



Liraglutide Reduces Carotid Intima-Media Thickness by Reducing Small Dense Low-Density Lipoproteins in a Real-World Setting of Patients with Type 2 Diabetes: A Novel Anti-Atherogenic Effect

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ABSTRACT

Introduction: Liraglutide has several non-glycemic effects, including those on plasma lipids and lipoproteins, contributing to its cardiovascular benefit; however, the exact underlying mechanisms remain unclear. We investigated a

novel anti-atherogenic effect of liraglutide in a real-world prospective study on patients with type 2 diabetes (T2DM).

Methods: Sixty-two patients with T2DM (31 men, 31 women; mean age \pm standard deviation 61 ± 9 years) naïve to incretin-based therapies were treated with liraglutide (1.2 mg/day) as add-on therapy to metformin (1500–3000 mg/day) for 4 months. Laboratory analyses included the assessment of lipoprotein subclass profile by gel electrophoresis (Lipoprint; Quantimetrix Corp., Redondo Beach, CA, USA). Carotid intima-media thickness (cIMT) was assessed by Doppler ultrasonography. Statistical analyses included the paired *t* test, Spearman correlation and multiple regression analysis.

Results: The addition of liraglutide to metformin monotherapy resulted in significant reductions in fasting glycemia, hemoglobin A1c, body mass index, waist circumference, total cholesterol, triglycerides and low-density lipoprotein (LDL)-cholesterol, as well as in cIMT. There was an increase in the large LDL-1 subfraction, with a concomitant reduction in atherogenic small dense LDL-3 and LDL-4 subfractions. Correlation analysis revealed a significant association between changes in cIMT and changes in small dense LDL-3 subfraction ($r = 0.501$; $p < 0.0001$). Multivariate analysis, including all of the measured anthropometric and laboratory parameters, revealed that only changes in the small dense LDL-3 subfraction

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were independent predictors of changes in cIMT ($p < 0.0001$).

Conclusion: Our findings are the first to show that the vascular benefit of liraglutide in patients with T2DM is associated with reductions in atherogenic small dense LDL. This effect is independent of glycemic control and body weight reduction and may represent one of the key mechanisms by which liraglutide is able to reduce cardiovascular events.

Trial Registration: ClinicalTrials.gov: NCT01715428.

Keywords: Cardiovascular risk; Carotid intima-media thickness; Liraglutide; Lipoproteins; Small dense low-density lipoproteins; Type 2 diabetes

Key Summary Points

Liraglutide has significant cardiovascular benefit; however, the exact underlying mechanisms remain unclear.

We investigated a novel anti-atherogenic effect of liraglutide in a real-world prospective study on patients with type 2 diabetes mellitus (T2DM).

Our study shows that the vascular benefit of liraglutide in patients with T2DM is associated with reductions in atherogenic small dense low-density lipoproteins.

This vascular effect is independent of glycemic control and body weight reduction and may represent one of the key mechanisms by which liraglutide is able to reduce cardiovascular events.

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to <https://doi.org/10.6084/m9.figshare.13142954>.

INTRODUCTION

Liraglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA) with high analogy to human native GLP-1, is widely prescribed for the treatment of type 2 diabetes mellitus (T2DM). Its non-glycemic effects are well known and include reduction in body weight and waist circumference and improvement in several metabolic parameters, such as inflammatory markers, blood pressure and plasma lipids [1]. Liraglutide is also the first glucose-lowering drug in this class of medications that has shown cardiovascular (CV) benefit, as demonstrated in the LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) study [2], and its beneficial microvascular and macrovascular effects have been seen in subjects with and without T2DM, including those with type 1 diabetes, overweight and obesity, prediabetes and the metabolic syndrome [3, 4].

However, despite increasing evidence of liraglutide's CV benefit, the exact mechanism(s) have not yet been fully elucidated [5]. Subjects with T2DM have increased risk of developing atherosclerosis and the presence of small dense low-density lipoproteins (sdLDL) is strongly associated with CV risk in these patients [6]. LDL particles are heterogeneous and consist of several distinct subclasses, with sdLDL representing an established CV marker due to their highly atherogenic nature compared to their larger, more buoyant counterparts [7]. SdLDL are strongly associated with carotid atherosclerosis, as assessed by carotid intima-media thickness (cIMT), and are also predictive of changes in cIMT [8]; indeed, oxidation of LDL and its deposition in the endothelial wall is an early event in atherosclerosis, and oxidized LDL (oxLDL) is a predictor of subclinical and clinical atherosclerosis [9].

