GLP-1 receptor agonism to improve cardiometabolic health

Eyk, H.J. van

Chapter 3

Effect of liraglutide on cardiovascular function and myocardial tissue characteristics in type 2 diabetes patients of South Asian descent living in the Netherlands: a double-blind randomized placebo-controlled trial

Elisabeth H.M. Paiman[a*], Huub J. van Eyk[b,c*], Minke M.A. van Aalst[a], Maurice B. Bizino[a], Rob J. van der Geest[a], Jos J.M. Westenberg[a], Petronella H. Geelhoed-Duijvestijn[d], Aan V. Kharagjitsing[e], Patrick C.N. Rensen[b,c], Johannes W.A. Smit[f], Ingrid M. Jazet[b,c], Hildo J. Lamb[a]

[a]Department of Radiology, Leiden University Medical Centre, Leiden, the Netherlands, [b]Department of Medicine, Division of Endocrinology, Leiden University Medical Centre, Leiden, the Netherlands, [c]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Centre, Leiden, the Netherlands, [d]Department of Medicine, Haaglanden Medical Centre, The Hague, the Netherlands, [e]Department of Diabetology and Endocrinology, University Hospital Brussels, Brussels, Belgium, [f]Department of Medicine, Radboud University Medical Centre, Nijmegen, the Netherlands.

[*]These authors contributed equally to this work.

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ABSTRACT

Background: The glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide may be beneficial in the regression of diabetic cardiomyopathy. South Asian ethnic groups in particular are at risk of developing type 2 diabetes.

Purpose: To assess the effects of liraglutide on left ventricular (LV) diastolic and systolic function in South Asian type 2 diabetes patients.

Study Type: Prospective double-blind randomized placebo-controlled trial.

Population: 47 type 2 diabetes patients of South Asian ancestry living in the Netherlands, with or without ischemic heart disease, who were randomly assigned to 26-week treatment with liraglutide (1.8 mg/day) or placebo.

Field strength/Sequence: 3T (bSSFP cine MRI, 2D and 4D velocity-encoded MRI, ^{1}H-MRS, T1 mapping).

Assessment: Primary endpoints were changes in LV diastolic function (early deceleration peak (Edec), ratio of early and late peak filling rate (E/A), estimated LV filling pressure (E/Ea)) and LV systolic function (ejection fraction). Secondary endpoints were changes in aortic stiffness (aortic pulse wave velocity (PWV), myocardial steatosis (myocardial triglyceride content) and diffuse fibrosis (extracellular volume (ECV)).

Statistical Tests: Data were analyzed according to intention-to-treat. Between-group differences were reported as mean (95%CI) and were assessed using ANCOVA.

Results: Liraglutide (n=22) compared with placebo (n=25) did not change Edec (+0.2 mL/s^2x10^{-3} (-0.3;0.6)), E/A (-0.09 (-0.23;0.05)), E/Ea (+0.1 (-1.2;1.3)) and ejection fraction (0% (-3;2)), but decreased stroke volume (-9 mL (-14;-5)) and increased heart rate (+10 bpm (4;15)). Aortic PWV (+0.5 m/s (-0.6;1.6)), myocardial triglyceride content (+0.21% (-0.09;0.51)) and ECV (-0.2% (-1.4;1.0)) were unaltered.

Data Conclusion: Liraglutide did not affect LV diastolic and systolic function, aortic stiffness, myocardial triglyceride content or extracellular volume in Dutch South Asian type 2 diabetes patients with or without coronary artery disease.

INTRODUCTION

Type 2 diabetes is associated with a two- to five-fold increased risk of heart failure[1]. Diabetic cardiomyopathy, which is characterized by left ventricular (LV) diastolic dysfunction, may eventually progress to heart failure with preserved ejection fraction[1]. A potential anti-hyperglycemic agent with cardioprotective effects is the glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide[2].

Recently, the LEADER trial has demonstrated a reduced total cardiovascular mortality as a result of liraglutide in patients with type 2 diabetes and high cardiovascular risk, presumably because of a lower risk of ischemic events[3]. Similar reductions in cardiovascular mortality have been reported in response to treatment with the GLP-1 receptor agonists semaglutide and dulaglutide[4]. However, it is largely unknown whether liraglutide in the management of type 2 diabetes is advantageous for heart function in asymptomatic diastolic dysfunction[2]. It is conceivable that the favorable metabolic impact of liraglutide on lipid profiles and inflammatory markers[5], in addition to the natriuretic and vasodilatory actions[6,7], has indirect beneficial effects on diastolic function. Liraglutide has been assumed to exert direct actions on the myocardium that may amend myocardial metabolism, although preclinical and clinical studies have not been conclusive[2]. The effects of liraglutide on diastolic function may be mediated by regression of type 2 diabetes-related myocardial steatosis, diffuse fibrosis and aortic stiffening[1,8]. Notably, clinical studies have consistently reported an increase in heart rate in individuals using liraglutide[2,5,9]. In this regard, the actual effect of liraglutide on heart function, taken into account the wide range of cardiovascular actions, is uncertain.

