Circulation: Cardiovascular Imaging

ORIGINAL ARTICLE

Effect of Liraglutide on Arterial Inflammation Assessed as [^{18}F]FDG Uptake in Patients With Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial

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BACKGROUND: The mechanism behind the cardiovascular protection observed with human GLP-1 RA (glucagon-like peptide-1 receptor agonists) in type 2 diabetes is unknown. We hypothesized that treatment with the GLP-1 RA liraglutide had a positive effect on vascular inflammation.

METHODS: LIRAFLAME (Effect of liraglutide on vascular inflammation in type-2 diabetes: A randomized, placebocontrolled, double-blind, parallel clinical PET/CT trial) was a double-blind, randomized controlled trial performed at a single university hospital clinic in Denmark. Patients with type 2 diabetes were via computer-generated randomization list assigned (1:1) liraglutide up to 1.8 mg or placebo once daily for 26 weeks. The primary end point was change in vascular inflammation over 26 weeks assessed by [^{18}F]-fluorodeoxyglucose positron emission tomography/computed tomography. Analyses were based on intention-to-treat. Key secondary outcomes included change in other indices of atherosclerosis.

RESULTS: Between October 26, 2017, and August 16, 2019, 147 patients were screened and 102 were randomly assigned to liraglutide (n=51) or placebo (n=51) and 99 (97%) completed the trial. Change in the [^{18}F]-fluorodeoxyglucose positron emission tomography measure of vascular inflammation (active-segment target-to-background ratio) did not differ between treatment groups: change from baseline to 26 weeks was −0.04 (95% CI, −0.17 to 0.08) in the liraglutide group compared with −0.09 (−0.19 to 0.01) in the placebo group (mean difference, 0.05 [95% CI, −0.11 to 0.21], *P*=0.53). Secondary analyses restricted to [^{18}F]-fluorodeoxyglucose positron emission tomography of the carotid arteries as well as other indices of atherosclerosis confirmed the primary result. We performed an explorative analysis of interaction between treatment group and history of cardiovascular disease (*P*=0.052).

CONCLUSIONS: In this low to moderate risk population with type 2 diabetes, liraglutide did not change vascular inflammation assessed as [^{18}F]-fluorodeoxyglucose uptake compared with placebo. An explorative analysis indicated a possible effect in persons with history of cardiovascular disease, in line with current guidelines where liraglutide is recommended to patients with history of cardiovascular disease.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT03449654.

Key Words: atherosclerosis ■ cardiovascular diseases ■ carotid arteries ■ glucagon-like peptide 1 ■ inflammation ■ Type 2 Diabetes

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For Sources of Funding and Disclosures, see page 642.

CLINICAL PERSPECTIVE

Treatment with the human glucagon-like peptide-1 receptor agonists liraglutide protects against cardiovascular disease by an unknown mechanism. We investigated in a double-blind, randomized controlled trial if liraglutide affects vascular inflammation in patients with type 2 diabetes. The primary end point was change in vascular inflammation over 26 weeks assessed by [18F]-fluorodeoxyglucose positron emission tomography/computed tomography. We found, in a type 2 diabetes population with an overall moderate risk of cardiovascular disease, that liraglutide did not change the [18F]-fluorodeoxyglucose uptake compared with placebo. An explorative analysis indicated a possible effect in persons with history of cardiovascular disease, in line with guidelines where liraglutide is recommended to these patients.

Nonstandard Abbreviations and Acronyms

CACS	coronary artery calcium score
CIMT	carotid intima-media thickness
CT	computed tomography
CVD	cardiovascular disease
GLP-1	glucagon-like peptide 1
HO	Harmony Outcomes
ICC	intraclass correlations coefficient
LDL	low-density lipoprotein
PET	positron emission tomography
TBR	target-to-background ratio

Large cardiovascular outcome trials have demonstrated that treatment with 4 different human GLP-1 (glucagon-like peptide 1) receptor agonists reduces the high risk of cardiovascular disease (CVD) in type 2 diabetes.[1–4] These trials included patients with established CVD (HO [Harmony Outcomes] testing albiglutide[3]) or with a high prevalence of CVD (81% in LEADER [Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results] testing liraglutide[1] and 83% in SUSTAIN-6 [Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes] testing semaglutide[2]). Recently, the positive outcome of the REWIND trial (Researching Cardiovascular Events With a Weekly Incretin in Diabetes) using dulaglutide (including 32% with CVD) extended the cardiovascular protection observed with GLP-1 receptor agonists beyond high risk to a broader population of patients with type 2 diabetes and cardiovascular risk factors.[4] Evidence from these cardiovascular outcome trials have changed clinical practice guidelines[5] and fueled a debate regarding the mechanism behind the cardiovascular protection observed with human GLP-1 receptor agonists.

GLP-1 receptor agonists have well-established beneficial effects on several cardio-renal risk markers, including reduction in body weight, HbA_{1c}, blood pressure, LDL (low-density lipoprotein)-cholesterol, and albuminuria.[6] An interesting observation from the HO trial was that the magnitude of the cardiovascular protection was comparable to what has been demonstrated for liraglutide, semaglutide, and dulaglutide; however, in the HO trial, there were no major differences between the placebo- and the albiglutide-treated group in the effect on blood pressure, body weight, or renal function over time and only a modest reduction in HbA_{1c} with albiglutide-treatment.[3] Thus, it appears unlikely that the cardiovascular protection observed with GLP-1 receptor agonists is mediated solely by an effect on the classic cardio-renal risk markers. Results from atherosclerotic mice models have suggested that GLP-1 receptor agonists reduce the aortic plaque areas and change gene expression in the aorta related to proteins representing inflammatory pathways associated with leucocyte recruitment, adhesion, and migration.[7] Inflammation plays a key role in all phases of atherosclerotic plaque development. Treatment with liraglutide reduces inflammatory markers in the blood in humans,[8] but the direct effect on vascular inflammation has not been investigated.

The LIRAFLAME trial (Effect of liraglutide on vascular inflammation in type-2 diabetes: A randomized, placebo-controlled, double-blind, parallel clinical PET/CT trial) was designed to investigate the effect of liraglutide on vascular inflammation in patients with type 2 diabetes. We used state-of-the art positron emission tomography/computed tomography (PET/CT) with the radiolabeled glucose analog fluorodeoxyglucose ([18F]FDG) for specific in vivo evaluation of vascular inflammation. We hypothesized that 26 weeks treatment with liraglutide would reduce vascular inflammation assessed as [18F]FDG uptake compared with placebo. Likewise, we investigated changes in coronary artery calcium score (CACS), carotid intima-media thickness (CIMT), endothelial function, circulating biomarkers, as well as collected information on adverse events.

METHODS

Data Sharing

Individual, deidentified participant data are not freely available due to the risk of patient reidentification.