Our group has previously reported that liraglutide use is associated with reduced oxidative stress [10] and cIMT [11] in patients with T2DM. Therefore, we hypothesized that liraglutide would reduce cIMT in the arteries through its specific reduction of plasma sdLDL,

ultimately reducing the amount of LDL particles prone to be oxidized in the endothelial wall. We undertook a prospective study to further explore the antiatherogenic effect of liraglutide in patients with T2DM in the real-world clinical practice setting.

METHODS

Patients and Methods

Sixty-two patients with T2DM (31 men and 31 women, mean \pm standard deviation age 61 ± 9 years) referred for clinical evaluation to the Unit of Diabetes and Cardiovascular Prevention, University Hospital of Palermo, Palermo, Italy were consecutively recruited for the study. All subjects were naïve to incretin-based therapies and on stable metformin treatment (doses ranging from 1500 to 3000 mg daily). All patients included in the present study were treated with metformin only, but some of them had a medical history of previously using sulfonylureas. There were no insulin users. Liraglutide was added to the metformin monotherapy at a dosage of 0.6 mg/day for the first 2 weeks, followed by 1.2 mg/day for the rest of the study.

All subjects underwent a medical examination, biochemical analyses, including analysis of lipoprotein subclasses, and Doppler ultrasonography of the carotid arteries. Exclusion criteria were the presence of severe liver dysfunction, renal failure, heart failure, previous major CV events (within 12 weeks before screening); cancer; and severe infections, such as human immunodeficiency virus, hepatitis B virus or hepatitis C virus. All subjects were in primary CV prevention program. Anthropometric parameters (waist circumference, height and body weight) were recorded, and body mass index (BMI) was calculated in kg/m^2 .

The enrolled subjects were seen at baseline and after 4 months of treatment; similar clinical and laboratory procedures were performed at both time points. Compliance with the study regimen was high, and the dose of metformin and liraglutide did not need to be changed during the study since none of the participants

experienced significant side effects. A few mild, transient gastrointestinal symptoms were reported in the first weeks of liraglutide administration, and there were no severe hypoglycemic episodes. All concomitant therapies, such as antihypertensive, lipid-lowering agents, and aspirin, remained unchanged throughout the study in order to minimize potential confounding effects, and the doses of such therapies had been stable for at least 4 weeks before enrollment. In addition, all subjects were advised not to change their Mediterranean-style eating habits and their lifestyle habits, which commonly consist of a sedentary lifestyle with little physical activity. Monthly follow-up telephone calls were made in order to ensure that there were no changes in their diet/lifestyle habits as well as to check compliance to treatment and assess for adverse events.

All subjects provided informed written consent prior to enrollment in the study. All procedures performed were in accordance with the 1964 Helsinki declaration and its later amendments. The present study was approved by the Ethics Committee of the University Hospital, Palermo, Italy, and was registered with clinicaltrials.gov (NCT01715428).

Biochemical Analyses

Blood samples were taken after a 14-h overnight fast and centrifuged within 30 min of collection. Aliquots of both serum and plasma were collected, and unfrozen samples were used for all biochemical analyses except for the analysis of lipoprotein subclasses, for which the samples were stored at $-80\text{ }^\circ\text{C}$ immediately after collection. In all cases, samples were analyzed blinded and simultaneously at baseline and after 4 months of treatment. Fasting serum glucose, glycated hemoglobin A1c (HbA1c), and lipids (total cholesterol [TC], triglycerides [TG], and high-density lipoprotein-cholesterol [HDL-C]) were measured by standard laboratory methods enzymatically, while LDL-cholesterol (LDL-C) was calculated using the Friedewald formula.

We analyzed a total of 11 distinct lipoprotein subclasses, including very-low-density lipoproteins (VLDL), three intermediate-density lipoprotein (IDL-A, IDL-B and IDL-C) subfractions and seven LDL subclasses [12–14]. LDL subclasses were assessed by non-denaturing, linear polyacrylamide gel electrophoresis (Lipoprint; Quantimetrix Corp., Redondo Beach, CA, USA), which is the only diagnostic tool approved by the U.S. Food and Drug Administration for lipoprotein subfraction testing [7], as previously reported [15]. Briefly, electrophoresis was performed for 60 min at 3 mA in a gel tube, and each chamber had two quality controls. Following electrophoresis, the gel tubes were scanned using the ArtixScan F1 densitometric flatbed scanner (Microtek Int. Corp., Hsinchu, Taiwan) combined with a Mac personal computer (Apple Inc, Cupertino, CA, USA) with installed ScanWizard Pro software (Microtek Int. Corp.). The relative area for each lipoprotein band was determined and multiplied by the total cholesterol concentration of the sample, and LDL subclasses were defined as seven bands (LDL1 to LDL7, respectively), from larger and less atherogenic LDL-1 and -2 subclasses to smaller denser LDL-3 to -7 subclasses.

