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Efficacy of dulaglutide to improve Vascular Health indexes in subjects with Type 2 Diabetes: A Randomized Trial

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Abstract

Background: Recent cardiovascular outcome trials have shown significant reductions of major cardiovascular (CV) events with glucagon-like peptide (GLP)-1 receptor agonists. Adjunctive surrogates for cardiovascular risk more recently validated by some studies (23,24) are arterial stiffness and endothelial function indexes. No randomized trials have yet addressed the possible effects of antidiabetic interventional drugs such as GLP1 agonists on endothelial and arterial stiffness indexes as surrogate markers of vascular damage

Aims: A randomized trial to once-weekly dulaglutide (1.5 mg) added to traditional antidiabetic treatment compared to traditional treatment alone to evaluate some metabolic efficacy endpoints and surrogate vascular efficacy endpoints such as endothelial function and arterial stiffness indexes.

Methods: Men and women (aged ≥50 years) with established or newly detected type 2 diabetes whose HbA1c was 9.5% or less on stable doses of up to two oral glucoselowering drugs with or without basal insulin therapy were eligible. Subcutaneous dulaglutide was initiated at the full dose (1.5 mg/day weekly). Arterial stiffness indexes (pulse wave velocity and augmentation index) and endothelial function index (reactive hyperemia index) were evaluated at baseline and at a three-month and nine-month examination visit. At each visit (at 3 and 9 month follow-up) were also evaluated glycemic variables such as fasting plasma glucose (FPG), HbA1c and lipid variables such as total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride.

Results: at a three month follow up subjects treated with dulaglutide in comparison with subjects treated with conventional therapy showed significantly lower serum levels of FBG and significantly lower percentage of HBa1c. At a 9 month follow up subjects treated with dulaglutide in comparison with control subjects treated with conventional therapy showed a significant lowering of diastolic blood pressure, BMI, mean total serum cholesterol, LDL cholesterol, FPGa significantly lower percentage of HBa1C and PWV and higher mean RHI values.

Discussion: These findings are consistent with previous studies indicating the strict relationship between cardiovascular risk factors such as systolic blood pressure, total serum cholesterol and LDL levels and cardiovascular events and vascular health surrogate markers.

Background

Glucagon-like peptide-1 (GLP-1) is a member of an endogenous class of incretin hormones synthesized in intestinal epithelial-cells as a response to gastrointestinal nutrients (1). GLP-1 enhances glucose-dependent secretion of insulin (2,3), inhibits glucagon secretion (4), slows gastric emptying (5) and reduces food intake (6).

Dulaglutide (Eli Lilly and Co., Indianapolis, IN, USA), a long-acting GLP-1 receptor agonist (7), mimics some endogenous GLP-1 effects. Dulaglutide has been approved in the USA and European Union at once-weekly doses of 0.75 and 1.5 mg as a subcutaneous injection to improve glycaemic control in patients with type 2 diabetes.

In global clinical trials completed to date, dulaglutide (1.5mg) is superior to metformin, sitagliptin and exenatide twice daily and non-inferior to liraglutide (1.8mg) for glycated haemoglobin (HbA1c) changes (8-11).

Endothelial dysfunction and abnormal vascular structure may represent a possible complication of diabetes. Substantial clinical and experimental evidences suggest that both diabetes and insulin resistance cause a combination of endothelial dysfunctions, which may diminish the anti-atherogenic role of the vascular endothelium (12-14).

The initiation and progression of atherosclerosis may have its origins in impaired endothelial function that can be detected at the earliest stages of development of the syndrome. The elements of the metabolic syndrome and accelerated phase of atherogenesis are often silent partners that present many years before the onset of type 2 diabetes mellitus. The ability to detect subclinical vascular disease, as a reflection of the multiple factors that impair arterial wall integrity, offer new tools to refine cardiovascular risk stratification (15). Endothelial dysfunction in peripheral arteries is evaluated by forearm flow-mediated vasodilation (FMD) (18). However, this technique offers findings that can widely vary due to technical problems, thus making it a not fully standardized method (19).

Kuvin et al. (20) described a new method to evaluate endothelial dysfunction called reactive hyperemia peripheral arterial tonometry (RH-PAT). This method measures hyperemic response in a non-invasive way. The Framingham Heart Study reported that RHI (reactive hyperemia index) is inversely related to various cardiovascular risk factors (21) confirming usefulness of this test.

Arterial stiffness indexes, such as augmentation index (Aix) and pulse wave velocity (PWV), have been reported as surrogate cardiovascular markers (22), whereas endothelial dysfunction has been reported as an associated factor (21).

Adjunctive surrogates for cardiovascular risk more recently validated by some studies (23, 24) are arterial stiffness and endothelial function indexes.

Recent cardiovascular outcome trials have shown significant reductions of major cardiovascular (CV) events with these glucagon-like peptide (GLP)-1 receptor agonists (13-16).

Progressive separation of the treatment and placebo curves, starting clearly between 12 and 18 months of the trial period, and significant reductions in the risk of myocardial infarction and stroke, indicate that the beneficial CV effects observed with GLP-1 receptor agonists could be due to an antiatherogenic effect (17).

Glucose-lowering is an effective strategy for preventing vascular complications, but the extent to which it can reduce CV complications is less certain. Glucagon-like peptide-1 (GLP-1) agonists are potent glucose-lowering agents, but also have potentially beneficial effects on other traditional (body weight, blood pressure (BP), and LDL cholesterol) and non-traditional risk factors (low grade inflammation and endothelial dysfunction).