Deidentified participant data or anonymized clinical study reports can be obtained from the first author upon reasonable request. Necessary data protection agency and ethical committee approvals must be provided in compliance with relevant legislation.

Study Design

We performed a randomized, double-blind, placebo-controlled, parallel-group trial in 102 patients with type 2 diabetes. Participants were recruited from the outpatient clinic at the Steno Diabetes Center Copenhagen in Denmark and through

newspaper advertisements. Patients were assigned in a 1:1 ratio to receive subcutaneous injections of liraglutide or placebo (with matched administration device, diluent and volume injected) once daily for 26 weeks. The protocol was approved by the local ethics committee (H-16044546) and the Danish Medicines Agency (2016110109). The study was performed in compliance with the principles of the Declaration of Helsinki and according to Good Clinical Practice guidelines. The statistical analysis plan is available in the Data Supplement.

Participants

Patients were eligible if they met the inclusion criteria: type 2 diabetes (World Health Organization criteria); age >50 years; HbA_{1c} ≥48 mmol/mol (6.5%); eGFR (estimated glomerular filtration rate) ≥30 mL/min/1.73 m^2 (estimated by CKD-EPI formula); stable glucose- and cholesterol-lowering treatment for a minimum of 4 weeks before the baseline PET/CT. Main exclusion criteria were type 1 diabetes; treatment (90 days before screening) with oral glucocorticoids, calcineurin inhibitors, dipeptidyl peptidase 4 inhibitors, GLP-1 receptor agonists and other agents which, in the investigator's opinion, could interfere with the effect of liraglutide; cancer or any other clinically significant disorder, except for conditions associated with type 2 diabetes history which, in the investigator's opinion, could interfere with the results of the trial (detailed in and exclusion criteria are in the Data Supplement). All the patients provided written informed consent before participation.

Randomization and Masking

Identical liraglutide and placebo pens as well as the random liraglutide/placebo allocation sequence were provided by Novo Nordisk A/S (Bagsvaerd, Denmark). Two persons not otherwise involved in the study had access to the computer-generated random liraglutide/placebo allocation sequence. They assigned and verified study medication numbers to the participants before the randomization visit. All study medication was numbered sequentially. The investigators, participants, and treating physicians were blinded to treatment allocation.

Procedures

All patients received a starting dose of 0.6 mg/d. The dose was escalated, if tolerated, as follows: first week 0.6 mg/d; second week 1.2 mg/d; and third week 1.8 mg/d. Maintenance dose was 1.8 mg/d; however, we allowed a flexible dose-escalation procedure to reach the maximum tolerated dose for each patient.

The study had 6 visits: (1) screening; (2) baseline [18F]FDG-PET/CT; (3) baseline randomization; (4) 13 weeks follow-up; (5) 26 weeks follow-up [18F]FDG-PET/CT, and (6) 26 weeks end of study. The [18F]FDG-PET/CT imaging was performed at Rigshospitalet, Denmark at Department of Clinical Physiology, Nuclear Medicine & PET. All other visits took place at the Steno Diabetes Center Copenhagen.

Arterial inflammation was examined using [18F]FDG-PET/CT of the carotid arteries and the aorta. Details of the procedure and analyses are described in the Data Supplement. In brief, [18F]FDG-PET/CT imaging of the carotid arteries and aorta was undertaken according to recommended methods.[9]

Maximum standardized uptake value of [18F]FDG was measured along the carotids and ascending aorta in axial orientation. Target-to-background ratio (TBR) was calculated as the ratio of standardized uptake value of the artery compared with background venous activity (Figure 1). Three established methods of uptake quantification were employed[9]: mean of maximum TBR in (1) all active segments (TBR >1.6), (2) most diseased segments, and (3) the whole vessels. PET/CT images were analyzed by a masked, experienced reader.

Reproducibility of [18F]FDG TBR measurements was tested using N=10 scans selected at random with >30 days between the readings.

Coronary atherosclerosis was assessed using CT-based CACS, and vascular anatomy was further evaluated by ultrasound CIMT. The endothelial function was examined by reactive hyperemia index evaluated with EndoPat (Itamar Medical, Israel), and glycocalyx integrity evaluated with the GlycoCheck device (Maastricht, the Netherlands).

Physical examination including height, weight, and blood pressure as well as blood samples for quantification of glucose metabolism, and other laboratory measures were performed at visit 3, 4, and 6. Adverse events were recorded at each visit. Details of the imaging protocols and methods are described in the Data Supplement.

Outcomes

The prespecified primary end point was change in [18F]FDG uptake in both the carotid arteries and the aorta using active segments analysis.[9]

Secondary end points were (1) change in arterial [18F]FDG uptake in the carotid arteries and the aorta using most diseased segment and mean uptake in the whole vessels; (2) change in CACS; (3) change in CIMT; (4) change in endothelial function; and (5) change in circulating biomarkers.

Statistical Analysis

The trial was powered to detect a clinically relevant 10% reduction in [18F]FDG uptake. We assumed a baseline [18F]FDG value (TBR mean of max) of 2.8, an absolute difference of 0.28 between groups (corresponding to a 10% reduction) and a SD of the change of 0.43.[10] Using these assumptions in a 2-sample *t* test power calculation, we would require 40 participants in each treatment group, with 80% power and an α of 0.05 (2-sided). A difference of 0.28 between groups is feasible to detect using FDG-PET.[11] We aimed to recruit at least 100 participants to account for drop-outs and deviations from the expected baseline [18F]FDG value.

As per the original specification in the trial protocol, results are primarily presented for (1) a modified intent-to-treat efficacy population, that consist of all included participants, for whom any aspect of treatment was initiated, and who had follow-up PET data available. Subjects were used in the analysis as randomized, and (2) an optimal efficacy population, consisting of all treated patients who were compliant (assessed by investigator) and completed the trial on maximum protocol treatment (1.8 mg/d) without change in lipid-lowering intervention during the study. We also present blood glucose corrected values of the vascular [18F]FDG uptake.[9] Only observed data were part of the analyses, no imputation was made and if data was missing exclusion was case-wise.