South Asian ethnic groups in particular are at increased risk of developing type 2 diabetes[10]. South Asians appear to have a strong genetic predisposition for insulin resistance, while differences in lifestyle factors seem to have a smaller role in the increased risk of type 2 diabetes as compared with other ethnic groups[11]. The impaired insulin sensitivity in South Asians has been related to the relatively high total body fat percentage and high fat storage in the visceral compartments[12]. In addition, adipocytes may be dysfunctional, as reflected by the increased release of free fatty acids, adipokines and proinflammatory cytokines among South Asian individuals[13,14]. Previously, it has been demonstrated that hyperglycemia is more detrimental for cardiac function in South Asians than in Europeans[15]. As the pathogenesis of type 2 diabetes but also the impact of type 2 diabetes on cardiac function appears to be different, the cardiometabolic effects of liraglutide in the treatment of type 2 diabetes may be more pronounced in South Asians compared with individuals of other ethnicities.

In this study, we aimed to assess the effects of 26-week liraglutide treatment among South Asian type 2 diabetes patients on LV diastolic and systolic function and, secondary, myocardial steatosis and diffuse fibrosis. We used cardiovascular magnetic resonance, as this imaging modality enables the measurement of LV diastolic and systolic function and aortic stiffness[16,17] and also the assessment of myocardial tissue characteristics[18,19].

MATERIALS AND METHODS

Study Design And Participants

This study is a 26-week double-blind randomized controlled trial (ClinicalTrials.gov NCT02660047)[20]. Written informed consent was obtained prior to inclusion. This study complied with the revised Declaration of Helsinki and was approved by the institutional research board and the Central Committee on Research Involving Human Subjects.

Patients were recruited from the outpatient clinic of the Leiden University Medical Center (Leiden, the Netherlands), local hospitals and general practices in Leiden and The Hague, and by advertisements in local newspapers. Individuals aged 18-75 years of South Asian ancestral origin with type 2 diabetes treated with metformin, sulfonylurea derivatives and/or insulin for at least 3 months in stable dose were eligible for participation. South Asian descent was defined as both biological parents and their ancestors being South Asian (i.e. South Asian Surinamese, Indian, Pakistani, Bangladeshi or Sri Lankan origin). Inclusion criteria were: BMI ≥23 kg/m^2; HbA1c ≥6.5 and <11.0% (≥47.5 and <96.5 mmol/mol); estimated glomerular filtration rate (eGFR) >30 mL/min/1.73 m^2; blood pressure <180/110 mmHg. Main exclusion criteria were: use of GLP-1 receptor agonists; dipeptidyl peptidase 4 inhibitors or thiazolidinediones within the past 6 months; heart failure New York Heart Association (NYHA) class III-IV; acute coronary or cerebrovascular accident in the preceding 30 days; pancreatitis or medullary thyroid carcinoma; gastric bypass surgery; pregnant or lactating women; any contra-indication for magnetic resonance imaging (MRI). Due to the insufficient number of eligible patients, several criteria were adjusted (initial inclusion criteria: age 18-70 years; HbA1c ≥7.0 and <10.0% (≥53 and <86 mmol/mol); eGFR >60 mL/min/1.73 m^2; blood pressure <150/85 mmHg; no history of cardiovascular disease).

Randomization, Blinding And Intervention

Patients were randomized to once-daily subcutaneous injections of liraglutide (Victoza®, Novo Nordisk A/S, Bagsvaerd, Denmark) or placebo added to standard care during 26 weeks (randomization with block size 4, with 1:1 stratification for sex and insulin use). A randomization code list was generated by the institutional research pharmacist. If necessary to prevent hypoglycemia, the concomitant glucose-lowering medication was

adjusted at study entry. Starting dose of the trial medication was 0.6 mg/day, which was increased every 7 days up to 1.8 mg/day. The dose was reduced upon poor tolerance. Investigators and patients were blinded to treatment allocation. Furthermore, the MRI data were stripped of any information on the participant's identity and measurement date.

Study Procedures

Study days at baseline and after 26 weeks consisted of clinical measurements and MRI. Baseline and follow-up measurements were both scheduled either in the morning or evening. Patients were asked to fast overnight or for 6 hours, when measurements were in the morning or evening, respectively. To prevent hypoglycemia during fasting, insulin dose was adjusted and other anti-diabetic medications were temporarily discontinued. Patients were instructed to adhere to their usual diet and physical activity. During the trial, patients received a weekly telephone call for glycemic control based on their self-monitored blood glucose levels. At week 4 and 12, routine blood tests and clinical measurements were performed. Glycemic control and blood pressure management was according to the current guidelines [21,22]. Patients were asked for adverse events once a week. Study drug pens were collected during the trial as a surrogate marker of compliance.