Our group recently reported that in some cohorts of patients such as diabetic patients with diabetic foot (25), subjects with NAFLD (26) and in patients with acute ischemic stroke (27), higher mean values of arterial

stiffness markers (PWV, Aix) and lower reactive hyperemia indexes (RHI) mean values may be considered as surrogate markers of vascular damage.

A previous recent study on a little sample of patients showed that once-weekly dulaglutide was comparable to once-daily liraglutide in terms of its effects on oxidative stress and endothelial function measured by RHI (28).

Nevertheless, no randomized trials have yet addressed the possible effects of antidiabetic interventional drugs such as GLP1 agonists on endothelial and arterial stiffness indexes as surrogate markers of vascular damage.

Study Hypothesis

Our study hypothesis is that the antiatherogenic effect and the consequent beneficial cardioprotective profile of dulaglutide may be related to its metabolic efficacy and to its possible efficacy on vascular health indexes such as endothelial dysfunction and arterial stiffness markers.

Aim of the study

In the present randomized trial, we compared once-weekly dulaglutide (1.5 mg) added to traditional antidiabetic treatment compared to traditional treatment alone with regard to some metabolic efficacy endpoints and surrogate vascular efficacy endpoints such as endothelial function and arterial stiffness indexes.

Materials And Methods

Study Design and Participants

This study was a 9-month randomized trial designed to evaluate the efficacy of antidiabetic traditional treatment + dulaglutide when compared with traditional antidiabetic treatment alone in patients with type 2 diabetes.

Traditional antidiabetic treatment was considered treatment with metformin or metformin plus sulfonylurea or basal insulin plus metformin.

Men and women (aged ≥50 years) with established or newly detected type 2 diabetes whose HbA1c was 9.5% or less on stable doses of up to two oral glucoselowering drugs with or without basal insulin therapy.

The key exclusion criteria for patients screened were: type 1 diabetes, previous GLP-1 receptor agonist treatment, treatment with more than half of the sulphonylurea maximum dose at screening, current insulin or thiazolidinedione use, chronic systemic glucocorticoid use, or gastric emptying abnormality.

Further exclusion criteria were: an eGFR18 less than 15 mL/min per 1·73 m^2, cancer in the last 5 years, severe hypoglycemia in the last year, life expectancy less than 1 year, a coronary or cerebrovascular event within the last 2 months, and plans for revascularization

A common protocol was approved by the institutional review board, and the study was performed in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice (29).

Each patient provided written informed consent before participation.

The trial has been registered on Clinical Trials.gov [ClinicalTrials.gov Identifier: NCT03824002].

We used the revised criteria of the American Diabetes Association to diagnose type 2 diabetes (T2DM), using a value of fasting blood glucose ≥126 mg/dl or a clinically based algorithm that considered, presenting weight and symptoms, age at onset, onset of insulin treatment, family history and history of ketoacidosis (30) .

Hypertension was diagnosed according to the 2013 ESC-ESH criteria (31).

Dyslipidemia was defined as TG level ≥150 mg/dl and HDL cholesterol level (<40 mg/dl) regardless of the patient's gender (32)

Randomization

After a 2-week placebo screening and run-in period, eligible patients were randomly assigned to undergo traditional antidiabetic treatment plus dulaglutide or traditional antidiabetic treatment alone using a computer-generated random sequence (1:1) with dulaglutide administered once weekly for 9 months. Investigators involved in clinical data collection and measurement of outcome variables were not directly involved in the patients' treatment and were masked to the randomization process. The randomization code was maintained only at the central data facility and was not broken until all data analysis was complete.

Procedures

Subcutaneous dulaglutide was initiated at the full dose (1.5 mg/day weekly).

During the treatment period, participants in both groups were instructed to inject study drug on the same day at approximately the same time, each week. Participants were visited at 2 weeks, 3 months, and 9 months. At every visit, assessments included cardiovascular events, adverse events, vital signs, and periodic questionnaires, laboratory tests electrocardiograms (ECGs) and vascular damage index assessment (see above). Investigators were advised to promote a healthy lifestyle and to manage glucose concentrations according to local guidelines. Management of blood pressure, lipids, other cardiovascular risk factors, and medical conditions was at the discretion of the study investigator.

Hypoglycemia was defined as a blood glucose concentration ≤3.9mmol/l. Severe hypoglycemia was defined as an episode that required the assistance of another person to actively administer carbohydrate, glucagon or other resuscitative actions. Patients were allowed to initiate rescue therapy for severe, persistent hyperglycemia according to predefined thresholds on fasting blood glucose for at least 2weeks with no readily identifiable cause.

PWV Measurement

Carotid-femoral PWV was assessed in the supine position using the automatic device (SphygmoCor version 7.1) that evaluated the time delay between the rapid upstroke of the carotid and femoral artery pulse waves. The distance between the two arterial points was measured using a tape measure on the surface of the body. PWV was valuated as the distance traveled by the arterial pulse wave (meters) divided by the time delay between the two arterial points (seconds), thus expressed as meters per second.

Pulse Wave Analysis

We used the Applanation tonometry to record radial artery pressure waveform continuously, and mean values of ≥2 screens of pulse waves of good quality were used for analysis. In consideration of the collected data, an averaged radial pressure waveform was created and a corresponding aortic pressure waveform and BP calculated by the validated transfer function (SphygmoCor version 7.1). The aortic pressure waveform was utilized to calculate the AIx (difference in height between the first and second systolic peaks expressed as a percentage of PP).

