.6	186.8 ± 11.1	188.5 ± 11.2
Duration of DM (months)	Range	--	0*-36	0*-24	0*-30
	Mean ± SEM	--	14.8 ± 2.98	13.6 ±1.89	14.4 ± 2.54

M: Male; F: Female; BMI: Body Mass Index; FBG: Fasting Blood Glucose; DM: Diabetes Mellitus
*: Newly diagnosed (naïve)

Table 1: Demographic data of patients and controls.

Parameter		Control Group	Metformin Group		
			Pre-treatment	Post-treatment	% Change
FBG (mg/dL)	Range	70-118	160-390	90-140	↓ 48.59 %
	Mean ±SEM	100.47 ± 3.74	229.90 [a] ± 15.6	118.20 [a,b] ± 4.03	
Total Cholesterol (mg/dL)	Range	160-210	176-249	192-253	↓ 1.46 %
	Mean ±SEM	178.50 ± 5.44	204.80 [a] ± 5.15	201.8 [a] ± 4.52	
Triglycerides (mg/dL)	Range	62-100	66-150	80-135	↑ 6.78 %
	Mean ±SEM	80.80 ± 3.64	110.07[a] ± 7.61	118.07 [a] ± 5.22	
LDL-C (mg/dL)	Range	101-110	105-160	95-156	↓ 7.84 %
	Mean ±SEM	106.40 ± 0.79	124.93 [a] ± 5.18	115.13 [b] ± 4.33	
HDL-C (mg/dL)	Range	54-60	41-51	46-53	↑ 9.20 %
	Mean ±SEM	58.00 ± 0.68	44.40 [a] ± 0.78	48.66 [b] ± 0.69	
Insulin (µIU/mL)	Range	15.5-19.1	29.5-33	16.4-18.4	↓ 42.12 %
	Mean ±SEM	17.37 ± 0.23	31.60 [a] ± 0.39	18.29 [a,b] ± 0.34	
HOMA-IR	Range	2.18-4.24	12.2-28.7	3.64-6.84	↓ 70.11 %
	Mean ±SEM	3.65 ± 0.19	17.90 [a] ± 1.18	5.35 [a,b] ± 0.23	
HbA_{1c} %	Range	5.13-6.1	7.82-12.00	7.8-11.9	↓ 0.57 %
	Mean ±SEM	5.79 ± 0.11	9.42 [a] ± 0.33	9.37 [a] ± 0.32	
Leptin (ng/mL)	Range	5.3-10.8	8.0-61.0	4.0-51.0	↓ 23.38 %
	Mean ±SEM	8.62 ± 0.51	27.67 [a] ± 4.14	21.20 [a,b] ± 3.16	
FFA (ng/mL)	Range	213-310	380-572	377-401	↓ 21.24 %
	Mean ±SEM	275.07 ± 7.59	474.3 [a] ± 13.1	373.60 [a,b] ± 6.61	
GLP-1 (ng/mL)	Range	6.3-9.5	3.1-7.5	3.0-7.0	↓ 8.66 %
	Mean ±SEM	7.67 ± 0.33	5.95 [a] ± 0.36	5.43 ± 0.32	

a: Significant *versus* control; b: Significant *versus* pretreatment; FBG: Fasting Blood Glucose;
LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; HOMA-IR: Homeostatic Model of Assessment of insulin resistance; FFA: Free Fatty Acids; GLP-1: Glucagon-Like Peptide-1

Table 2: Biochemical results of Metformin group.

a significant decrease in LDL-C and a significant increase in HDL-C compared to pretreatment values. Although FBG showed a greater decrease (-48%) in metformin group, the marked improvement of plasma insulin (-47.98%) and HOMA-IR (-70%) were observed in the co-treatment group. GLP-1 recorded the highest increase in co-treatment group (45%) followed by the vildagliptin group (30%). A significant positive correlation was found after treatment of diabetic patients between FBG and each of HbA1c (r=0.439) and HOMA-IR (r=0.881). Also, a significant positive correlation was found between plasma FFA and each of insulin (r=0.443) and leptin (r=0.352).

Discussion

Data from the Landmark UK Prospective Diabetes Study indicates that loss of beta-cell function is progressive and leads to the clinical impression of failure of therapy in T2DM patients. This is the main reason why many patients with T2DM are not within target ranges of glycemic control [21]. Only ~25% of adult diabetic patients achieve adequate glycemic control on monotherapy and the majority of patients will eventually require the addition of a second drug to achieve acceptable glycemic control [8].