Normal distributed data are presented as mean±SD. Non-normal distributed data were presented as median with interquartile range and were log2 transformed before analysis. A value of 0.1 was added to the CACS before log2 transformation, since

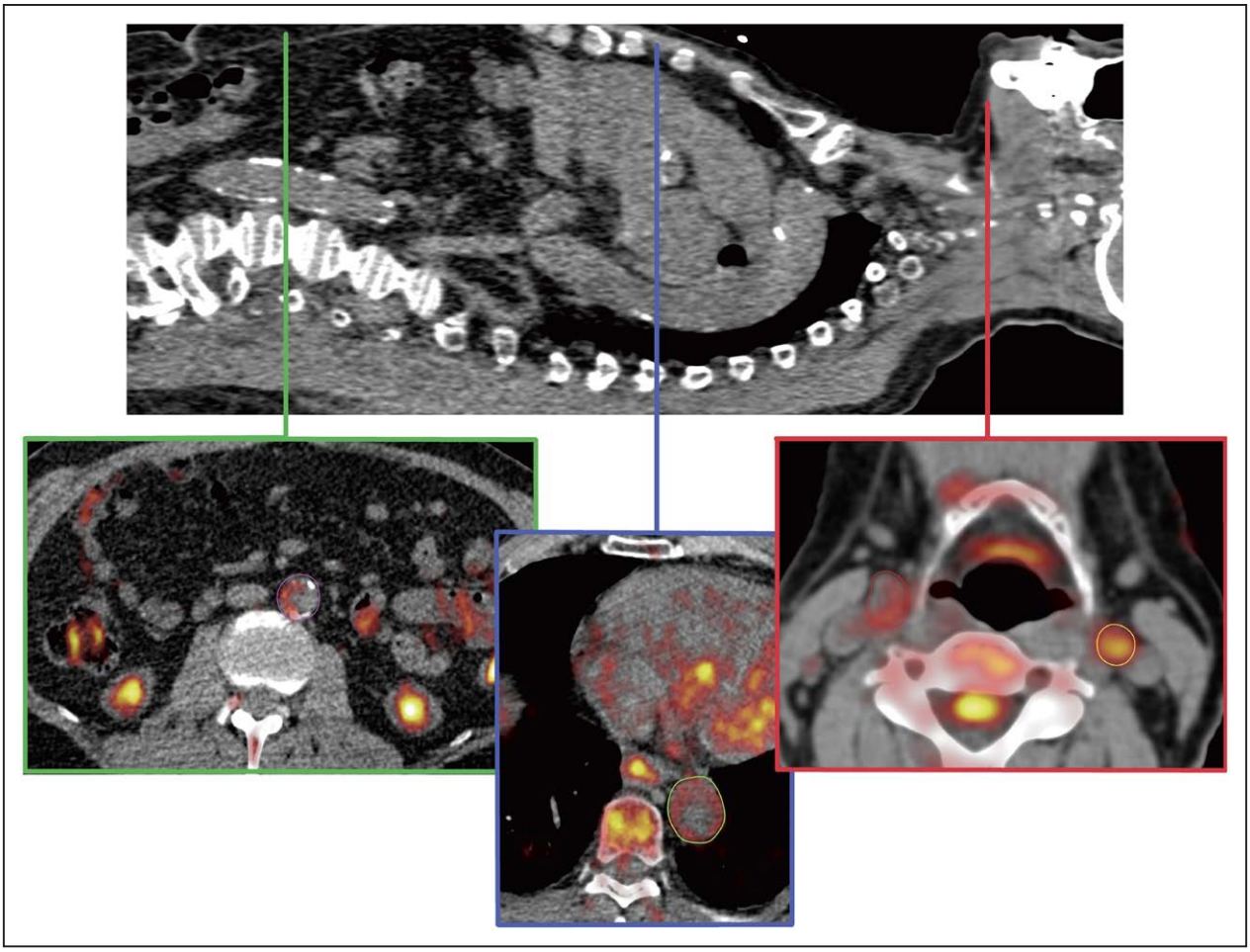

Figure 1. [18F]fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computed tomography (CT) imaging approach. The abdominal aorta, the thoracic aorta, and the carotid arteries were identified and manually traced with free hand or ellipse regions of interest (ROI) on all axial CT images without use of the PET images. Afterwards the ROIs were copied onto the spatially aligned PET examination as shown in the figure. The FDG uptake was quantified in each ROI as the standardized uptake value (SUV) by measuring a maximum pixel activity value (SUVmax). Target-to-background ratio was finally calculated as a ratio of SUVmax and the average blood SUV estimated from venous blood in the superior cava vein or the jugular vein.

the unequal distribution included values of zero. Differences in baseline characteristics between the liraglutide and the placebo group were tested using unpaired *t* test, the χ^2 test, or Fisher exact test as appropriate. Differences between the liraglutide and the placebo group were tested using (1) paired *t* test for comparisons between baseline and end-of-treatment within groups (descriptive) and (2) unpaired *t* test for comparison of the change from baseline to end-of-treatment between the 2 groups (primary analysis). For TBR analysis, a mean value was used for each patient in all analysis, and multiple segments was not considered separately for the same patient. Adjustment for blood glucose and interaction analysis with CVD were analyzed in separate between-group regression models. Correlations were tested using Pearson correlation coefficient.

Intraclass correlations coefficients (ICC) with 95% CIs were calculated using a 2-way mixed model to test the intraobserver agreement of [18F]FDG TBR measurements.

Adverse events and serious adverse events are reported in tabulated form without significance testing.

All statistical analyses were performed using SAS software (version 9.4; SAS Institute, NC) and IBM SPSS statistics (version 25, IBM, NY). A 2-sided *P* value <0.05 was considered significant.

The trial is registered with the EU Clinical Trials Register, 2016-001523-31.

Role of Funding Source

Novo Nordisk A/S supplied the liraglutide and matching placebo and was the main funder the study by an unrestricted grant. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Between October 26, 2017, and August 16, 2019, 102 patients were randomly assigned to receive liraglutide (n=51) or placebo (n=51; Figure 2). Clinical characteristics at baseline are presented in Table 1. The population consisted mainly of overweight men above 60 years of age. The median diabetes duration was 10.9 years