RH-PAT

The principle of RH-PAT has been described previously by some researchers (19). In sum, a blood pressure cuff was positioned on 1 upper arm, while the contralateral arm served as a control. PAT probes were positioned on one finger of each hand. After a 5-min equilibration period, the cuff was inflated to 60 mm Hg above the systolic pressure or 200 mm Hg for 5 min and then deflated to induce reactive hyperemia. Reactive hyperemia is a temporary increase of blood flow on an area as result of induced ischemia and express “the health state” of endothelium.

The RH-PAT data were digitally analyzed online by Endo-PAT2000 software version 3.0.4. The RH-PAT index (RHI: reactive hyperemia index) reproduces the range of reactive hyperemia and was measured as the ratio of the mean amplitude of PAT signal over 1 min starting 1.5 min after cuff deflation (control arm, A; occluded arm, C) divided by the mean amplitude of PAT signal of a 2.5-min time period before cuff inflation (baseline) (control arm, B; occluded arm, D). Thus RH-PAT index (RHI) = (C/D)/(A/B) x baseline correction. A value of RHI < 1,67 indicated endothelial dysfunction

Vascular damage markers evaluation

PWV, Aix and RHI were measured at admission and at every three- and nine-month follow-up visit assessment.

Endpoints of the study

Metabolic Efficacy endpoints were:

- *change from baseline in body weight at three and nine-month follow-up;*
- *change from baseline in BMI at three and nine-month follow-up*
- *change from baseline in FPG at three and nine- month follow-up*
- *change frombaseline in HBa1C at three and nine-month follow-up*
- *change frombaseline in serum total cholesterol at three and nine-month follow-up*

- *change from baseline in serum LDL cholesterol at three and nine-month follow-up*
- *change from baseline in serum HDL cholesterol at three and nine-month follow-up*

Vascular Efficacy endopoints were:

- *change from baseline in RHI value at three and nine-month follow-up*
- *change from baseline in PWV at three and nine-month follow-up*
- *change from baseline in Aix at three and nine-month follow-up*

Outcomes

The first primary objective was to show the superiority of dulaglutide vs traditional treatment on metabolic variable change (*fasting plasma glucose, HBa1C, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides*) from baseline at a three- and a nine-month follow-up.

The second primary objective was to show the superiority of dulaglutide vs traditional treatment on some vascular health indexes such as markers of arterial stiffness (PWV, Aix) and of endothelial function such reactive hyperemia index (RHI) change from baseline at three- and nine-month follow-up.

Statistical Analysis

The sample-size of at least 40 randomized patients was selected to provide >99% power to demonstrate superiority of dulaglutide to placebo. This assumed a true mean difference in metabolic and vascular variable mean value change from baseline between dulaglutide and placebo of 0.8%, a common standard deviation of 1.1%, a one-sided significance level of 0.025, and a 9% drop-out rate between randomization and week (26). Moreover, the given sample-size provided at least 90% power to confirm non-inferiority of dulaglutide to liraglutide with a margin of 0.4%.

Continuous data are expressed as mean ±SD, unless otherwise specified. Intergroup differences were assessed by the chi-square test or Fisher exact test, as needed for categorical variables, and by the independent Student t test for continuous parameters if the data were normally distributed. Intragroup differences were performed by repeated measures analysis of variance (ANOVA) and post hoc analysis with the Tukey test was used to determine if there were any intra-group differences in pairs.

Data were analysed by IBM SPSS Software 22 version (IBM Corp., Armonk, NY, USA). All p-values were two-sided and $p<0.05$ was considered statistically significant.

Results

Between april 2 2017 and april 12 2019, 124 patients entered the study, 60 were randomized and were treated with study drug (56 completed 3 months and 9 months of treatment), 64 control subjects were enrolled, 12 patients (4 in the group traditional treatment plus dulaglutide and 8 in the control group) discontinued the study with ‘withdrawal by subject’ being the most common reason.

Patient demographics and baseline characteristics were similar between the two groups (Table 1).

At the end of the randomization phase 56 patients completed the study for each group .

At baseline, patients treated with dulaglutide plus traditional therapy vs controls treated with traditional therapy showed no significant difference with regard of age (69.7 ± 8.6 years vs 67. 6 ± 5.1 years ; p=0.111), duration of diabetes (10.4 ±3·3 years vs 10.2±4.0 years; p=0.773), mean fasting plasma glucose (FPG) (147.6±32.5 mg/dl vs 144.6±31.5 mg/dl p=0.626), mean HBa1C (7.4± 0.7 % ± 7,2 ± 0.6 %; p=0.101), mean total cholesterol (160.9 ± 20.5 mg/dl vs 167.8 ± 25 mg/dl mmol/L; p=0.121), mean HDL cholesterol (37.4 ± 4.3 mg/dl vs 37.5±4.5 mg/dL/p=0.961), mean LDL cholesterol (102.7±11.6 mg/dl /2.65±0.3 mmol/L vs 104.5±13.6 mg/dl/2.7±0.35 mmol/L; p=0.439), mean triglycerides (139.2 ± 13.6 mg/dl vs 138.90 ± 13.58 mg/dl; p=0.910), mean RHI (1.7 ± 0.4 vs 1.8 ± 0.7; p=0.275), PWV (11.2±0.91m/sec vs 10.9±0.8 m/sec; p=0.193). (see table 1)

Subjects treated with dulaglutide and control subjects treated with traditional therapy showed no significant differences for the other clinical and laboratory variables at baseline.