Ultrasonography of Carotid Arteries

Carotid artery wall thickness was evaluated by B-mode real-time ultrasound at baseline and after 4 months of treatment. A single examiner (AMP) performed all of the examinations in a blinded manner using the SonoAce Pico Ultrasound System (Samsung Medison Co., Gyeonggi-do, Korea) with a probe of 7.5–10.0 MHz. When follow-up studies were completed, the previous scans were not accessible to the examiner. The ultrasound examination was performed in a standardized manner with fixed angles of insonation, as previously reported and as routinely used in our Unit of Diabetes and Cardiovascular Prevention [11, 16]. For the reliability of the results, we calculated that the coefficient of variation for repeat scans was < 5.0% for all scans, which was consistent with values found in previous studies using of different GLP-1RAs [11, 16].

Statistical Analysis

The SPSS software (V.17.0 for Windows; IBM Corp., Armonk, NY, USA) was used for statistical analyses. First, the Kolmogorov–Smirnov normality test was performed in order to investigate normal versus non-normal distribution of all assessed parameters. The paired *t* test was used to estimate the difference between baseline and the final measurements after 4 months. Spearman correlation analysis was performed to assess potential associations between changes in cIMT and changes in all of the evaluated parameters after liraglutide treatment. Multiple regression analysis that included all of the measured anthropometric and laboratory parameters was performed to assess potential independent predictors of changes in cIMT.

RESULTS

Baseline characteristics of all patients are shown in Table 1. After 4 months of liraglutide therapy (Table 2), we found a significant reduction in all anthropometric parameters, including body weight ($p < 0.0001$), waist circumference ($p < 0.0001$) and BMI ($p < 0.0001$). Glycemic parameters (fasting glycemia and HbA1c) also decreased significantly ($p < 0.0001$ for both), as did TC, TG and LDL-C ($p = 0.0020$, $p = 0.0061$ and $p = 0.0089$, respectively). In addition, cIMT significantly reduced ($p < 0.0001$), while HDL-C concentrations remained unchanged.

As shown in Table 3, liraglutide treatment was able to modify lipoprotein subclasses towards a more favorable profile, with a reduction in the total VLDL subclass ($p < 0.0001$), an increase in the LDL-1 ($p < 0.0001$) subclass and a concomitant reduction in the LDL-3 and LDL-4 subclasses ($p < 0.0001$ and $p = 0.0233$, respectively). The lipoprotein profile of a representative subject at baseline and after therapy is shown in Fig. 1: liraglutide favorably modulated the LDL subclass profile by a reduction in smaller, denser and more atherogenic LDL particles (LDL subclasses 3–7; see Fig. 1a) with a concomitant increase in larger, more buoyant and less atherogenic subspecies (LDL subclasses 1–2; Fig. 1b).

Table 1 Baseline characteristics of all subjects enrolled in the study ($n = 62$)

Baseline characteristics	Values
Age (years)	61 ± 9
Women	31 (50%)
Diabetes duration (years)	9 ± 8
Smoking habit	24 (39%)
Family history of cardiovascular diseases	35 (56%)
Systolic blood pressure (mmHg)	128 ± 18
Diastolic blood pressure (mmHg)	77 ± 8
Hypertension	45 (72%)
Obesity	36 (58%)
Dyslipidemia	47 (76%)
Use of antihypertensive therapies	
Beta-blockers	22 (35%)
Angiotensin-converting enzyme inhibitors)	20 (32%)
Calcium channel blockers	16 (26%)
Diuretics	17 (27%)
Use of lipid-lowering drugs	
Statins	31 (50%)
Fibrates,	4 (6%)
Omega-3 fatty acids	15 (24%)
Aspirin use	11 (18%)

Values in table are presented as the mean ± standard deviation (SD) or as a number with the percentage in parentheses

The results of the correlation analysis for changes in cIMT and changes in all the evaluated parameters after 4 months of liraglutide treatment are shown in Table 4. A significant association was observed only between changes in cIMT and those in the sdLDL-3 subfraction ($r = 0.501$; $p < 0.0001$; Fig. 2). Multiple regression analysis (data not shown) found that the only independent predictor of changes in cIMT were changes in the sdLDL-3 particles ($p < 0.0001$).