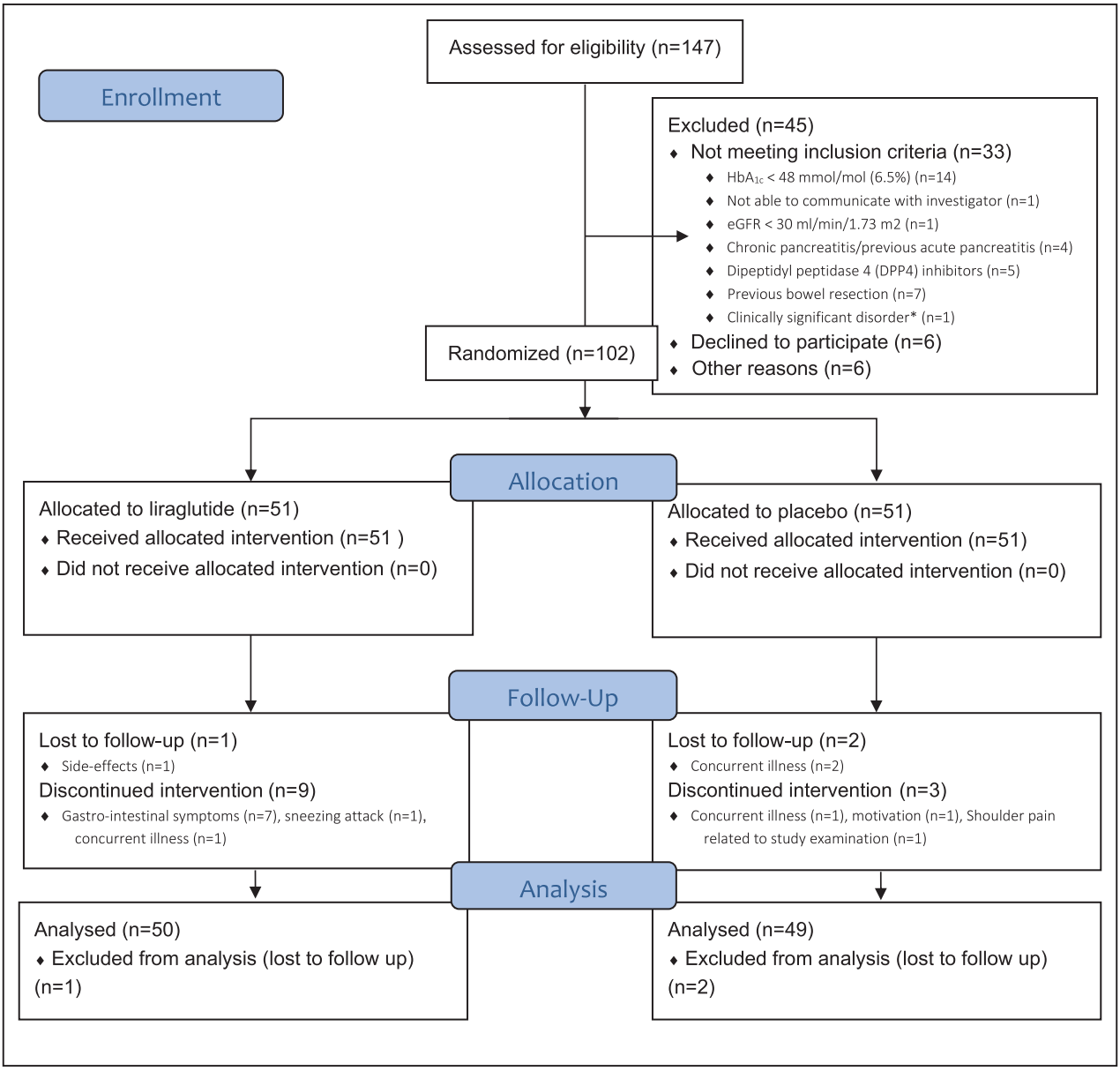

Figure 2. Trial profile.
eGFR indicates estimated glomerular filtration rate.

(interquartile range, 5.7–18.2), mean HbA_{1c} was 58.4 mmol/mol (SD, 10.1; 7.5% [0.92]), and eGFR was 83.2 mL/min/1.73 m^2 (SD, 16.3). At baseline 23 (22.6%) reported history of CVD (history of acute myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, peripheral arterial thrombosis [ie, arterial blot clot in the legs or arms], claudication, and nitroglycerin requiring angina pectoris).

Clinical characteristics at baseline were balanced between the liraglutide and placebo group, apart from a slightly higher concentration of triglycerides and more patients receiving beta-blockers in the group randomized to liraglutide (Table 1).

The intention-to-treat population (excluding those who did not complete the final [18F]FDG-PET/CT, n=3 [Figure 2]) consisted of 50 patients treated with liraglutide and 49 with placebo. The optimal efficacy population consisted of 63 patients; 24 treated with liraglutide and 39 with placebo, Table I in the Data Supplement.

For the group treated with liraglutide compared with placebo, HbA_{1c} and weight were reduced as follows: mean change in HbA_{1c} was −5.1 mmol/mol (95% CI, −8.1 to −2.0) [−0.47% (−0.74 to −0.18)] compared with −0.1 mmol/mol (−1.9 to 1.7) [−0.01% (−0.17 to 0.16)] in the placebo group (mean difference −5.0 mmol/mol [95% CI, −8.5 to −1.5] [−0.46% (−0.78 to −0.14)], P=0.006) and mean change in body weight was −3.7 kg (95% CI, −4.8 to −2.6) compared with −0.2 kg (−0.8 to 0.4) in the placebo group (mean difference −3.5 kg [95% CI, −4.8 to −2.3], P<0.001). The LDL-cholesterol concentrations and systolic blood pressure did not change significantly in any of the treatment groups (Table 2).

Table 1. Characteristics of the Patients at Baseline

	Total (n=102)	Liraglutide (n=51)	Placebo (n=51)	*P* value
Sex (woman)	16 (15.7%)	6 (11.8%)	10 (19.6%)	0.28
Age, y	66.4 (8.2)	65.9 (8.6)	66.9 (7.8)	0.56
Body mass index, kg/m^2	29.9 (4.6)	30.5 (5.3)	29.3 (3.8)	0.16
Type 2 diabetes				
Known duration, y	10.9 (5.7–18.2)	12.2 (5.4–18.2)	10.2 (5.7–19.2)	0.65
HbA_{1c}, mmol/mol	58.4 (10.1)	58.7 (9.6)	58.0 (10.6)	0.73
HbA_{1c}, %	7.5 (0.92)	7.5 (0.88)	7.5 (0.97)	0.73
Kidney function				
Estimated glomerular filtration rate, mL/(min·1.73 m^2)	83.2 (16.3)	82.7 (17.6)	83.7 (15.0)	0.75
Urinary albumin creatinine ratio, mg/g	6.0 (3.5–14.5)	6.0 (3.5–15.0)	6.0 (3.5–14.5)	0.65
Cardiovascular risk factors				
Systolic blood pressure, mm Hg	135.3 (17.3)	133.4 (14.5)	137.3 (19.7)	0.25
Diastolic blood pressure, mm Hg	79.3 (7.8)	79.8 (7.0)	78.9 (8.4)	0.57
Total cholesterol, mmol/L	4.1 (0.8)	4.1 (0.8)	4.1 (0.8)	0.85
LDL-cholesterol, mmol/L	2.1 (0.7)	2.1 (0.7)	2.1 (0.6)	0.48
HDL cholesterol, mmol/L	1.2 (0.4)	1.2 (0.4)	1.3 (0.3)	0.14
Triglycerides, mmol/L	1.8 (1.0)	2.1 (1.2)	1.6 (0.8)	0.01
Current smoker	14 (13.7%)	10 (19.6%)	4 (7.8%)	0.08
Hypertension	79 (77.5%)	43 (84.3%)	36 (70.6%)	0.10
History of cardiovascular event				
Myocardial infarction	13 (12.8%)	8 (15.7%)	5 (9.8%)	0.37
Stroke	6 (5.9%)	3 (5.9%)	3 (5.9%)	1.00
Peripheral arterial thrombosis	2 (2.0%)	2 (3.9%)	0 (0.0%)	0.50
History of cardiovascular symptoms				
Claudication	4 (3.9%)	3 (5.9%)	1 (2.0%)	0.62
Angina pectoris	5 (4.9%)	4 (7.8%)	1 (2.0%)	0.36
Any cardiovascular disease	23 (22.6%)	14 (27.5%)	9 (17.7%)	0.24
Glucose lowering medication				
Insulin use	39 (38.2%)	20 (39.2%)	19 (37.3%)	0.84
SGLT2 inhibitors	20 (19.6%)	8 (15.7%)	12 (23.5%)	0.32
Cardiovascular medication				
Angiotensin converting enzyme inhibitors	30 (29.4%)	15 (29.4%)	15 (29.4%)	1.00
Angiotensin II receptor blockers	45 (44.1%)	22 (43.1%)	23 (45.1%)	0.84
Mineralocorticoids	5 (4.9%)	2 (3.9%)	3 (5.9%)	1.00
Calcium channel antagonists	35 (34.3%)	17 (33.3%)	18 (35.3%)	0.83
β blockers	19 (18.6%)	14 (27.5%)	5 (9.8%)	0.02
Aspirin treatment	37 (36.3%)	16 (31.4%)	21 (41.2%)	0.30
Lipid-lowering treatment	88 (86.3%)	46 (90.2%)	42 (82.4%)	0.25