Efficacy

At a three-month follow up subjects treated with dulaglutide in comparison with control subjects treated with conventional therapy showed no significant difference with regard of SBP, DBP, BMI, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, GFR CKD-EPI, Microalbuminuria, but they showed significantly lower body weight (73.1± 7.6 kg vs 76.6±7.1 Kg, P=0.014), significantly lower serum levels of FPG (131.2 ± 21.95 mg/dl vs 146.2 ±29.5 mg/dl; p=0.003) and significantly lower percentage of mean HBa1c (6.5 ± 0.5 % vs 7.0 ± 0.5 %; p<0.0005) (see table 2).

At a nine-month follow up subjects treated with dulaglutide in comparison with control subjects treated with traditional therapy showed a significant lowering of mean DBP values (71.8 ± 9.2 mm/Hg vs 75.9 ± 9.7 mm/Hg; p=0.026), body weight (68.9 ± 5.6 Kg vs 76.3 ± 13.84 mm/Hg; p=0.015), BMI (25.2 ± 3.6 Kg/m^2 vs 27.8 ± 5.1 ; p=0.03**)**, mean total serum cholesterol (152.3 ± 22.4 vs 163.3±21.5 mg/dl 4.22±0.56; p=0.009), mean LDL serum cholesterol (96.0±9.1 mg/dl vs 103.2±10.8 mg/dl p<0.0005), FPG (119.3 ± 14.3 mg/dl vs 145.5 ± 29.6 mg/dl; p <0.0005), Hba1C (6.2 ± 0.3% vs 6.9 ± 0.5 %; p <0.0005), microalbuminuria (79.1 ± 9.0 vs 82.5 ± 9.3 mg/dl; p=0.05), mean PWV (10.6 ±0.8 m/sec vs 11.0 ±0.6 ; p=0.015**)** and higher mean RHI values **(**2.0 ± 0.4 vs 1.8 ± 0.4 ; p=0.023**).** (see table 2).

Intragroup analysis showed that subjects treated with once-weekly dulaglutide at a 9 month follow-up showed in comparison to baseline and three month follow-up, significantly lower mean SBP (129.7 ± 10.71 mm/Hg vs 137.8 ± 12.1 mm/Hg vs 134.9 ± 10.9 mm/Hg; p**<**0.0005), mean DBP (71.9 ± 9.29 mm/hg vs 76.3 ± 12.2 vs 73.2 ± 9.99; p=0.03), weight (68.9 ± 8.8 Kg vs 73.1± 7.6 Kg vs 75.8± 8.5 Kg ; p **<**0.0005**),** BMI (25.2 ± 3.6 Kg/m^2 vs 26.9 ± 3.4 Kg/m^2 vs 27.6 ± 3.4 Kg/m^2 ; p**<**0.0005), mean FPG values (119.3 ± 14.3 mg/dL vs 147.6 ±32.5 mg/dl vs 131.2 ± 21.9 mg/dl; p**<**0.0005), mean HBa1C percentage (6.2± 0.3% vs 6.5 ± 0.4 vs 7.3± 0.7%; p**<**0.0005), total cholesterol (152.3 ± 22.4 mg/dl vs 160.3 ±21.2 mg/dl vs 160.9± 20.5 mg/dl p=0.04), mean LDL cholesterol (96.0 ± 9.1 mg/dl vs 102.7 ±11.6 mg/dl vs 101.8±10.6 mg/dl p=0.001), mean PWV (10.6±0.8 m/sec vs 11.2±0.9 m/sec vs 10.8±0.8m/sec; p**<**0.0005), mean Aix (101.8±5.3% vs 105.6 ± 6.9% vs

103.1±6.7%; p= 0.001) and significantly higher median RHI values (2.0 ± 0.4 vs 1.7 ± 0.44 vs 1.8 ± 0.4; p= <0.0005) (see table 3).

Discussion

This study aimed to examine the efficacy in ameliorating endothelial and arterial stiffness markers of once-weekly dulaglutide (1.5 mg) plus traditional therapy in diabetic patients with type 2 diabetes.

This present study is the first that analyzed the efficacy of dulaglutide therapy on surrogate vascular endpoints such as endothelial and arterial stiffness indexes in parallel with its metabolic effects.

Consistent with previous studies, the study reports that subjects treated with traditional therapy plus dulaglutide showed at three and nine-month follow-up significantly lower mean fasting plasma glucose and glycated hemoglobin. Thus, our study furtherly confirms previous findings concerning the reduction of glucose serum levels and Hba1C serum levels (33-34).

Grunberger et al (35) demonstrated a dose-dependent HbA1c reduction for dulaglutide and greater reduction compared to placebo and similar results were seen for daily plasma glucose and fasting plasma glucose (FPG) with dose-dependent reductions for all.

Furthermore, randomized, placebo-controlled REWIND study (36) recruited participants who were at least 50 years old with type 2 diabetes, a hemoglobin A_{1c} (HbA_{1c}) level of 9.5% or less, and a body mass index of more than 23 kg/m^2 and concerning to diabetes outcomes, authors reported that HbA_{1c} levels fell in the drug group by a mean −0.61% compared with placebo (34).

Our study also reported that subjects treated with traditional therapy plus dulaglutide at a nine-month follow-up showed a significantly lower mean body weight, BMI and significantly lower mean LDL cholesterol and total cholesterol serum levels.