MRI Protocol

MRI scans were acquired on a 3 Tesla MR scanner (Ingenia, Philips Healthcare, Best, the Netherlands). For contrast-enhanced MRI, 0.15 mmol gadoterate meglumine (0.5 mmol/mL Dotarem; Guerbet, Villepinte, France) per kilogram of body weight was administered intravenously. LV systolic and diastolic function parameters were assessed by short-axis and 4-chamber cine balanced steady-state free precession (bSSFP) and whole-heart gradient-echo 4D velocity-encoded MRI, with retrospective ECG (electrocardiography) gating. To determine aortic stiffness, the aortic pulse wave velocity (PWV) was calculated from a scout view of the aorta and two 2D velocity-encoded scans at the ascending and abdominal aorta. Myocardial steatosis was quantified as the myocardial triglyceride content, examined by proton-magnetic resonance spectroscopy (^{1}H-MRS) in the mid-ventricular septum and expressed as the amplitude of triglyceride methylene divided by the amplitude of unsuppressed water, multiplied by 100%. Myocardial diffuse fibrosis was assessed using native and post-contrast modified Look-Locker inversion (MOLLI) recovery T1 mapping. Native T1 and the extracellular volume (ECV) were measured in the mid-ventricular septum. To identify ischemic scarring, late gadolinium enhancement (LGE) MRI was acquired. If septal delayed enhancement was present, myocardial triglyceride content data was excluded and diffuse fibrosis was measured outside the region with scar. LGE-MRI was assessed visually by a radiologist (H.J.L.) and clinical investigator (E.H.M.P.) with 25 and 4 years of experience in cardiovascular MRI, respectively. A detailed description of the MRI protocol is provided as **Supplementary Material**.

Study Endpoints

Primary endpoints were LV diastolic function (peak deceleration slope of the transmitral early peak filling rate (Edec), ratio of transmitral early and late peak filling rate (E/A), early peak diastolic mitral septal tissue velocity (Ea), estimated LV filling pressure (E/Ea)) and LV systolic function (ejection fraction, stroke volume, cardiac output, cardiac index, peak ejection rate). Secondary endpoints included myocardial triglyceride content, ECV, aortic PWV, LV dimensions and clinical parameters (heart rate, blood pressure, body weight and HbA1c).

Statistical Analysis

Statistical analyses were performed with SPSS version 23 (IBM Corporation, Chicago, Illinois, USA), according to intention-to-treat. Within-group differences from baseline to 26 weeks were reported as means ± SD. Between-group differences for liraglutide vs. placebo were analyzed using ANCOVA with the baseline values as covariate to reduce within- and between-group variability and were reported as means (95%CI). Statistical tests were 2-sided and $P<0.05$ was considered significant. The power calculation is described in the **Supplementary Material**.

RESULTS

Baseline Characteristics

Patients were recruited between July 16, 2015 and December 6, 2017. A total of 47 patients were randomized to liraglutide (n=22) or placebo (n=25) (**Figure 1**). Between October 7, 2015 and March 9, 2018, all participants completed the trial. There were no clinically relevant differences between the treatment groups regarding demographics and clinical, laboratory and MRI parameters (**Table 1**). The total study population (40% men) had a mean (SD) age of 55 ± 10 years, a diabetes duration of 18 ± 10 years and HbA1c of 8.4 ± 1.0% (68 ± 11 mmol/mol), whilst 77% of the patients was using insulin.

Drug Compliance And Clinical Parameters

Study drug compliance was high (95 ± 8% and 99 ± 5% for liraglutide and placebo treatment, respectively) and the dose could be titrated up to 1.8 mg/day in most patients (in 86% and 96% of the patients treated with liraglutide and placebo, respectively). For glycemic control, in some patients in the placebo group, concomitant medication was started (metformin (n=1) or sulfonylurea derivates (n=3)) or the insulin dose was adjusted (1 ± 23 and -11 ± 34 units/day in the placebo and liraglutide group, respectively). For blood pressure management, in some patients in the placebo and liraglutide group, antihypertensive mediation was started or the dose was elevated (n=5 vs. n=3) or the dose was reduced (n=1 vs. n=2).

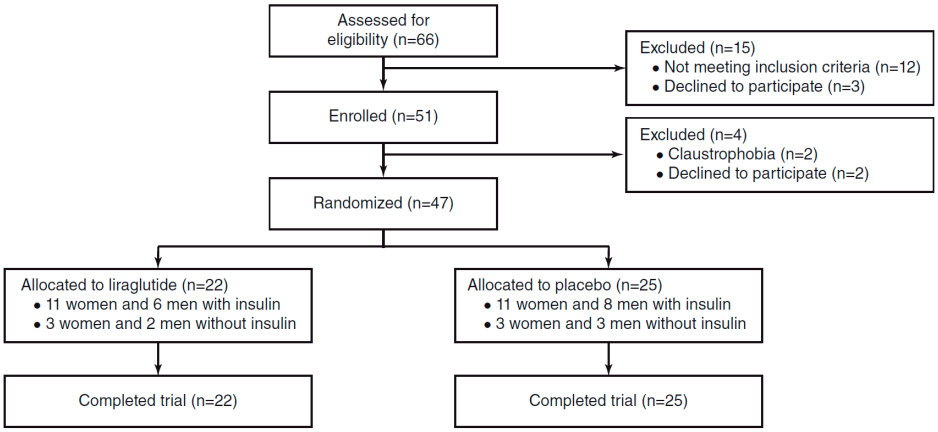

Figure 1. Trial profile.