In the present study, obese T2DM patients were kept on vildagliptin and/or metformin for two months. The mean values of BMI and HOMA-IR of the T2DM patients enrolled in our study indicated that they were obese and having insulin resistance. The pretreatment levels of HbA1c % indicated that the patients had a bad glycemic control in the previous two to four months. Kelly [22] demonstrated that there is a likes between obesity and T2DM involving pro-inflammatory cytokines (tumor necrosis factor-α and interleukin-6), insulin resistance, deranged fatty acid metabolism, and cellular processes such as mitochondrial dysfunction and endoplasmic reticulum stress. Hyperglycemia and hyperinsulinemia represent the whole mark of insulin resistance [23].

The hyperinsulinemia observed in T2DM patients at the start of study was reduced after two months of treatment in all groups, with a parallel reduction in FBG and HOMA-IR. Glycated hemoglobin, although it was insignificantly changed in T2DM patients administered different treatments, it tends to decrease, and the greatest reduction of HbA1c % was achieved with vildagliptin monotherapy. A significant positive correlation was found between HOMA-IR and each of FBG and HbA1c % in diabetic patients.

The present results were supported by the work of Pospisilik [24],

Parameter		Control Group	Vildagliptin Group		
			Pre-treatment	Post-treatment	% Change
FBG (mg/dL)	Range	70-118	150-292	110-222	↓ 21.88 %
	Mean ±SEM	100.47 ± 3.74	186.8 [a] ± 11.1	145.93 [a,b] ± 8.48	
Total Cholesterol (mg/dL)	Range	160-210	157-251	150-230	↓ 5.63 %
	Mean ±SEM	178.50 ± 5.44	202.60 [a] ± 6.05	191.2 [b] ± 4.52	
Triglycerides (mg/dL)	Range	62-100	80-135	70-120	↓ 10.76 %
	Mean ±SEM	80.80 ± 3.64	98.53 [a] ± 3.76	87.93 [b] ± 3.29	
LDL-C (mg/dL)	Range	101-110	110-161	92-150	↓ 12.56 %
	Mean ±SEM	106.40 ± 0.79	139.67 [a] ± 3.81	122.13 [a,b] ± 3.66	
HDL-C (mg/dL)	Range	54-60	41-49	49-57	↑ 20.30 %
	Mean ±SEM	58.00 ± 0.68	42.93 [a] ± 0.62	52.33 [b] ± 0.70	
Insulin (µIU/mL)	Range	15.5-19.1	28.4-33.3	15.5-19.3	↓ 44.49 %
	Mean ±SEM	17.37 ± 0.23	30.41 [a] ± 0.40	16.88 [b] ± 0.35	
HOMA-IR	Range	2.18-4.24	10.50-23.00	4.64-9.37	↓ 56.82 %
	Mean ±SEM	3.65 ± 0.19	14.00 [a] ± 0.83	6.04 [a,b] ± 0.32	
HbA_{1c} %	Range	5.13-6.1	7.31-10.69	7.30-10.55	↓ 5.00 %
	Mean ±SEM	5.79 ± 0.11	8.32 [a] ± 0.30	8.27 [a] ± 0.29	
Leptin (ng/mL)	Range	5.3-10.8	5.0-70.0	5.0-70.0	↓ 15.02 %
	Mean ±SEM	8.62 ± 0.51	22.83 [a] ± 5.01	19.40 [a,b] ± 4.64	
FFA (ng/mL)	Range	213-310	380-560	301-401	↓ 21.42 %
	Mean ±SEM	275.07 ± 7.59	440.4 [a] ± 13.5	346.07 [a,b] ± 8.91	
GLP-1 (ng/mL)	Range	6.30-9.50	3.0-7.0	5.0-8.5	↑ 30.16 %
	Mean ±SEM	7.67 ± 0.33	5.08 [a] ± 0.30	6.61 [b] ± 0.33	

a: Significant *versus* control; b: Significant *versus* pretreatment; FBG: Fasting Blood Glucose;
LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-density lipoprotein cholesterol; HOMA-IR: Homeostatic Model of Assessment of Insulin Resistance; FFA: Free Fatty Acids; GLP-1: Glucagon-Like Peptide-1

Table 3: Biochemical results of Vildagliptin group.