Table 2 The effect of 4-month liraglutide therapy on evaluated parameters in all patients

Parameters evaluated	Baseline	After 4 months	p
Body weight (kg)	81 ± 16	78 ± 14	< 0.0001*
BMI (kg/m ²)	30 ± 5	29 ± 5	< 0.0001*
Waist circumference (cm)	106 ± 13	103 ± 12	< 0.0001*
Fasting glycemia (mmol/l)	9.1 ± 4.0	7.3 ± 2.0	0.0010*
HbA1c (%)	8.4 ± 1.5	6.9 ± 1.1	< 0.0001*
Total cholesterol (mmol/l)	4.7 ± 1.1	4.2 ± 0.9	0.0020*
Triglycerides (mmol/l)	1.9 ± 1.1	1.6 ± 0.7	0.0061*
HDL-cholesterol (mmol/l)	1.1 ± 0.2	1.1 ± 0.2	0.2970
LDL-cholesterol (mmol/l)	2.7 ± 1.1	2.3 ± 0.8	0.0089*
Carotid IMT (mm)	1.13 ± 0.29	0.92 ± 0.24	< 0.0001*

Values in table are presented as the mean ± SD

BMI Body mass index, *HbA1c* hemoglobin A1c, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

* p values that reached statistical significance between baseline and follow-up evaluations

We also tested whether there was any difference between statin users and non-users in all evaluated parameters. As shown in Table 5, in both groups HbA1c, VLDL and cIMT changed significantly after 4 months, in addition to significant changes in body weight, BMI, waist circumference, fasting glucose, TG, LDL-1 and LDL-2 in statin non-users and TC, LDL-C, LDL-1, LDL-2 and LDL-3 in statin users. However, comparison of these two groups showed that the differences reached statistical significance

Table 3 Change in lipoprotein subclasses after 4 months of liraglutide therapy in all subjects

Lipoprotein subclasses	Baseline (%)	After 4 months (%)	<i>p</i>
VLDL	23.4 ± 4.1	20.9 ± 4.3	< 0.0001*
Large IDL particles	10.7 ± 2.4	10.7 ± 3.2	0.8252
Intermediate IDL particles	9.6 ± 1.9	9.9 ± 1.8	0.2806
Small IDL particles	8.4 ± 3.2	8.6 ± 2.4	0.6486
LDL-1	17.4 ± 4.7	23.6 ± 5.6	< 0.0001*
LDL-2	17.5 ± 4.0	18.9 ± 3.8	0.0644
LDL-3	9.8 ± 4.7	5.3 ± 2.8	< 0.0001*
LDL-4	3.1 ± 1.2	1.7 ± 1.8	0.0233*
LDL-5	0.2 ± 0.6	0.3 ± 0.9	0.5665
LDL-6	0.01 ± 0.01	0.03 ± 0.02	0.3213
LDL-7	–	–	–

Values in table are presented as the mean ± SD
IDL Intermediate-density lipoprotein, *VLDL* very-low-density lipoprotein

**p* values that reached statistical significance between baseline and follow-up evaluations

only for body weight and BMI in favor of statin non-users.

DISCUSSION

Type 2 diabetes mellitus is linked to a high prevalence of atherosclerosis and increased CV risk [17], with some of this risk persisting despite comprehensive pharmacological treatment (including anti-diabetic, anti-hypertensive, lipid-lowering, anti-platelet agents); this constitutes the so-called residual CV risk [18]. Recent CV outcome trials performed in subjects with T2DM have shown CV benefit in terms of adverse CV events (MACE) for some (liraglutide, albiglutide, dulaglutide, semaglutide) but not all

(lixisenatide, exenatide) GLP-1 RAs beyond “standard of care” use of cardiometabolic medications. This suggests that GLP-1 analogues have greater CV benefit than exendin-based agents [19] and, therefore, the CV benefit of GLP-1RAs seems not to be a class effect. Although the exact mechanisms involved are still largely unknown, several hypotheses highlight the role of endothelial changes [20]. In this 4-month prospective real-world study of subjects with T2DM, we demonstrate a novel anti-atherogenic effect of liraglutide of reducing sdLDL and resulting vascular benefit; this may represent one of the key mechanisms by which this agent is able to reduce CV events.