Table 1. Baseline Characteristics

	Liraglutide (n=22)	Placebo (n=25)
Demographic and clinical characteristics		
Age, years	55 (11)	55 (9)
Men, no.	8 (36%)	11 (44%)
Diabetes duration, years	19 (10)	17 (10)
Diabetes complications, no.	15 (68%)	16 (64%)
Coronary artery disease, no.		
Non-significant coronary artery stenosis	4 (18%)	0 (0%)
Percutaneous coronary intervention	2 (9%)	3 (12%)
Coronary artery bypass grafting	1 (5%)	2 (8%)
Smoking, no.		
Currently	2 (9%)	5 (20%)
Previously	6 (27%)	0 (0%)
Never	14 (64%)	20 (80%)
Medication		
Metformin, no.	22 (100%)	23 (92%)
Sulfonylurea derivatives, no.	3 (14%)	5 (20%)
Insulin, no.	17 (77%)	19 (76%)
Metformin dose, g/day	1.8 (0.7)	1.7 (0.6)
Insulin dose, units/day	77 (34)	67 (30)
Lipid-lowering drugs, no.	17 (77%)	20 (80%)
Anti-hypertensive drugs, no.	16 (73%)	18 (72%)
Beta-blockers, no.	8 (36%)	9 (36%)
Diuretics, no.	9 (41%)	8 (32%)
ACE-inhibitors, no.	6 (27%)	7 (28%)

Table 1. Baseline Characteristics (*continued*)

	Liraglutide (n=22)	Placebo (n=25)
Angiotensin II receptor-blockers, no.	7 (32%)	9 (36%)
Calcium-antagonists, no.	2 (9%)	5 (20%)
Clinical parameters		
Weight, kg	82 (11)	78 (12)
BMI, kg/m^2	30.4 (3.8)	28.6 (4.0)
Waist circumference, cm	104 (8)	98 (10)
Waist-hip ratio	1.00 (0.07)	0.95 (0.09)
Heart rate, bpm	73 (13)	77 (11)
Systolic blood pressure, mmHg	149 (25)	141 (18)
Diastolic blood pressure, mmHg	85 (11)	85 (10)
Laboratory parameters		
HbA1c, %	8.1 (0.9)	8.6 (1.1)
HbA1c, mmol/mol	65 (10)	70 (12)
Triglycerides, mmol/L	1.6 (0.9)	2.1 (1.8)
Total cholesterol, mmol/L	4.0 (0.6)	4.5 (1.1)
HDL-cholesterol, mmol/L	1.2 (0.3)	1.2 (0.3)
LDL-cholesterol, mmol/L	2.0 (0.7)	2.2 (1.0)
LV diastolic function		
Edec, mL/s^2 x10^{-3}	-2.5 (1.3)	-2.7 (1.2)
E, mL/s	305 (99)	328 (118)
A, mL/s	316 (75)	306 (58)
E/A	0.99 (0.31)	1.11 (0.43)
E, cm/s	34 (9)	37 (9)
Ea, cm/s	5.3 (2.1)	5.7 (1.9)
E/Ea	7.4 (3.9)	7.4 (3.3)
LV systolic function		
Stroke volume, mL	70 (12)	67 (15)
Ejection fraction, %	56 (8)	57 (7)
Cardiac output, L/min	4.7 (0.9)	4.7 (1.1)
Cardiac index, L/min/m^2	2.4 (0.4)	2.5 (0.4)
Peak ejection rate, mL/s	338 (82)	345 (84)
LV structure		
End-diastolic volume, mL	128 (25)	120 (36)
End-systolic volume, mL	57 (21)	53 (24)
Mass, g	98 (22)	96 (24)
Aortic stiffness		
Aortic pulse wave velocity, m/s	8.8 (2.4)	8.3 (2.4)
Myocardial tissue characteristics		

Table 1. Baseline Characteristics (*continued*)

	Liraglutide (n=22)	Placebo (n=25)
Myocardial triglyceride content, %	0.92 (0.43)	1.00 (0.58)
Native T1 relaxation time, ms	1264 (45)	1254 (33)
Extracellular volume, %	25.9 (3.1)	27.0 (2.6)

Data are presented as mean (SD) or no. (%). Diabetes complications: retinopathy, neuropathy, nephropathy or macrovascular complications. A: late transmitral peak filling rate; E: early transmitral peak filling rate; Ea: early peak diastolic mitral septal tissue velocity; E/Ea: estimation of LV filling pressure; Edec: early deceleration peak.

In both the liraglutide and placebo group there was a decrease (mean ± SD) after 26 weeks in HbA1c (-0.8 ± 1.0 vs. -0.6 ± 0.8% (-9 ± 11 vs. -7 ± 9 mmol/mol) and systolic blood pressure (-14 ± 18 vs. -7 ± 15 mmHg), but not in diastolic blood pressure (-3 ± 11 vs. -3 ± 9 mmHg). However, between-group differences for liraglutide vs. placebo in HbA1c (-0.4% (95%CI: -0.9 to 0.2); -4 mmol/mol (95%CI: -10 to 2), *P*=0.16) and systolic blood pressure (-3 mmHg (95%CI: -9 to 3, *P*=0.36)) were non-significant. Liraglutide compared with placebo decreased body weight (-3.9 ± 3.6 vs. -0.6 ± 2.2 kg; between-group difference: -3.5 kg (95%CI: -5.3 to -1.8, *P*<0.001)) and increased heart rate (9 ± 11 vs. -2 ± 8 bpm; between-group difference: 10 bpm (95%CI: 4 to 15, *P*=0.001)).