Parameter		Control Group	Co-treatment Group		
			Pre-treatment	Post-treatment	% Change
FBG (mg/dL)	Range	70-118	140-270	90-170	↓ 32.27%
	Mean ±SEM	100.47 ± 3.74	188.5 [a] ± 11.2	127.67 [a,b] ± 5.63	
Total Cholesterol (mg/dL)	Range	160-210	194-250	170-230	↓ 8.07 %
	Mean ±SEM	178.50 ± 5.44	214.87 [a] ± 4.72	197.53 [b] ± 4.50	
Triglycerides (mg/dL)	Range	62-100	87-154	80-130	↓ 15.76 %
	Mean ±SEM	80.80 ± 3.64	133.27 [a] ± 5.43	112.27 [a,b] ± 4.20	
LDL-C (mg/dL)	Range	101-110	115-171	100-152	↓ 12.89 %
	Mean ±SEM	106.40 ± 0.79	141.73 [a] ± 4.75	123.53 [b] ± 4.05	
HDL-C (mg/dL)	Range	54-60	41-47	45-57	↑ 14.98 %
	Mean ±SEM	58.00 ± 0.68	43.13 ± 0.61	50.07 [b] ± 0.79	
Insulin (µIU/mL)	Range	15.5-19.1	28.8-34.0	12.3-19.0	↓ 47.98 %
	Mean ±SEM	17.37 ± 0.23	30.89 [a] ± 0.41	16.07 [a,b] ± 0.49	
HOMA-IR	Range	2.18-4.24	7.63-22.31	3.43-6.97	↓ 70.57 %
	Mean ±SEM	3.65 ± 0.19	17.90 [a] ± 1.18	5.35 [a,b] ± 0.23	
HbA_{1c} %	Range	5.13-6.1	7.73-10.75	7.7-10.78	↓ 2.80 %
	Mean ±SEM	5.79 ± 0.11	8.54 ± 0.40	8.52 [a] ± 0.40	
Leptin (ng/mL)	Range	5.3-10.8	4.0-29.0	4.0-25.0	↓ 19.55 %
	Mean ±SEM	8.62 ± 0.51	13.30 [a] ± 1.86	10.70 [b] ± 1.50	
FFA (ng/mL)	Range	213-310	388-510	210-401	↓ 27.74 %
	Mean ±SEM	275.07 ± 7.59	424.7 [a] ± 10.2	306.9 [a,b] ± 12.5	
GLP-1 (ng/mL)	Range	6.3-9.5	2.0-6.0	5.3-9.0	↑ 45.39 %
	Mean ±SEM	7.67 ± 0.33	4.93 [a] ± 0.27	7.79 [b] ± 0.29	

a: Significant *versus* control; b: Significant *versus* pretreatment; FBG: Fasting Blood Glucose;
LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; HOMA-IR: Homeostatic model of Assessment of Insulin Resistance; FFA: Free Fatty Acids; GLP-1: Glucagon-Like Peptide-1

Table 4: Biochemical results of Co-treatment group.

who found that subcutaneous administration of DPP-4 inhibitor for 12 weeks improved insulin sensitivity in a diabetes model in rats. Moreover, Zander et al. [25] demonstrated that subcutaneous administration of GLP-1 for 6 weeks has been shown to enhance insulin sensitivity in subjects with T2DM, along with improved glucose metabolism. Lowering of blood glucose by vildagliptin is attributed to the potentiation of release of the incretin hormones, GLP-1 and GIP, from the small intestine into the vasculature. Vildagliptin acts as incretin enhancer by preventing the inactivation of endogenous incretin by DPP-4, thereby elevating active incretin levels, which then regulate insulin secretion in a glucose-dependent manner [26]. Vildagliptin has been shown to inhibit circulating DPP-4 activity by about 80% [27].

Measurement of GLP-1 in the current study provided an additional support, where GLP-1 showed low pretreatment levels in T2DM patients reflecting a defective incretin response, but increased post-treatment levels in both vildagliptin and co-treatment groups. Such finding goes with Charbonnel et al. [28], who reported that DPP-4 inhibitors increase the biologically active pool of GLP-1, raising endogenous GLP-1 levels, by up to 2-3 fold, and improving glucose tolerance and insulin secretion. On the other hand, metformin reduces the quantity of insulin required to control hyperglycemia in human diabetics [29]. Metformin appears to augment the hypoglycemic effectiveness of circulating insulin, possibly by enhancing its stimulation of glucose utilization in muscle [30]. Metformin, also, reduces hepatic glucose output by inhibiting gluconeogenesis. The "average" person with T2DM has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one third [31].

Other investigators have demonstrated the inhibition of mitochondrial respiration by metformin, which may reduce the energy supply required for gluconeogenesis [32]. In counterbalance, metformin stimulates glucose entry into the liver and glycolysis through the activation of glycolytic enzymes, such as hexokinase (glucokinase) and pyruvate kinase [29].