We found that almost all the traditional CV risk factors evaluated in our subjects with T2DM, including BMI, waist circumference and plasma lipids, improved after 4 months of liraglutide treatment independently of glycemic control; this result is consistent with previous reports [21]. We also observed a relatively rapid vascular benefit on cIMT as a marker of subclinical atherosclerosis after only 4 months of liraglutide as an add-on to metformin and other cardiometabolic medications [22]. Liraglutide’s effect on the regression of cIMT appeared to be much stronger than that of exendin-based GLP1-RAs or dipeptidyl peptidase 4 (DPP4) inhibitors [23], which can be linked to its superior impact on CV morbidity and mortality (MACE) compared to other incretin-based therapies. Liraglutide has shown better efficacy and safety in relation to both DPP4-inhibitors and other GLP-1 RA, such as lixisenatide [19, 24].

In our study liraglutide also significantly reduced atherogenic sdLDL, confirming similar observations in patients with T2DM [23], although here we have used high-quality methodology to assess the full spectrum of apolipoprotein (ApoB)-containing particles (VLDL, IDL and LDL), as well as the lowest dosage of liraglutide approved for treatment of patients with T2DM. Here we report for the first time that this favorable modulation of the LDL subclass profile by liraglutide is a strong and independent predictor of vascular benefit, as assessed by cIMT regression. It has also been suggested that liraglutide and incretin-based