LV Function, Aortic Stiffness And Myocardial Tissue Characteristics

LV diastolic function parameters were unaltered by liraglutide. Also, LV systolic function was unaffected upon liraglutide, as ejection fraction and peak ejection rate were unchanged and, despite the decreased stroke volume, cardiac output and cardiac index were preserved (**Table 2 and Figure 2**). The decrease in stroke volume was in parallel with the reductions in end-diastolic and end-systolic volume, which persisted when adjusting for body surface area. Whereas liraglutide significantly reduced end-diastolic volume, the decrease in LV mass was not significant. Also, liraglutide did not change aortic stiffness, myocardial triglyceride content or diffuse fibrosis. A total of 6 patients had delayed enhancement at baseline, but in only one patient the ventricular septum was involved. In all patients, the extent of delayed enhancement was unchanged at follow-up. Details on missing values are provided as **Supplementary Material**.

Adverse Events

There was one serious adverse event in the placebo group (admission for acute coronary syndrome symptoms without requiring further treatment). More patients with treatment with liraglutide compared with placebo had complaints of nausea (73% vs. 40%) and vomiting (27% vs. 8%). There were no cases of severe hypoglycemia.

Table 2. Study Endpoints: Mean Change Over 26 Weeks

	Mean change (SD) from 0 to 26 weeks		Mean change (95%CI) from 0 to 26 weeks	
	Liraglutide (n=22)	**Placebo (n=25)**	**(Liraglutide vs. Placebo)**	***P* value**
Primary				
LV diastolic function				
Edec, mL/s^2 x10^{-3}	0.2 (1.1)	0.1 (0.7)	0.2 (-0.3 to 0.6)	0.46
E, mL/s	-36 (84)	-18 (55)	-24 (-60 to 12)	0.18
A, mL/s	17 (77)	-2 (45)	18 (-21 to 56)	0.35
E/A	-0.11 (0.24)	-0.05 (0.24)	-0.09 (-0.23 to 0.05)	0.21
E, cm/s	-2 (7)	-1 (7)	-2 (-6 to 1)	0.20
Ea, cm/s	-0.1 (1.1)	-0.1 (1.1)	-0.1 (-0.7 to 0.5)	0.73
E/Ea	-0.4 (2.4)	-0.3 (2.6)	0.1 (-1.2 to 1.3)	0.89
LV systolic function				
Ejection fraction, %	0 (5)	0 (3)	0 (-3 to 2)	0.86
Stroke volume, mL	-10 (9)	0 (7)	-9 (-14 to -5)	<0.001
Cardiac output, L/min	-0.2 (0.5)	-0.1 (0.5)	-0.1 (-0.4 to 0.2)	0.44
Cardiac index, L/min/m^2	-0.1 (0.3)	-0.1 (0.3)	0.0 (-0.2 to 0.1)	0.87
Peak ejection rate, mL/s	-9 (60)	-7 (45)	-3 (-34 to 27)	0.83
Secondary				
LV structure				
Mass, g	-4 (9)	0 (7)	-4 (-9 to 0)	0.07
End-diastolic volume, mL	-19 (13)	-1 (11)	-17 (-24 to -10)	<0.001
End-systolic volume, mL	-9 (9)	-1 (7)	-7 (-11 to -3)	0.001
Aortic stiffness				
Aortic pulse wave velocity, m/s	0.2 (2.1)	-0.2 (1.7)	0.5 (-0.6 to 1.6)	0.35
Myocardial tissue characteristics				
Myocardial triglyceride content, %	0.14 (0.47)	-0.09 (0.56)	0.21 (-0.09 to 0.51)	0.16
Native T1 relaxation time, ms	-6 (36)	6 (26)	-7 (-21 to 7)	0.35
Extracellular volume, %	0.5 (2.6)	0.4 (1.3)	-0.2 (-1.4 to 1.0)	0.76

Abbreviations as in Table 1.