Data are n (%) or mean (SD) or median (IQR). Differences in baseline characteristics between the liraglutide and the placebo group were tested using unpaired *t* test, the χ^2 test, or Fisher exact test as appropriate. Hypertension was defined as treatment with antihypertensive medication. HDL indicates high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; and SGLT2, sodium glucose transporter 2.

The intraobserver reliability of [18F]FDG TBR measurements were excellent for both all arteries combined (intraclass correlation coefficient [ICC], 0.97 [95% CI, 0.86–0.99]) and the individual territories: carotid arteries (ICC, 0.96 [95% CI, 0.83–0.99]), thoracic aorta (ICC, 0.97 [95% CI, 0.87–0.99]), and abdominal aorta (ICC, 0.98 [95% CI, 0.91–0.99]).

An overview of missing data for primary and secondary end points and key clinical characteristics is presented in Table II in the Data Supplement.

[18F]FDG-PET After GLP-1 Treatment

The primary end point was unchanged in both groups as mean change in active segments TBR from baseline to

Table 2. Changes in Clinical Characteristics and Secondary End Points

Group	Mean (SD) or median (IQR)		*P* value	Change mean (95% CI) or median [IQR]	*P* value
	Baseline	End-of-treatment			
Clinical characteristics					
HbA_{1c}, mmol/mol, [HbA_{1c} %]					
Liraglutide, n=49	58.8 (9.6) [7.5 (0.88)]	53.8 (14.7) [7.1 (1.34)]	0.002	−5.1 (−8.1 to −2.0) [−0.47 (−0.74 to −0.18)]	0.006
Placebo, n=48	57.3 (8.8) [7.4 (0.81)]	57.2 (8.5) [7.4 (0.78)]	0.9	−0.1 (−1.9 to 1.7) [−0.01 (−0.17 to 0.16)]	
Body weight, kg					
Liraglutide, n=49	94.5 (20.3)	90.8 (19.2)	<0.0001	−3.7 (−4.8 to −2.6)	<0.0001
Placebo, n=48	87.8 (13.8)	87.6 (14.2)	0.54	−0.18 (−0.76 to 0.40)	
Systolic blood pressure, mm Hg					
Liraglutide, n=49	134 (15)	133 (13)	1.0	0 (−5 to 5)	1.0
Placebo, n=48	138 (19)	137 (17)	1.0	0 (−4 to 4)	
LDL-cholesterol, mmol/L					
Liraglutide, n=45	2.1 (0.7)	2.2 (1.0)	0.6	0.1 (−0.2 to 0.3)	0.4
Placebo, n=46	2.2 (0.6)	2.1 (0.8)	0.5	−0.1 (−0.3 to 0.1)	
Secondary end points					
Vascular anatomy					
Coronary artery calcium score					
Liraglutide, n=44	150 [3 to 359]	145 [10 to 439]	0.41*	8 [0 to 41]	0.62*
Placebo, n=46	185 [52 to 399]	211 [60 to 405]	0.53*	7 [−1 to 31]	
Carotid intima-media thickness, mm					
Liraglutide, n=50	0.77 (0.17)	0.76 (0.17)	0.33	0.009 (−0.010 to 0.027)	0.51
Placebo, n=48	0.75 (0.14)	0.75 (0.14)	0.89	−0.001 (−0.025 to 0.023)	
Endothelial function					
Reactive hyperemia index					
Liraglutide, n=46	1.8 (0.40)	1.8 (0.38)	0.18	−0.08 (−0.19 to 0.04)	0.80
Placebo, n=44	1.8 (0.40)	1.8 (0.39)	0.43	−0.06 (−0.20 to 0.08)	
Glycocalyx integrity, µm					
Liraglutide, n=21	2.0 (0.25)	2.0 (0.23)	0.38	−0.05 (−0.18 to 0.07)	0.27
Placebo, n=23	1.9 (0.29)	2.0 (0.25)	0.48	0.05 (−0.10 to 0.20)	
Biomarkers					
High-sensitivity C-reactive protein, mg/L					
Liraglutide, n=47	1.6 [0.99 to 3.9]	1.7 [0.9 to 2.7]	0.13*	−0.11 [−0.91 to 0.27]	0.22*
Placebo, n=48	1.5 [0.75 to 2.9]	1.3 [0.7 to 4.0]	0.99*	0.03 [−0.22 to 0.42]	
Pro-B-type natriuretic peptide, pmol/L					
Liraglutide, n=34	11.2 [6.7 to 20.6]	8.4 [6.3 to 22.4]	0.41*	−2.3 [−6.4 to 4.1]	0.98*
Placebo, n=35	10.1 [6.9 to 15.7]	10.5 [3.0 to 16.4]	0.36*	−2.1 [−4.0 to 5.3]	
Interleukin-6, ng/L					
Liraglutide, n=43	1.3 [0.87 to 2.5]	1.2 [0.78 to 1.8]	0.049*	−0.19 [−0.62 to 0.28]	0.86*
Placebo, n=39	1.1 [0.87 to 1.7]	0.78 [0.66 to 1.3]	0.06*	−0.21 [−0.38 to 0.16]	
Monocyte chemoattractant protein-1, ng/L					
Liraglutide, n=49	20.6 [16.8 to 23.1]	11.6 [8.5 to 16.2]	<0.0001*	−8.2 [−11.5 to −4.1]	0.052*
Placebo, n=48	19.4 [14.1 to 23.0]	12.1 [10.0 to 15.5]	<0.0001*	−6.1 [−11.1 to −3.2]	

Data are mean (SD), median (IQR), or mean (95% CI) change. Paired *t* test for comparisons between baseline and end-of-treatment within groups and unpaired *t* test for comparison of the change from baseline to end-of-treatment between the 2 groups. IQR indicates interquartile range; and LDL, low-density lipoprotein.