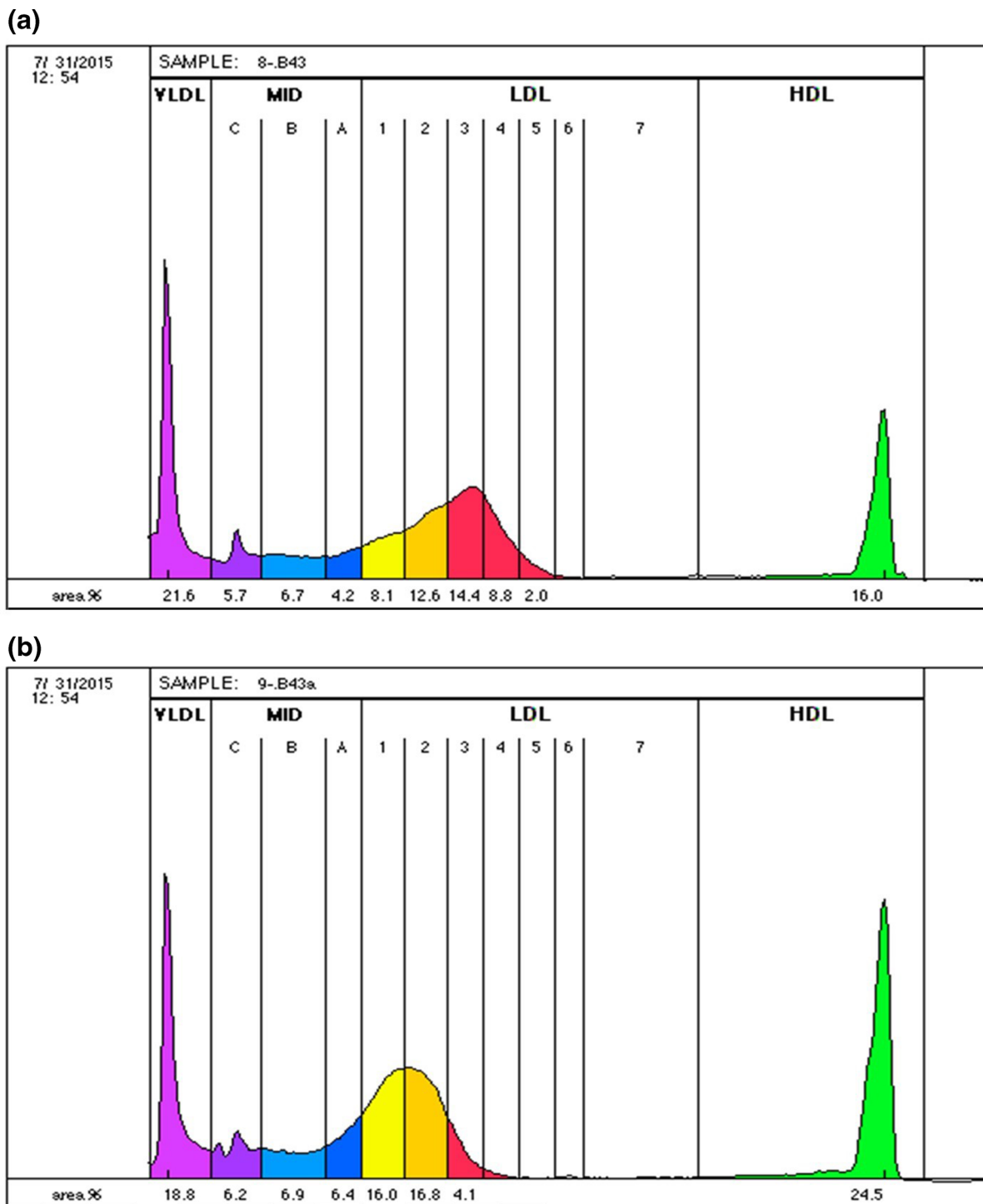


Fig. 1 Lipoprotein profile of a representative subject at baseline (a) and after 4 months of liraglutide therapy (b). *LDL* Low-density lipoprotein, *cIMT* carotid intima-media thickness

therapeutic agents are beneficial for augmenting HDL-particle functionality, beyond raising

HDL-cholesterol concentrations [25]; however, this remains to be confirmed in future studies.

Table 4 Spearman correlation analysis in all patients between changes in carotid intima-media thickness and changes in all the evaluated parameters after 4 months of liraglutide treatment

Parameter	Correlation coefficient (r^2)	<i>p</i> value
Weight	0.046	0.7222
BMI	0.046	0.7247
Waist circumference	0.143	0.2691
HbA1c	0.030	0.8160
Fasting glucose	0.155	0.2451
Total cholesterol	0.050	0.6972
Triglycerides	0.064	0.6210
LDL-cholesterol	0.059	0.6469
HDL-cholesterol	− 0.105	0.4187
cIMT	−	−
LDL-1	− 0.216	0.0917
LDL-2	− 0.184	0.2254
LDL-3	0.501	< 0.0001*
LDL-4	0.171	0.3590
LDL-5	0.224	0.7177
LDL-6	−	−
LDL-7	−	−

cIMT Carotid intima-media thickness

**p* value that reached statistical significance between baseline and follow-up evaluations

Accumulating evidence suggests that the anti-inflammatory and antiatherogenic actions of liraglutide contribute to plaque stability, thereby lowering inflammation, improving endothelial function and reducing the number of foam cells [23]. The favorable effects of liraglutide on sdLDL found in the present study provide possible explanations for understanding the direct beneficial actions of liraglutide beyond glycemic control and weight loss, through exerting significant effects at the very early stage of atherosclerosis and impacting plaque formation and its progression [23].

SdLDL are more susceptible to oxidation than larger, more buoyant particles [9]; by reducing sdLDL, there is less substrate available to be oxidized, and thus liraglutide may help slow down or prevent the atherosclerotic processes at an early stage. In addition, liraglutide is able to largely reduce per se the oxidative stress present in patients with T2DM after just 8 weeks of treatment [10].

Previous studies [26, 27] have shown that ketogenic diets have very strong effects in terms of reliably reducing sdLDL. Such diets are nonetheless criticized for their limited effect on total LDL. The importance of sequential or joint effects of diet and drugs is stated often but rarely advanced. In one case in which this did occur, the authors reported that liraglutide further improved benefits from a low-calorie diet [28]. However, the effect of that diet per se was not impressive compared to the effects reported by Hallberg et al. [29]. Interestingly in the work of the latter authors, whereas most participants substantially reduced or eliminated pre-existing drugs, responses to GLP-1 were mixed. Practically, of course, not everybody will undertake a low-carbohydrate or ketogenic diet, and it may be that liraglutide and these diets are either/or. On a research level, it is possible that liraglutide is actually contraindicated for patients who opt for that kind of intervention.