In the multicenter, randomized, double-blind, placebo-controlled trial, REWIND, subjects with type 2 diabetes who had either a previous cardiovascular event or cardiovascular risk factors randomly assigned to either weekly subcutaneous injection of dulaglutide (1.5 mg) or placebo showed at follow-up lower total cholesterol and lower LDL cholesterol (36)

The atherogenic dyslipidemia in diabetes in type 2-diabetes is characterized by an overproduction and/or delayed catabolism of triglycerides-rich particles (TRLs), including apolipoprotein apo-B-48 containing chylomicrons and apo-B100-containing VLDLs, cholesterol rich remnant particles, small dense LDLs and reduction in circulating HDLs (37). It has been reported that a reduction of postprandial TRLs in response to induction of GLP-1 signalling, GLP-2 receptor activation is involved in promotion of lipid absorption (38). In experimental hamsters an infusion of GLP1 and GLP2 causes an initial increase in lipid absorption and increased plasma concentrations of TRL-apo B48 in plasma and this response in terms of increased postprandial lipid response has been reported as enhanced in condition of insulin resistance such as in type 2 diabetes (39).

We also reported that subjects treated with traditional therapy plus dulaglutide showed at a nine month follow-up a significantly lower mean body weight and SBP, DBP values. Furthermore at nine months we also observed a significant reduction of microalbuminuria levels that is a well known marker of vascular damage in diabetes (38,39).

With regard of patients enrolled in REWIND trial, their weight decreased by a mean –1.5 kg while systolic blood pressure and LDL cholesterol levels were slightly lower in the drug group (40).

In our trial the metabolic effects on FPG and Hb1aC have been observed earlier at three-months in contrast, at a nine-month follow-up, we observed more delayed effects on cholesterol serum variables and vascular health indexes such as RHI and arterial stiffness indexes such as PWV and Aix, thus underlying a possible more strict relationship between vascular damage markers and cholesterol serum levels.

These findings are consistent with previous studies indicating the strict relationship between cardiovascular risk factors such as systolic blood pressure, total serum cholesterol and LDL levels (41-43) and cardiovascular events and vascular health surrogate markers (44).

Furthermore, as observed in our trial, and similarly in recent studies (36,45), weekly subcutaneous injection of 1.5 mg of dulaglutide was associated with weight loss and consequently a reduction in the BMI.

Chronic inflammatory state linked to obesity leads to a dysregulation of the endocrine and paracrine actions of adipocyte-derived factors, which disrupt vascular homeostasis and contribute to endothelial vasodilator dysfunction by determining, among other effects, an imbalance in endothelin-1/nitric oxide pathway (46).

In consideration of these premises, the reduction of body-weight and BMI, observed in the dulaglutide-arm at nine-months follow-up, might lead to a reduction of systemic inflammatory state, to a resumption of the correct mechanisms of vascular homeostasis and therefore, as observed in our trial, to an improvement of the vascular health indexes.

Several studies indicated that in addition to lowering glucose, dulaglutide had an effect on the cardiovascular system (41-44). These investigators reported that patients with T2DM found that dulaglutide could lower systolic blood pressure for 2.8mmHg compared with placebos. Tuttle et al. in a study of 6005 patients with T2DM showed that dulaglutide could slightly lower urine protein, but did not lower the glomerular filtration rate (42).

Furthermore, Ceriello et al. showed a beneficial effect of a combination of GLP-1-RA and insulin on hyperglycemia-induced oxidative stress and endothelial function for patients with T2DM (43). The antioxidant properties of GLP-1-RA increases intracellular antioxidant defenses (44). This mechanism may be due to the fact that the use of a GLP-1-RA affected oxidative stress and vascular endothelial function characteristics. Another possible reason is that glycemic control was better in patients treated with dulaglutide added on conventional therapy and possibly related to an improvement in glucose variability as reported by some studies (47).

Consistently to previous studies (36, 50), our findings underlined the efficacy of dulaglutide on glycemic control, as showed by its precocious action on lowering FPG and HB1AC not only at nine month but also at

three month.

A previous research (47) evidenced that the reduction of FPG and the improvement of glycemic variability is correlated to the reduction of oxidative stress on vascular endothelial.

In particular an increase of FPG and glucose variability are able to induce a worsening of oxidative stress thought many mechanisms such as overproduction of ROS mediated by many pathways like increase of AGEs levels, vasomotor imbalance mediated by reduction of NO availability and increase of Oxidant peroxynitrite, increase of inflammation molecules and overexpression of cellular adhesion molecules on endothelial surface like ICAM, VCAM, E-selectin which promote inflammation by leukocyte rolling (48). All these pathways may contribute to endothelial dysfunction and accelerated the process of atherosclerosis which lead to increase cardiovascular events (49).

Moreover our study showed at nine month an improvement of endothelial markers such as RHI, AIX and PWV. It is suggestive that our findings of precocious reduction of FPG and HB1AC at three month, owing to their possible negative action on modulation of oxidative stress, should provide an interesting explanation on improvement of endothelial function, as suggested by an increase of RHI and a decrease of PWV and AIX at nine months.

Furthermore, since in the Rewind trial the separation of the treatment and placebo curves, start clearly between 12 and 18 months of the trial treatment period indicating that the beneficial vascular effect with GLP-1 receptor agonists it could be due to an antiatherogenic effect (17,36). Our findings are consistent with these results and may offer a possible pathogenetic explanation of the delayed effects on cardiovascular events indicating in amelioration of metabolic and vascular markers observed at nine month of treatment this basis.

Our study is the first one that evaluated the beneficial effects of dulaglutide on both the vascular health indexes such as arterial stiffness and endothelial dysfunction markers. These novel findings may represent a possible explanation of the interplay between improvement of metabolic variables and reduction of cardiovascular outcome reported by trial on dulaglutide effectiveness (45) on cardiovascular outcomes. Our findings concerning surrogate vascular markers may also represent an explanation of the cardiovascular positive effects of other Glucagon-like peptide 1 receptor agonists (GLP-1-RAs) such as liraglutide and semaglutide (50-57).