DISCUSSION

In this double-blind, randomized controlled trial in type 2 diabetes patients of South Asian descent living in the Netherlands, with or without coronary artery disease, 26-week treatment with 1.8 mg/day liraglutide had no effect on LV diastolic and systolic function, aortic stiffness, myocardial triglyceride content or extracellular volume, as compared with placebo when added to standard care. Our results imply that liraglutide does not amend cardiovascular remodelling in diabetic cardiomyopathy in a Dutch

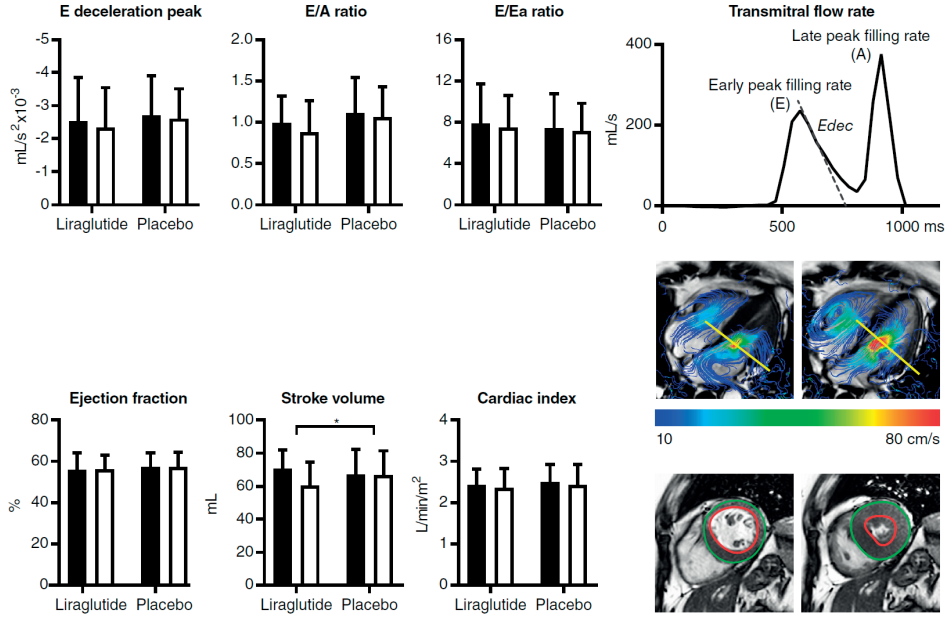

Figure 2. Liraglutide does not alter left ventricular (LV) diastolic and systolic function in South Asian type 2 diabetes patients with or without coronary artery disease and without advanced heart failure. LV diastolic and systolic outcome measures (mean ± SD) before (black bars) and after (white bars) treatment with liraglutide and placebo are presented. An example of a transmitral flow rate curve, 4D velocity-encoded and short-axis cine magnetic resonance is provided for illustration. E/A: ratio of transmitral early and late peak filling rate; E/Ea: estimation of LV filling pressure; Ea: early peak diastolic mitral septal tissue velocity; Edec: early deceleration peak.

South Asian type 2 diabetes population including patients with pre-existing ischemic heart disease, at least not upon a treatment period of 26 weeks.

Mechanisms

Whether liraglutide exerts direct actions on the ventricles, such as enhancement of coronary blood flow and myocardial glucose uptake, has been debated[2]. The GLP-1 receptor has been demonstrated to be present on the sinoatrial node and atrial cardiomyocytes[23], but its function as well as its presence on ventricular cardiomyocytes and blood vessels in humans is still uncertain. Furthermore, it has been suggested that the cardioprotective effects of native GLP-1 as described in earlier studies may be related to actions of degradation products of GLP-1, which are not produced by GLP-1 analogues[2]. We hypothesized that liraglutide may reverse diabetic cardiomyopathy, partly as a result of its indirect cardiovascular actions[5-7]. However, based on our findings, at least large immediate effects on LV function in South Asian type 2 diabetes patients can be excluded.

The reductions in LV end-diastolic volume and stroke volume in our study were not explained by liraglutide-induced body weight loss. It is conceivable that the decreased end-diastolic volume and stroke volume were related to the increased heart rate and consequent reduced ventricular filling time. Notably, the elevation in heart rate in our study was relatively large compared with other trials with the same dose and of similar duration[2,5,9]. Proposed mechanisms for the heart rate acceleration upon treatment with GLP-1 receptor agonists include enhancement of the sympathetic activity[24] and inhibition of the cardiac vagal neurons[25] as well as direct sinoatrial node stimulation[9]. Our study population included patients with prevalent coronary artery disease. It has been suggested that individuals with pre-existing cardiac disease may be more susceptible to heart rate acceleration upon GLP-1 receptor agonists[2]. Furthermore, South Asians may have an altered balance in the autonomic nervous system[26], which may have contributed to the profound heart rate elevation by liraglutide treatment in our study population.

Previous Studies

Only a few previous studies, including two open-label randomized controlled trials[27,28] and one small double-blind randomized controlled trial[29], assessed the effect of liraglutide on diastolic function in type 2 diabetes, during an intervention period of 4 to 6 months. One study demonstrated an improvement in myocardial relaxation in response to liraglutide, with amelioration of aortic stiffening[28], whereas others reported no improvement of diastolic function[27,29]. Large trials on the impact of liraglutide on systolic function have been previously performed in heart failure with reduced ejection fraction, where no effect was reported[30,31]. Regarding the impact of GLP-1 receptor agonists on myocardial tissue characteristics, most research has been limited to preclinical studies. In animal models of type 2 diabetes, liraglutide has been shown to reduce cardiac fibrosis[32], possibly by inhibition of the endoplasmic reticulum (ER) stress pathway via activation of the AMP-activated protein kinase (AMPK) system[33,34]. Activation of AMPK, which acts as a regulator of cellular energy status, has also been proposed as the underlying mechanism for improved cardiac function in type 2 diabetes after liraglutide as observed in preclinical research[33]. Furthermore, GLP-1 receptor agonists have been shown to relieve the intramyocardial lipid deposition in diabetic mice, in association with ameliorated levels of plasma cholesterol[35]. However, attenuation of myocardial steatosis by treatment with GLP-1 receptor agonists in type 2 diabetes has not been confirmed in human studies[36].