Some published results suggest that short-term consumption of the Mediterranean-style diet leads to a favorable redistribution of LDL subclasses from smaller to larger LDL [30]. It should be highlighted that all of the participants in the present study have been on a Mediterranean diet for all their life since birth and have been always living within the same geographical area of Southern Italy, and that they did not change their eating habits. Also, it should be mentioned that liraglutide per se may improve eating behavior in obese patients with T2DM (especially the urge for fat intake) and that this improvement may be maintained for 6 months after discontinuation of the treatment [31, 32]; this mechanism could further explain liraglutide's effect on weight loss. There is also a potential association of genetic variability of GLP-1 receptor and inter-individual

Table 5 Effect of 4-month liraglutide therapy on evaluated parameters in non-statin versus statin users

Parameters	Non-statin users (<i>n</i> = 30)			Statin users (<i>n</i> = 32)			<i>p</i> value between groups
	Baseline	After 4 months	<i>p</i> value	Baseline	After 4 months	<i>p</i> value	
Body weight (kg)	86 ± 17	81 ± 16	< 0.0001*	77 ± 13	75 ± 12	0.0871	0.0125*
BMI (kg/m ²)	31 ± 5	30 ± 5	< 0.0001*	29 ± 5	28 ± 5	0.0779	0.0125*
Waist circumference (cm)	109 ± 13	105 ± 13	0.0004*	103 ± 12	101 ± 11	0.0560	0.3015
HbA1c (%)	8.6 ± 1.9	6.9 ± 1.2	< 0.0001*	8.2 ± 0.8	6.9 ± 0.9	< 0.0001*	0.4922
Fasting glucose (mmol/l)	9.0 ± 2.8	7.1 ± 1.7	0.0002*	9.2 ± 5.0	7.5 ± 2.2	0.2451	0.1176
Total cholesterol (mmol/l)	4.6 ± 1.0	4.3 ± 0.8	0.1001	4.8 ± 1.3	4.2 ± 1.0	0.0009*	0.3325
Triglycerides (mmol/l)	1.8 ± 0.9	1.6 ± 0.7	0.0346*	2.0 ± 1.0	1.7 ± 0.6	0.0678	0.4974
LDL-cholesterol (mmol/l)	2.6 ± 1.0	2.4 ± 0.7	0.2326	2.8 ± 1.1	2.3 ± 0.8	0.0183*	0.2695
HDL-cholesterol (mmol/l)	1.1 ± 0.2	1.1 ± 0.1	0.3965	1.1 ± 0.3	1.2 ± 0.2	0.5271	0.8510
VLDL (%)	22.9 ± 3.6	19.9 ± 4.2	0.0015*	23.9 ± 4.5	21.7 ± 4.2	0.0297*	0.4201
Large IDL (%)	10.6 ± 2.5	11.3 ± 3.5	0.2546	10.7 ± 2.3	10.2 ± 2.9	0.2180	0.0841
Intermediate IDL (%)	9.6 ± 1.9	9.8 ± 1.8	0.6952	9.6 ± 1.9	10.0 ± 1.9	0.2126	0.9096
Small IDL (%)	8.5 ± 2.9	8.9 ± 2.6	0.4388	8.3 ± 3.5	8.3 ± 2.2	0.9885	0.8418
LDL-1 (%)	18.3 ± 4.3	25.3 ± 6.1	< 0.0001*	16.5 ± 4.9	22.0 ± 4.7	0.0002*	0.9018
LDL-2 (%)	17.7 ± 3.9	18.3 ± 3.5	0.6100	17.3 ± 4.1	19.5 ± 4.0	0.0426*	0.3712
LDL-3 (%)	9.7 ± 4.7	4.7 ± 2.8	< 0.0001*	9.8 ± 4.8	5.9 ± 2.7	< 0.0001*	0.5791
LDL-4 (%)	2.5 ± 4.5	1.5 ± 2.1	0.1903	3.7 ± 4.7	1.9 ± 3.4	0.0685	0.8221
LDL-5 (%)	0.1 ± 0.5	0.1 ± 0.4	0.7027	0.2 ± 0.7	0.3 ± 1.1	0.6690	0.7171
LDL-6 (%)	0.01 ± 0.01	0.03 ± 0.02	0.7215	0.01 ± 0.01	0.02 ± 0.01	0.8304	0.7942
LDL-7 (%)	–	–	–	–	–	–	–
Carotid IMT (mm)	1.17 ± 0.31	0.93 ± 0.25	0.0003*	1.09 ± 0.27	0.92 ± 0.23	0.0006*	0.5796