Glucagon-like peptide 1 receptor agonists (GLP-1 RAs) are emerging as an important therapy to consider for patients with type 2 diabetes (T2D) given this class of treatment's ability to reduce glycated hemoglobin and their associated weight loss and low risk for hypoglycemia. Additionally, some cardiovascular outcomes trials (CVOTs) found non-inferiority for cardiovascular outcomes, with many findings of superiority of these drugs. These findings have transformed our guidelines on pharmacological treatment of T2D. Future studies will be addressed to evaluate the relationship between the effects of GLP-1 RAs on vascular damage markers and incidence of new cardiovascular events on a prospective way.

Conclusions

Our randomized trial showed that diabetic subjects treated with conventional therapy plus 1.5 mg subcutaneous dulaglutide in comparison with subjects treated with conventional therapy alone showed favorable metabolic effects that are associated with positive effects on vascular health markers such as arterial stiffness and endothelial function markers. We furthermore reported that unlike some metabolic effects such as the positive effects on FPG, HbA1c have been observed at a three-month follow-up, in contrast, other metabolic effects such as body weight and BMI reduction and positive effects on lipid serum values have been observed only at a nine month follow up, similarly to the positive vascular effects on PWV, Aix, RHI.

Thus, it is intriguing to speculate that the positive vascular effects could be strictly linked to lipidemic metabolic positive effects of dulaglutide and to the reduction of body weight with consequent modulation of related adipose-derived inflammation.

Declarations

Ethics approval and consent to participate

This protocol study was approved by the Ethics Committee of the Policlinico P. Giaccone Hospital and all patients gave their written informed consent to participate in the study, as well as for sampling and banking of the biological material. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and written informed consent was obtained from all patients. a statement of ethics approval with the name of the ethics committee's and the reference number if appropriate.

Consent for publication

All enrolled patients released a consent to publish from the participant (or legal parent or guardian for children) to report individual patient data.

Availability of data and material

All data and material are available on figshare

Competing interest

All the authors have no competing interest

Funding

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Author’s contributions

- Antonino Tuttolomondo. designed the research study and wrote the paper , collected and analysed the data
- Anna Cirrincion e: designed the research study, performed the research, collected and analysed the data
- Alessandra Casuccio: performed the research,

- Alessandro Del Cuore : performed the research,
- Mario Daidone : performed the research,
- Tiziana Di Chiara: performed the research,
- Irene Simonetta : performed the research,
- Stefania Scaglione : performed the research,
- Antonio Pinto designed the research study

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Abbreviations

CV: cardiovascular events

(GLP): glucagon-like peptide 1 receptor agonists.

FPG: fasting plasma glucose

HbA1c: *glycated hemoglobin*

LDL : low density lipoprotein

HDL :_ high density lipoprotein

BMI: body mass index

PWV: pulse wave velocity

RHI: reactive hyperaemia index

FMD: flow-mediated vasodilation (RH-PAT reactive hyperemia peripheral arterial tonometry

NAFLD: Non-alcoholic fatty liver disease (

eGFR: extimated glomerular filtration rate

ESC-ESH criteria : European Society of Cardiology-European Society of Hypertension

BP: blood pressure

AIx : augmentation index

PP: pulsatory pressure

SBP: systolic blood pressure

DBP:diastolic blood pressure

TRLs: triglycerides-rich particles (),

VLDLs: very low density lipoprotein

T2DM : type 2 diabetes mellitus

GLP-1-RA*: glucagon-like peptide 1* receptor agonist

ROS: *Reactive oxygen species*

AGEs : advanced glycation end-products

NO: nitric oxide

ICAM-1: Intercellular Adhesion Molecule 1

VCAM-1: v*ascular cell adhesion molecule 1*

CVOTs : cardiovascular outcomes trials

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Tables

Table 1: general, demographic and laboratory variables in subjects treated with dulaglutide vs controls (conventional therapy)

Variables	subjects treated with conventional therapy + dulaglutide (n=56)	Controls (conventional therapy) (n=56)	P
Age (years) (mean± SD)	69.7 ± 8.6	67.6 ± 5.1	0.111
M/F (n/%)	24/32 (42.8/57.2)	21/35 (37.5/62.5)	0.700
SBP (mm/Hg) (mean± SD)	137.8 ± 12.1	133.9 ± 12.3	0.087
DBP (mm/Hg) (mean± SD)	76.3 ± 12.3	79.8 ± 7.7	0.071
Weight (Kg) (mean± SD)	75.7 ± 8.5	76.6 ± 7.5	0.562
BMI (kg/m2) (mean± SD)	27.6 ± 3.4	27.9 ± 3.2	0.563
HR (bpm) (mean± SD)	76.6 ± 11.1	79.3± 15.8	0.299
Total cholesterol (mg/dl) (mmol/L) (mean± SD)	160.9± 20.5/4.2±0.5	167.8 ±25.8/4.3±0.7	0.121
HDL cholesterol (mmol/L) (mean± SD)	37.4 ± 4.3/0.9±0.1	37.5 ± 4.5/0.9±0.2	0.961
LDL cholesterol (mmol/L) (mean± SD)	102.7 ± 11.6/2.6±0.3	104.5 ± 13.5 /2.7±0.4	0.439
Triglycerides (mmol/L) (mean± SD)	139.2 ± 13.6/1.6±0.2	138.9 ± 13.6/1.6±0.2	0.910
FPG (mg/dl/ mmol/L (mean± SD)	147.6±32.6 /8.2 ±1.2	144.7±31.5 /8.1±1.8	0.626
HBa1C (%) (mean± SD)	7.4± 0.7	7.2± 0.6	0.101
eGFR CKD-EPI (mL/min per 1·73 m²) (mean± SD)	77.4 ± 11.2	76.1± 16.9	0.640
Microalbuminuria (mg/24 h) (mean± SD)	80.9 ± 20.9	73.1± 29.2	0.107
RHI (mean± SD)	1.7 ± 0.4	1.8 ± 0.7	0.275
PWV (m/sec) (mean± SD)	11.2±0.9	10.9 ±0.8	0.193
Aix (%) (mean± SD)	105.6± 6.9	103.9±4.4	0.121
Hypertension (n/%)	45/80.3	35/62.5	0.700
Diabetes (n/%)	56/100	56/100	-
Duration of diabetes (years) (mean± SD)	10.4 ±3.3	10.2±4.0	0.773
dyslipidaemia (n/%)	32(57.1)	29 (51.8)	0.704
Hypercholesterolaemia (n/%)	31 (55.3)	27 (48.2)	0.570
Smoking (n/%)	12 (21.4)	13 (23.2)	1.0
Microalbuminuria	12 (21.4)	11 (19.6)	1.0