In a recent double-blind randomized controlled trial on the effect of liraglutide on cardiac function in European type 2 diabetes patients[37], liraglutide decreased the LV filling pressure, presumably through natriuresis and vasorelaxation, whereas myocardial relaxation was unaltered. Apart from ethnicity, the present South Asian cohort was distinct

from this European study group regarding sex (40% vs. 59% men), diabetes duration (18 ± 10 vs. 11 ± 7 years), insulin use (77% vs. 65%) and ischemic heart disease (17% vs. 0%). As there have been no large-scale clinical studies, it remains unknown whether certain patient characteristics have a modifying role in the cardiovascular actions of liraglutide.

Strengths And Limitations

The most important strengths of the present study are related to its double-blind, randomized controlled design, the absence of drop-outs and high study drug compliance. Liraglutide was added to standard care, mimicking the real-world setting. There are some limitations which need to be addressed. This trial comprised South Asian individuals living in a high-income country and included predominantly South Asian Surinamese, who originate from the northern part of India. Extrapolation of our results to other South Asian ethnic groups should be performed with caution. Furthermore, we did not use echocardiography, which is the routine clinical approach for evaluating diastolic function. Nonetheless, MRI is widely used in clinical studies for the assessment of diastolic function and, importantly, it has been validated with echocardiography[38]. It has to be noted that in individuals with high heart rate (>100 bpm), early and late diastolic filling cannot be separated. As a consequence, two participants in the liraglutide group had missing data for diastolic function at follow-up, which might have introduced bias. The LV diastolic function parameters in our study population, as well as aortic pulse wave velocity, were approximately one standard deviation from the mean in healthy individuals[39]. However, in contrast to the clear impairments in LV diastolic function, the myocardial triglyceride content was 0.92-1.00% in this type 2 diabetes cohort, whereas the values in healthy controls are approximately 0.58% and 0.84% among Europeans and South Asians, respectively[39]. The type 2 diabetes patients in the present study did not demonstrate abnormalities in extracellular volume, possibly as a result of angiotensin-converting enzyme (ACE) inhibitors which may relieve fibrotic remodelling[39]. Hence, we cannot exclude a beneficial effect of liraglutide on extracellular volume in type 2 diabetes patients with marked cardiac fibrosis. Also, we cannot preclude cardiovascular benefits after prolonged (>26 weeks) therapy with liraglutide. Nevertheless, in animal studies, improved myocardial function has been reported already after brief (1 week) treatment with liraglutide[33].

Implications

In our study, liraglutide did not enhance heart function and may therefore have no specific role in the prevention of heart failure with preserved ejection fraction in South Asian type 2 diabetes patients. In contrast, recent studies have indicated that the sodium-glucose co-transporter 2 (SGLT2) inhibitors empagliflozin, canagliflozin and dapagliflozin have a benefit on the incidence of heart failure[4], potentially because of di-

rect improvement of myocardial relaxation in addition to diuretic effects[40]. Conversely, the previously reported reduced cardiovascular mortality rate in response to liraglutide among patients with type 2 diabetes and high cardiovascular risk is probably primarily related to slowed progression of atherosclerosis[3,41]. Following the results from recent cardiovascular outcome trials[3,4], SGLT2 inhibitors have been recommended as part of type 2 diabetes management among individuals with co-existing heart failure or at risk of heart failure, and either GLP-1 receptor agonists or SGLT2 inhibitors should be considered in type 2 diabetes patients with established atherosclerotic disease and no specific concerns of heart failure[22]. Our study did not demonstrate regression of LV diastolic dysfunction in response to liraglutide. Nonetheless, because of its presumed anti-atherosclerotic actions, GLP-1 receptor agonists remain worth considering especially in South Asian type 2 diabetes patients given their disadvantageous cardiometabolic profile and high risk of ischemic heart disease[10].

Conclusion

In conclusion, in this 26-week double-blind randomized placebo-controlled trial in Dutch South Asian type 2 diabetes patients with or without coronary artery disease, liraglutide had no effect on LV diastolic and systolic function, nor on aortic stiffness, myocardial triglyceride content and extracellular volume. A previous study reported a reduced LV filling pressure after liraglutide therapy in a European cohort of type 2 diabetes patients without ischemic heart disease, who were predominantly men, with a shorter diabetes duration and less use of insulin as compared with the South Asian type 2 diabetes patients in the present study[37]. Further research should reveal whether the cardiovascular impact of liraglutide might be dependent on patient characteristics such as sex, ethnicity, diabetes duration, co-medication or history of ischemic heart disease.