Additional suggested mechanistic effects of metformin are inhibition of glucose absorption in the gut [33] and increase of plasma levels of GLP-1 [34]. The latter suggestion was not confirmed by our study; the present data revealed that treatment of T2DM patients with metformin for two months produced insignificant change in plasma level of GLP-1. Measurement of plasma leptin in the current work indicated that the elevated pretreatment levels in T2DM patients have been lowered by different treatments but remained higher than the control level. Metformin was more effective than vildagliptin in lowering plasma leptin level. The present results were supported by the work of Klein et al. [35], who found that metformin inhibited leptin secretion in a brown adipocyte model in a dose-dependent manner. Inhibition of leptin secretion by metformin may be through the stimulation of mitogen-activated protein kinase and is suggested to contribute to the anorexigenic effect of metformin [36]. Moreover, the observed decline in leptin level in T2DM patients treated with vildagliptin could be interpreted by activation of GLP-1 to its receptors in adipose tissues, resulting in decreased lipogenesis and, subsequently, decreased leptin synthesis [37].

Administration of vildagliptin and/or metformin in the present study produced a significant decrease of plasma FFA *versus* pretreatment values. Plasma FFA exhibited a significant positive correlation with each of plasma insulin and leptin in treated diabetic patients. It has been evidenced that elevation of plasma FFA produces peripheral and, probably also, hepatic insulin resistance in obese healthy and diabetic subjects [38]. Our results were in agreement with Matikainen et al. [39], who reported a tendency for the plasma FFA to decline upon treatment with DPP-4 inhibitor. They postulated that chronic DPP-4 inhibition would result in a substantial decline in plasma FFA and increase in insulin-stimulated glucose disposal. On the other hand, the antilipolytic action of metformin could be attributed to its insulin-sensitizing effect through the decrease of systemic FFA levels [40].

The decrease of plasma FFA observerd in metformin group could be explained on the basis that metformin suppresses Acetyl-CoA Carboxylase (ACC) activity [36]. ACC is an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is a critical precursor of fatty acid synthesis, and a potent inhibitor of mitochondrial fatty acid oxidation. Thus, the metformin-induced suppression of hepatic ACC appears to regulate the partitioning of fatty acids between oxidative and biosynthetic pathways. The net benefit is the diminution of intracellular hepatic lipid content, thus preventing hepatic steatosis, as well as, the reduction of plasma triglycerides [41]. The current results indicated that co-treatment with metformin and vildagliptin produced the greatest reduction of plasma total cholesterol, triglycerides, and LDL-C compared with either treatment alone. The greatest increase in HDL-C was observed in patients on vildagliptin monotherapy. Our results were

in line with the work of Ahren [42], who found that the combination of metformin with sitagliptin significantly decreased total cholesterol and triglycerides. Matikainen et al. [39] reported that treatment of T2DM patients with vildagliptin improved postprandial plasma triglycerides after a fat-rich meal, and this was achieved mainly through a decrease in intestinally-derived apoB-48-containing particles. Thus, vildagliptin reduces postprandial atherogenic triglycerides-rich lipoproteins in the circulation and may protect against weight gain in patients with T2DM by extracting less fat from the gut.

The co-treatment group also showed the greatest reduction in plasma insulin, FFA, and HOMA-IR; i.e. the greatest reduction in insulin resistance, in T2DM patients. It is also obvious that the greatest increase in GLP-1 (45%) was obtained with the combined therapy, and was greater than the sum of the % increase of GLP-1 in metformin and vildagliptin monotherapy groups. This observation may suggest a synergistic mechanism between the two drugs regarding their effect on GLP-1. He et al. [43] reported that the ability of vildagliptin to increase plasma level of both fasting and postprandial GLP-1 was clearly and consistently enhanced in patients receiving concomitant metformin. The rationale for combining metformin with DPP-4 inhibitor is the complimentary mechanism of action of the two strategies. Metformin acts primarily by reducing hepatic glucose output and improving insulin sensitivity in liver and muscle [44], whereas DPP-4 inhibitors act by increasing GLP-1 levels and, thereby, stimulating insulin secretion and inhibiting glucagon secretion [7,4]. Fortunately, the pharmacokinetics of metformin and a DPP-4 inhibitor do not change by combining the two, as shown for sitagliptin, which further indicates the feasibility of the combination [45]. Taken together, vildagliptin effectively reduced insulin resistance in mild and moderate T2DM patients. The overall experience is that this strategy is efficient, highly tolerable, and safe with a minimal risk for hypoglycemic events. The use of vildagliptin as add-on therapy to metformin in subjects with inadequate glycemic control produced a better achievement in decreasing insulin resistance and improving glucose handling. The strategy of the use of DPP-4 inhibitors is expected to be of increasing value in the future treatment of T2DM.

Conflict of Interest

We declare that there is no conflict of interest and, All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and,

1. There is no support from any organization for the submitted work.
2. No financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, and the research was financially supplied by the authors.
3. No other relationships or activities that could appear to have influenced the submitted work.

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