Macroalbuminuria	4 (7.1)	3 (5.4)	1.0
Metformin (n/%)	50(89.3)	49 (87.5)	1.0
Sulfoniluree (n/%)	31 (55.3)	30 (53.6)	1.0
Insulin (n/%)	12 (21.4)	11(19.6)	1.0
ACEi (n/%)	28(50.0)	32(57.1)	0.569
Beta blockers (n/%)	28(50.0)	24(42.8)	0.570
CCB (n/%)	21(37.5)	22(39.3)	1.0
Antiplatelets (n/%)	23(41.1)	18(32.1)	0.432
Anticoagulants (n/%)	30(53.6)	30(53.6)	1.0
Diuretics(n/%)	27(48.2)	30(53.6)	0.705
Statins (n/%)	22(39.3)	16(28.6)	0.318

Data are expressed as mean (SD), n (%),

SBP: systolic blood pressure; **DBP:** diastolic blood pressure; **BMI:** body mass index**; HR:** heart rate; LDL: low density lipoprotein; **HDL:** High Density Lipoprotein; **FPG:** fasting plasma glucose; **HBa1C:** glycated hemoglobin; **PWV:**pulse wave velocity, **AIX :** augmentation index ; **E-GFR:** Estimated Glomerular Filtration Rate

SGLT2:sodium-glucose co-transporter-2. **ACE=**angiotensin-converting enzyme. **ARB:**angiotensin-receptor blocker. HbA1c**:**glycated haemoglobin A1c. eGFR=estimated glomerular filtration rate.

Table 2: Intergroup analysis of metabolic and vascular variables in patients treated with dulaglutide and controls at a three and nine month follow-up

Variable	Three months follow-up	Nine months follow-up
SBP (mmHg) (mean±SD)		
subjects treated with dulaglutide	134.9 ± 10.9	129.7 ± 10.7
controls	133.7 ± 16.6	132.7 ± 16.7
P	0.653	0.264
DBP (mmHg) (mean±SD)		
subjects treated with dulaglutide	73.2± 9.9	71.8 ± 9.2
controls	75.9 ± 9.1	75.9 ± 9.7
	0.138	**0.026**
P		
Weight (Kg) (mean±SD)		
subjects treated with dulaglutide	73.1± 7.6	68.9 ± 5.6
controls	76.6± 7.1	76.3 ± 13.8
	0.014	**0.015**
P		
BMI (kg/m2) (mean±SD)		
subjects treated with dulaglutide	26.9 ± 3.4	25.2 ± 3.6
controls	26.6 ± 5.0	27.8 ± 5.1
	0.741	**0.003**
P		
HR (bpm) (mean±SD)		
subjects treated with dulaglutide	75.6 ± 6.0	75.1 ± 5.3
controls	78.7 ±12.5	77.7 ± 12.5
P	0.097	0.161
Total cholesterol (mg/dl) (mmol/L) (mean±SD)		
subjects treated with dulaglutide	160.3 ±21.2/ 4.3±0.7	152.3 ± 22.4/3.94±0.58
controls	165.2± 26.3/4.2±0.6	163.3 ± 21.5/4.22±0.56
P	0.284	**0.009**
HDL cholesterol (mg/dl) (mmol/L) (mean±SD)		
subjects treated with dulaglutide	36.4 ± 4.3/0.9±0.1	38.3 ± 5.7/ 0.99±0.15
controls	36.7 ±4.3/0.9±0.1	36.9 ±4.7/0.95±0.11