*Change in log2 values.

end-of-treatment was −0.04 (95% CI, −0.17 to 0.08) for the liraglutide group and −0.09 (−0.19 to 0.01) for the placebo group, with no difference between treatment groups (mean difference, 0.05 [95% CI, −0.11 to 0.21], *P*=0.53), Table 3, Figure 3.

For the secondary objective of assessing TBR in most diseased segment and in the whole vessels, results were consistent: liraglutide versus placebo group, mean change in TBR in most diseased segment was −0.22 (95% CI, −0.40 to −0.03) versus −0.24 (−0.44 to −0.04;

Table 3. Arterial Vascular Inflammation Evaluated With [18F]FDG-PET in 102 Type 2 Diabetes Subjects Assessed in 3 Ways: Active Segments, Most Diseased Segments, and Whole Vessels

Group	Mean TBR (SD)		*P* value	Δ TBR (95% CI)	*P* value
	Baseline	End-of-treatment			
Active segments					
Liraglutide, n=50	2.1 (0.30)	2.0 (0.39)	0.50	−0.04 (−0.17 to 0.08)	0.53*
Placebo, n=49	2.0 (0.29)	1.9 (0.29)	0.07	−0.09 (−0.19 to 0.01)	
Most diseased segments					
Liraglutide, n=50	2.7 (0.72)	2.5 (0.69)	0.02	−0.22 (−0.40 to −0.03)	0.87
Placebo, n=49	2.4 (0.63)	2.2 (0.55)	0.02	−0.24 (−0.44 to −0.04)	
Whole vessels					
Liraglutide, n=50	1.9 (0.33)	2.0 (0.37)	0.27	0.07 (−0.05 to 0.18)	0.46
Placebo, n=49	1.8 (0.35)	1.8 (0.29)	0.88	0.01 (−0.10 to 0.11)	

Arterial vascular inflammation evaluated as TBR. Data are mean (SD) or change (95% CI). Paired *t* test for comparisons between baseline and end-of-treatment within groups and unpaired *t* test for comparison of the change from baseline to end-of-treatment between the 2 groups. FDG indicates fluorodeoxyglucose; PET, positron emission tomography; and TBR, target-to-background ratio.
*Primary end point.

P=0.87 between treatment groups), and mean change in TBR in whole vessels was 0.07 (95% CI, −0.05 to 0.18) versus 0.01 (−0.10 to 0.11; *P*=0.46 between treatment groups; Table 3). Results were also consistent when analyzing the carotids, thoracic aorta, and abdominal aorta separately (Table III in the Data Supplement).

Secondary End Points of Atherosclerosis

Change in supportive secondary end points for liraglutide versus placebo were as follows: median change in CACS: 8 (IQR, 0; 41) versus 7 (−1; 33), *P*=0.62 between treatment groups; mean change in CIMT: 0.009 mm (95% CI, −0.010 to 0.027) versus −0.001 mm (−0.025 to 0.023), *P*=0.51 between treatment groups. Changes in endothelial function evaluated by reactive hyperemia index or glycocalyx integrity (Table 2) or biomarkers reflecting inflammation or atherosclerosis were not different between the 2 treatment groups (Table 2 and Table IV in the Data Supplement).

When analyzing the optimal efficacy population (n=63), we observed a nearly statistical significant increase in [18F]FDG uptake in the liraglutide group compared with placebo (*P*=0.052, Table V in the Data Supplement). This trend was driven by changes in the abdominal aorta and not reproduced when analyzing the carotid arteries only (Table VI in the Data Supplement).

Adverse Events

Adverse events are reported in Table 4, Tables VI and VII in the Data Supplement. Of the 102 participants, 83 (81%) reported at least one adverse event. Most common was gastrointestinal symptoms (Table VII in the Data Supplement). In the liraglutide group 8 (16%) participants discontinued study medication due to adverse

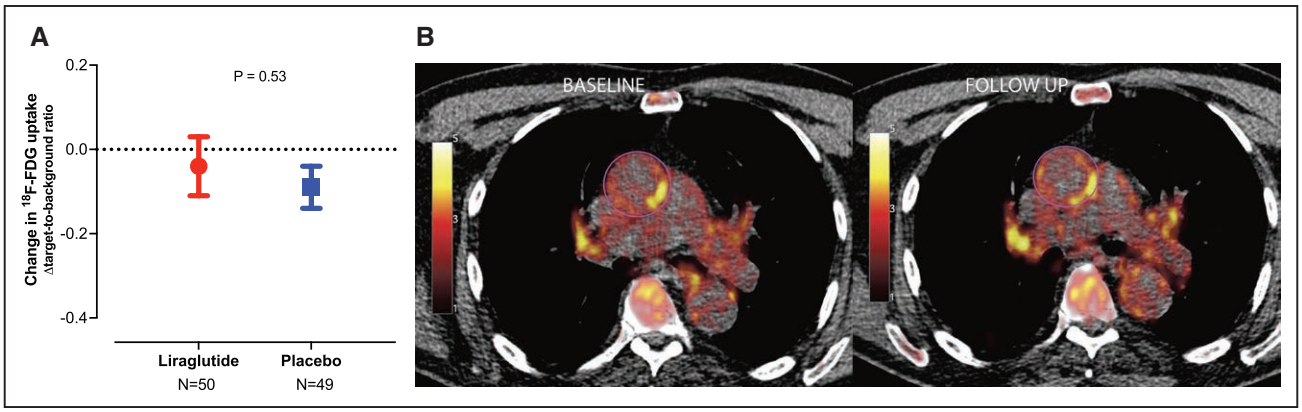

Figure 3. Arterial inflammation evaluated with [18F]fluorodeoxyglucose (FDG)-positron emission tomography (PET) in the liraglutide and the placebo-treated group.
A, Mean change from baseline to end-of-treatment in active segments target-to-background ratio for the liraglutide group and the placebo group (primary end point). Mean plots with SE. Unpaired *t* test for comparison. **B**, Representative [18F]FDG-PET/computed tomography images from a participant treated with liraglutide. The ascending thoracic aorta is outlined at baseline and follow-up examination.