Values in table are presented as the mean ± SD

**p* value that reached statistical significance

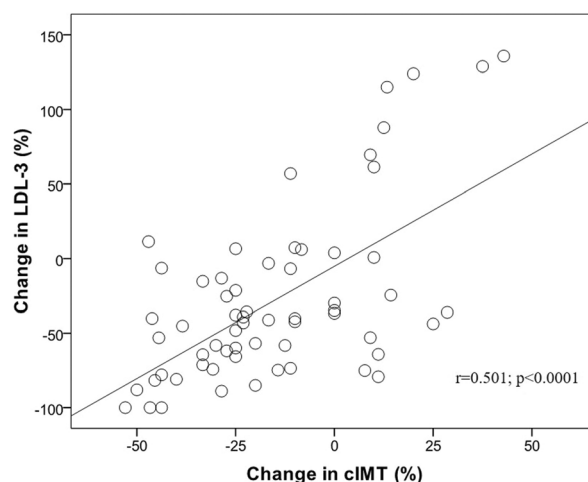


Fig. 2 Correlation plot of changes in cIMT and changes in small dense LDL-3 subclass after liraglutide therapy

differences in response to liraglutide related to a reduction in body weight [33].

This study is the first to show that liraglutide significantly reduced atherogenic sdLDL, leading to the regression of carotid atherosclerosis independently of glycemic control and body weight. A recent randomized, double-blind, placebo-controlled, cross-over trial over a 12 + 12-week period [34] has shown that liraglutide in combination with metformin alters the atherogenic lipid profile, including LDL subclasses by reducing the most atherogenic LDL subclasses the most. These findings are somewhat consistent with the results of our study, although we used high-quality methodology to assess the full spectrum of ApoB-containing particles (VLDL, IDL and LDL), as well as the lowest dosage of liraglutide approved for treatment of T2DM patients (1.2 mg/day).

The potential limitations of our study include the lack of a control group, the relatively small sample and the short duration of the treatment, but it is unlikely that the other cardiometabolic medications impacted the results since the doses of the latter remained unchanged during the course of the trial. Specifically, metformin has been shown to have a negligible impact on cIMT [35, 36], only a modest effect on waist circumference, body weight and BMI [37, 38], lipids [39, 40] and a only small [41] or null effect on sdLDL [42]. The significant correlation found between changes

in cIMT and those in sdLDL by multiple regression analysis indicated that a substantial reduction in cIMT was linked to liraglutide's favorable effects on atherogenic lipoproteins, beyond glycemic control. Although the impact of concomitant therapies cannot be disregarded, their doses were maintained unchanged during the study, and multivariate analysis showed no potential effect of concomitant therapy on changes in cIMT and sdLDL (data not shown). The anti-atherogenic effects of statins are well-known; however, the rapid regression of cIMT with the use of liraglutide is evident, and such a reduction was not even consistently reported for statin use in patients with T2DM. The effects of statins on LDL subclass distribution vary depending on the agent and dose used (rosuvastatin and atorvastatin are more associated with more beneficial effect on LDL subclass distribution, while simvastatin less) as well as baseline TG levels (the higher the baseline TG, the more benefit in terms of improving LDL distribution by statins) [7, 43]. The participants in the present study had normal or borderline high TG, while among statin users the majority have taken simvastatin (with stable doses at least 4 weeks before enrollment, and kept unchanged during the study). We cannot exclude the potential effects of statins, although the post-hoc analysis of the LEADER trial has shown that liraglutide's beneficial

effects are independent of both baseline LDL-C levels and statin use [44].

The strengths of our study include the real-world setting, with the effects of liraglutide evaluated in daily clinical practice, and our focus on understanding the mechanisms of this medication. Dietary, lifestyle habits and all concomitant therapies of the participants remained mainly unchanged during the study, while all measurements (including sdLDL and cIMT) were assessed in a blinded manner. The compliance was very high. Lastly, the study was performed independently of any pharmaceutical company and high-quality technology was used to assess the full LDL subclass profile.

CONCLUSIONS

Our findings contribute to a better understanding of liraglutide's mechanisms on CV outcomes that could pave the way for a more tailored and timely prevention and management of CV risk, even in subjects with normal LDL-C levels. SdLDL may become a new assessment tool and/or a therapeutic target in the management of atherosclerosis, particularly for patients with T2DM treated with GLP-1 analogues. Although further investigation is warranted to confirm our observations, it is likely that the findings represent one of the key mechanisms by which liraglutide is able to reduce CV events.

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Compliance with Ethics Guidelines. All subjects provided informed written consent prior to enrollment in the study. All procedures performed were in accordance with the 1964 Helsinki declaration and its later amendments. The present study was approved by the Ethics Committee of the University Hospital, Palermo, Italy, and was registered with clinicaltrials.gov (NCT01715428).

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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