P	0.607	0.135
LDL cholesterol (mg/dl) (mmol/L) (mean±SD)		
subjects treated with dulaglutide	101.8± 10.6 /2.6±0.2	96.0 ± 9.1/2.48± 0.24
controls	103.8 ±11.9/2.68±0.31	103.2 ± 10.8/2.67±0.28
P	0.339	**<0.0005**
triglycerides (mg/dl) (mmol/L) (mean±SD)		
subjects treated with dulaglutide	138.1 ± 13.2/1.5±0.1	137.5 ± 18.9/1.55±0.21
controls	138.0 ± 15.9/1.56±0.18	138.1 ± 18.9/2.72±0.42
	0.971	0.878
P		
FPG (mg/dl/mmol/l) (mean±SD)		
subjects treated with dulaglutide	131.2 ± 21.9/7.2±1.2	119.3 ± 14.3/6.65±1.05
controls	146.2 ± 29.5/8.13±1.64	145.5 ± 29.6/8.09±1.64
P	**0.003**	**<0.0005**
HBa1C (media± DS)		
subjects treated with dulaglutide	6.5 ± 0.5	6.2± 0.3
Controlli	7.0 ± 0.5	6.9 ± 0.5
P	**<0.0005**	**<0.0005**
GFR CKD-EPI (mean±SD)		
subjects treated with dulaglutide	76.7 ± 10.5	76.7 ± 9.8
controls	76.5 ± 16.2	76.5 ± 16.2
	0.954	0.959
P		
Microalbuminuria (mg/24H) (mean±SD)		
subjects treated with dulaglutide	80.1 ± 16.6	79.1 ± 9.0
controls	82.8 ± 18.5	82.5 ± 9.3
	0.432	**0.05**
P		
RHI (mean±SD)		
subjects treated with dulaglutide	1.8 ± 0.4	2.0 ± 0.4
controls	1.8 ± 0.4	1.8 ± 0.4

P	0.901	**0.023**
PWV (m/sec) (mean±SD)		
subjects treated with dulaglutide	10.8±0.8	10.6±0.8
controls	10.9±0.6	11.0±0.6
P	0.376	**0.015**
AIx (%)(media± DS)		
subjects treated with dulaglutide	103.1±6.7	101.8±5.3
controls	101.4±3.6	101.9±3.8
P	0.116	0.937

Data are expressed as mean (SD), n (%),

SBP: systolic blood pressure; **DBP:** diastolic blood pressure; **BMI:** body mass index**; HR:** heart rate; LDL: low density lipoprotein; **HDL:** High Density Lipoprotein; **FPG:** fasting plasma glucose; **HBa1C:** glycated hemoglobin; **PWV:**pulse wave velocity, **AIX :** augmentation index ; **E-GFR:** Estimated Glomerular Filtration Rate

Table 3: Intragroup analysis of patients treated with subjects treated with conventional therapy + dulaglutide at a three and nine month follow-up

Variabile	baseline	Three months	nine months	P
SBP (mm/Hg) (mean±SD)	137.8 ± 12.1	134.9 ± 10.9	129.7 ± 10.7	**<0.0005*** **0.012^**
DBP (mm/Hg) (mean±SD)	76.3 ± 12.3	73.2± 9.9	71.9 ± 9.2	**0.03***
Weight (Kg) (mean±SD)	75.7± 8.5	73.1± 7.6	68.9 ± 8.8	**0.02°** **<0.0005*** **0.008^**
BMI (kg/m2) (mean±SD)	27.6 ± 3.4	26.9 ± 3.4	25.2 ± 3.6	**<0.0005*** **0.01^**
HR (bpm) (mean±SD)	76.6 ± 11.1	75.6 ± 6.0	75.1 ± 5.3	0.58
Total cholesterol (mmol/L) (mean±SD)	160.9± 20.5 /4.2±0.5	160.3 ±21.2/ 4.3±0.7	152.3 ± 22.4/3.9±0.5	0**.04***
HDL cholesterol (mmol/L) (mean±SD)	37.4 ± 4.3/0.9±0.1	36.4 ± 4.3/ 0.9±0.1	38.3 ± 6.1/ 0.9±0.1	0.79
LDL cholesterol (mmol/L) (mean±SD)	102.7 ± 11.6/2.6±0.3	101.8± 10.6 /2.6±0.2	96.0 ± 9.1/2.4± 0.2	**0.001*** **0.002^**
Triglycerides (mmol/L) (mean±SD)	139.2 ± 13.6/1.6±0.2	138.1 ± 13.2/1.5±0.1	137.5 ± 18.9/1.5±0.2	0.1
FPG (mg/dl/mmol/l) (mean±SD)	147.6±32.6 /8.2 ±1.2	131.2 ± 21.9/7.2±1.2	119.3 ± 14.3/6.6±1.0	**0.002°** **<0.0005*** **0.001^**
HBa1C (%) (media± DS)	7.4± 0.7	6.5 ± 0.5	6.2± 0.3	**< 0.0005*°^**
GFR CKD-EPI (mean±SD)	77.4 ± 11.2	76.7 ± 10.5	76.7 ± 9.8	0.1
Microalbuminuria	80.9 ± 20.9	80.1 ± 16.6	80.1 ± 16.6	0.35

(mg/24h) (mean±SD)				
RHI (mean±SD)	1.7 ± 0.4	1.8 ± 0.4	2.0 ± 0.4	**<0.0005***
PWV (m/sec) (mean±SD)	11.2±0.9	10.8±0.8	10.6±0.8	**0.02°** **<0.0005***
Aix (%) (mean±SD)	105.6± 6.9	103.1±6.7	101.8±5.3	**0.001***

9 months vs baseline; ^ 9 months vs 3 months; ° 3 months vs baseline;

Data are expressed as mean (SD), n (%),

SBP: systolic blood pressure; **DBP:** diastolic blood pressure; **BMI:** body mass index**; HR:** heart rate; LDL: low density lipoprotein; **HDL:** High Density Lipoprotein; **FPG:** fasting plasma glucose; **HBa1C:** glycated hemoglobin; **PWV:**pulse wave velocity, **AIX :** augmentation index ; **E-GFR:** Estimated Glomerular Filtration Rate