Table 4. Adverse Events and Serious Adverse Events Leading to Discontinuation of the Study Drug

	Liraglutide, n=51	Placebo, n=51
Any adverse events (total number)	108	69
Any adverse events (patients with at least one)	48	35
Adverse events leading to discontinuation of study drug	8	1
Any serious adverse events (total number)	4	5
Any serious adverse events (patients with at least one)	4	4
Serious adverse events considered related to study drug	0	0
Death	0	0

Data are number of participants with a specified event. No participants died during the trial.

events (7 due to gastrointestinal symptoms, one due to sneezing attack) against 1 (2%) in the placebo group (back pain related to study examination). A total of 9 serious adverse events were registered (Table VIII in the Data Supplement); none were fatal or considered related to the study drug.

Impact of Blood Glucose

Capillary blood glucose was measured just before [18F]FDG injection and was similar in the 2 treatment groups at both baseline and follow-up (Table IX in the Data Supplement). The capillary blood glucose was not correlated with arterial [18F]FDG uptake at baseline (r=−0.03, P=0.8) or at follow-up (r=−0.1, P=0.2). Treatment group did not affect the primary end point by regression analysis either when controlling for capillary blood glucose (P=0.07) or HbA_{1c} (P=0.1). Finally, we performed adjustment for blood glucose on the quantification of arterial [18F]FDG uptake. This did not reveal any indications that liraglutide had a significant effect on arterial [18F]FDG uptake (Table X in the Data Supplement).

Impact of Previous CVD

Patients with CVD had a 10.4% higher carotid [18F]FDG uptake at baseline than the patients without CVD (P=0.16). In an exploratory analysis, we compared change in carotid [18F]FDG uptake in participants with (n=23) and without (n=79) a history of CVD and we observed a borderline significant interaction (P=0.052) between treatment group and history of CVD for predicting change in carotid [18F]FDG uptake (Figure 4). In explorative analysis including only participants with CVD, we observed a significant reduction in [18F]FDG uptake for the participants treated with liraglutide (−0.46 [95% CI, −0.89 to −0.02], P=0.04, n=13) but not for the participants treated with placebo (−0.16 [−1.03 to 0.71], P=0.68, n=9). However, the difference between

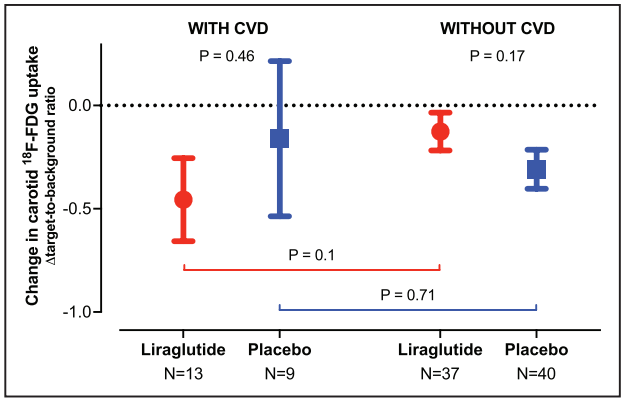

Figure 4. Arterial inflammation evaluated with [18F] fluorodeoxyglucose (FDG)-positron emission tomography (PET) in the subgroup of patients with and without cardiovascular disease.
Mean change from baseline to end-of-treatment in most diseased segment target-to-background ratio for the liraglutide group and the placebo group in subgroups of patients with and without cardiovascular disease (CVD). Mean plots with SE. Unpaired t test for comparison of the change from baseline to end-of-treatment between the groups.

participants with CVD treated with liraglutide and placebo was not statistical significant (P=0.46, Figure 4). Moreover, changes in the biomarkers reflecting inflammation or atherosclerosis were not different between the 2 treatment groups in analyses restricted to participants with a history of CVD (Table XI in the Data Supplement).

DISCUSSION

Treatment with liraglutide reduces cardiovascular events in high-risk patients,[1] but the mode of action is not fully understood. In this randomized, double-blind, placebo-controlled clinical trial, we demonstrated a significant effect of liraglutide on both HbA_{1c} and body weight, but we could not demonstrate an effect on arterial [18F]FDG uptake in this unselected type 2 diabetes population with the majority without known CVD. Similarly, we did not see any effect of liraglutide on CACS, CIMT, or endothelial function. In exploratory analyses, treatment with liraglutide in patients with manifest CVD did seem to reduce [18F]FDG uptake in the carotid arteries.

GLP-1 Treatment and Atherosclerosis

With positive results from four large cardiovascular outcome trials,[1–4] evidence has become strong for human GLP-1 receptor agonists to reduce CVD risk in type 2 diabetes patients with atherosclerotic CVD. The late separation of the Kaplan-Meier curves in the LEADER trial[1] could suggest a more indirect pleiotropic cardiovascular effect of liraglutide rather than a direct antithrombotic effect.

A number of animal studies support this hypothesis showing that GLP-1 inhibits the formation of

atherosclerotic plaques significantly,[12] inhibits the formation of macrophage-derived foam cells in the plaque,[13] and decreases the adhesion of mononuclear cells to the vessel wall.[14] This leads to an inhibition of atherogenesis by liraglutide in mice by decreased lipid deposition and reduction in intima-media thickness while the number of smooth muscle cells increase.[15] The attenuated development of plaque lesions in mice seems partly independent of weight and cholesterol lowering[7] and requires the endothelial GLP-1 receptor.[16] Similarly, a human study demonstrated that carotid plaques ex vivo were less inflamed in patients treated with GLP-1 receptor agonists, suggesting a more stable phenotype.[17] Also, a decrease in serum concentrations of the inflammatory marker sCD163 and fewer inflammatory macrophages were demonstrated in patients with type 2 diabetes treated for 6 months with liraglutide.[18] A similar effect is not seen with intense lowering of blood glucose without GLP-1 treatment.[19]

[18F]FDG-PET After GLP-1 Treatment

To our knowledge, this study is the first to investigate in vivo arterial [18F]FDG uptake, a marker of arterial inflammation, following treatment with GLP-1 receptor agonist in patients with type 2 diabetes. Inflammation plays a pivotal role in the development of atherosclerosis and recently 2 large randomized trials provided direct evidence that this risk is modifiable by anti-inflammatory therapy,[20,21] also in patients with type 2 diabetes.[22] The hypothesis of our study was that the known cardiovascular benefit of liraglutide[1] is at least in part caused by a reduction in arterial inflammation. [18F]FDG-PET/CT was chosen as primary end point since several studies, over the last decade, have shown that this method can be used to directly quantify arterial inflammation reliably and noninvasively.[11] [18F]FDG is actively taken up by cells with a high glycolytic activity, such as inflammatory cells, and the [18F]FDG signal correlates with metabolically active macrophages.[23] [18F]FDG-PET has been used to assess the direct impact of pharmaceutical interventions on arterial inflammation in a number of trials such as statin treatment.[24] The [18F]FDG-PET/CT method has similarly been used in a population with impaired glucose tolerance or type 2 diabetes to compare the effect of pioglitazone to glimepiride on plaque inflammation.[25] This study found an attenuation of arterial [18F]FDG uptake by pioglitazone but not by glimepiride despite equal effect on blood glucose in the 2 treatment arms[25]; these results are congruent with the known cardiovascular risk benefit of pioglitazone[26] as compared with no risk benefit of glimepiride.[27]

Based on the previous observations regarding the beneficial effect of liraglutide on CVD, our primary result is somewhat surprising. However, compared with the LEADER trial, we did not enrich the population with high-risk subjects and our study thus included only 23% with known CVD compared with 81% in the LEADER trial.[1] The majority of our population was, therefore, in a relatively lower risk of CVD, and it is our hypothesis that the low arterial inflammation resulted in a reduced potential for attenuation of the inflammatory signal. This hypothesis is supported by the fact that compared with previous studies assessing carotid uptake of [18F]FDG, our population had a low uptake at baseline.[10,24] In an exploratory analysis of the patients with known CVD, we observed that these subjects had a 10% higher carotid [18F]FDG uptake at baseline than the subjects without CVD. In an analysis of the total population, we observed a borderline significant interaction between presence of CVD and treatment supporting our hypothesis that the neutral results could have been caused by inclusion of relatively lower-risk patients. When only including subjects with known CVD in the analysis (n=23), we observed a numeric difference in the reduction of [18F]FDG uptake in subjects treated with liraglutide compared with placebo, but the difference did not reach statistical significance perhaps due to lack of power. However, as this was an explorative analysis the results are only hypothesis generating and need to be tested in a future study.

An alternative hypothesis for explaining the unchanged [18F]FDG uptake in our population is that [18F]FDG may not be a specific surrogate of inflammation in nonatherosclerotic arteries. The biological basis of cellular [18F]FDG uptake is complex and influenced by a number of metabolic factors. Small animal and clinical studies have investigated the biological link between arterial inflammation and [18F]FDG signal as reviewed by Sadeghi.[28] Several of these studies have demonstrated a correlation between [18F]FDG uptake and in vivo macrophage markers,[23] but without indications that inflammation should be the main determinant of arterial [18F]FDG uptake. This notion is supported by a study showing that uptake in medial smooth muscle cells was a significant contributor to the arterial [18F]FDG signal in minipigs.[29] However, at the same time positive results in clinical studies of anti-inflammatory drugs, like statins, in subjects with known CVD or at high risk for atherosclerosis[24] indicate that the arterial [18F]FDG signal does contain relevant clinical information despite its limited specificity.

Impact of Blood Glucose

A change in glucose metabolism could theoretically affect vascular [18F]FDG uptake and consequently influence the conclusion of our trial since [18F]FDG is a glucose analogue that is transported into the cells by the plasma membrane glucose transporters. However, a study of patients with diabetes or impaired glucose tolerance demonstrated no change in vascular [18F]

FDG uptake despite a significant reduction in both fasting plasma glucose and HbA_{1c} following treatment with glimepiride.[25] As expected, we found that liraglutide decreased HbA_{1c} compared with placebo, but when comparing the capillary blood glucose measured immediately before the [18F]FDG injection we found no difference between the 2 groups. Further support for the robustness of the [18F]FDG method in the presence of type 2 diabetes is that no correlation between arterial [18F]FDG uptake and blood glucose (either measured as HbA_{1c} or capillary blood glucose) was demonstrated in our study. This result is in line with observations in mice, where presence of type 2 diabetes does not confound evaluation of plaque inflammation with [18F]FDG.[30] In addition, patients with type 2 diabetes are routinely included in other studies of vascular inflammation using [18F]FDG-PET.[10,24] Glucose adjustment of [18F]FDG imaging is possible but generally not recommended for arterial [18F]FDG uptake.[9] Using blood glucose corrected [18F]FDG uptake in our study did not change the conclusions.

Secondary Supportive End Points

In support of our primary end point, we found that 26 weeks of treatment with liraglutide did not significantly change CACS, CIMT or measures of endothelial function compared with placebo. Circulating biomarkers of atherosclerosis and inflammation was not affected by liraglutide, and revealed that the included population had a low level of inflammation. Our study was not powered to detect changes in these secondary end points, and the treatment period of 26 weeks is probably too short for significant changes in the vascular anatomy, including CACS and CIMT, to occur.

As expected from previous studies with GLP-1 receptor agonists, gastrointestinal symptoms were the most common side effect and the most common reason to discontinue study treatment. More participants in the liraglutide group than in the placebo group discontinued the trial regimen due to adverse events. A total of 8 participants experienced a serious adverse event, none of which were fatal or considered related to liraglutide.

Study Limitations

Our trial has some limitations, and issues related to the design may have impacted our result. First, we included an unselected population of patients with type 2 diabetes rather than solely patients at very high CVD risk and we did not include a prescreening [18F]FDG-PET/CT to identify subjects with a [18F]FDG uptake indicating presence of vascular inflammation. The rationale for this was that type 2 diabetes is associated with increased cardiovascular risk,[31] and we thus expected a higher baseline [18F]FDG uptake than we observed. Second, we quantified the [18F]FDG uptake using active segments analysis of both aorta and the carotid arteries rather than using most diseased segment analysis of the carotid arteries only as in most of the recent studies. Recent studies indicate that the carotid artery [18F]FDG uptake has less day-to-day variation[11] and a study in pigs demonstrated [18F]FDG signal in the aorta irrespective of the presence of macrophage-containing lesions.[29] Based on these considerations, we performed the exploratory analysis in the subpopulation with known CVD using most diseased segment analysis of the carotid artery.

Conclusions

In conclusion, our study found no effect of liraglutide on arterial [18F]FDG uptake in patients with type 2 diabetes and low prevalence of vascular inflammation indicating that liraglutide did not attenuate arterial inflammation in these patients. An explorative analysis indicated a possible effect in the subgroup of patients with a history of CVD, in line with current guidelines where liraglutide is recommended to patients with history of CVD.

ARTICLE INFORMATION

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Disclosures

Dr Ripa, von Scholten, Hansen, and Rossing had shares in Novo Nordisk A/S, and Dr von Scholten is now an employee of Novo Nordisk A/S. Dr Rossing has received the following: Consultancy and speaking fees (to Steno Diabetes Center Copenhagen) from AbbVie, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, MSD, Novo Nordisk, and Sanofi Aventis; Research grants to institution from AbbVie, AstraZeneca, and Novo Nordisk. Dr Kjaer has received consultancy fees from Novo Nordisk. The other authors report no conflicts.

Supplemental Materials

Data Supplement Methods
Data Supplement Tables I–XI
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