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SUPPLEMENTARY MATERIAL

MRI acquisition and analysis

LV Systolic And Diastolic Function

MRI scans were acquired on a 3 Tesla magnetic resonance scanner (Ingenia, Philips Healthcare, Best, the Netherlands), using a dStream Torso anterior coil and a FlexCoverage posterior coil in the table top, resulting in up to 32 coil elements for signal reception. LV dimensions and systolic and diastolic function were quantified by standard short-axis and 4-chamber long-axis cine balanced steady-state free precession (bSSFP) and free-breathing whole-heart gradient-echo 4D velocity-encoded MR, with retrospective electrocardiography (ECG) gating. Typical imaging parameters of the bSSFP cines were: echo/repetition time (TE/TR) 1.5/3.0 ms, flip angle (FA) 45°, field-of-view (FOV) 350x350 mm^2 (4-chamber) and 400x352 mm^2 (short-axis), acquired voxel size 2.0x1.6 mm^2 (4-chamber) and 1.5x1.5 mm^2 (short-axis), slice thickness 8 mm, number of phases 30 (4-chamber) and 35 (short-axis). For short-axis bSSFP cine, the complete LV was imaged using 14-16 slices. 4D velocity-encoded (venc 150 cm/s) MR was acquired in parallel with the 4-chamber view, with typical imaging parameters: TE/TR 4.6/9.0 ms, FA 10°, FOV 360x360 mm^2, acquired voxel size 3.0x3.0 mm^2, slice thickness 3 mm, number of slices 41, number of phases 30, sensitivity encoding (SENSE) factor 2.

LV function and dimensions were assessed with MASS version 2015-EXP (Leiden University Medical Center, Leiden, the Netherlands). For LV diastolic function, the flow rate curves over the mitral valve were derived from the 4D velocity-encoded scans by retrospective mitral valve tracking, perpendicular to the streamlines of inflow across the mitral valve, at the location of peak flow velocity[1,2]. Subsequently, the ratio of the transmitral early (E) and late (A) peak filling rate (E/A ratio), the peak deceleration slope of the E wave (Edec) and the transmitral early peak velocity were calculated. The transmitral filling rate curves were corrected for the through-plane background velocity of the LV myocardial wall. The early peak diastolic mitral septal tissue velocity (Ea) was measured on 4-chamber cines. The mitral septal tissue velocity curves were calculated from the displacement of the semi-automatically tracked mitral valve, at the insertion to the LV septum, relative to the LV apex, throughout the cardiac cycle. The estimated LV filling pressure was defined as the ratio of the transmitral early peak velocity and Ea[3]. For LV dimensions and systolic function, LV endocardial and epicardial contours were semi-automatically drawn in the short-axis cines in the end-diastolic and end-systolic phase, to quantify the end-diastolic LV mass, LV end-diastolic and end-systolic volumes and subsequently LV stroke volume, ejection fraction, cardiac output and cardiac index.

The peak ejection rate was derived from the systolic flow rate curves over the aortic valve after retrospective valve tracking[4].

Aortic Stiffness

For aortic PWV a double-oblique sagittal scout view of the aorta and free-breathing through-plane 2D velocity-encoded MR scans were obtained (one transecting the proximal ascending aorta, with venc 200 cm/s, and one transecting the abdominal aorta above the bifurcation, with venc 150 cm/s). Typical imaging parameters were: TE/TR 2.5/4.4 ms, FA 20°, FOV 350x282 mm^2, slice thickness 8 mm, acquired voxel size 2.8x2.8 mm^2, temporal resolution 10 ms. Aortic PWV analyses were performed as previously described, using MASS version 2015-EXP (Leiden University Medical Center, Leiden, the Netherlands) and custom-made software for further analysis of the aorta velocity-time curves[5]. Aortic PWV was calculated by dividing the distance between ascending and abdominal aorta by the transit time of the onset of the systolic wave front.

Myocardial Tissue Characteristics

Myocardial triglyceride content was measured by proton-magnetic resonance spectroscopy (^{1}H-MRS), in a voxel of 40x15x25 mm^3 in the mid-ventricular septum, with ECG-triggering, using a respiratory navigator. Typical parameters were: TE 35 ms, TR 3.5 or 9 seconds (water-suppressed and non-water suppressed acquisition, respectively), acquired samples 2048 (spectral resolution 0.73 Hz/sample), number of signal averages 64 or 6 (water-suppressed and non-water suppressed acquisition, respectively). The signal-to-noise ratio was increased by a high permittivity pad on the thorax[6]. The myocardial triglyceride content was quantified using in-house developed software to assess the individual 64 water-suppressed and 6 non-water suppressed signals and the Java-based MR user interface (jMRUI v5.0; MRUI Consortium) to fit the averaged signal[7,8]. Prior knowledge for the fit included the following starting values: triglyceride methyl (CH_3) 0.9 ppm, triglyceride methylene $(CH_2)^n$ 1.3 ppm, $COO\text{-}CH_2$ 2.05 ppm, creatine 3.05 ppm, trimethylamines (TMA) 3.25 ppm. Myocardial lipid-to-water ratios were calculated as the signal of triglyceride methylene divided by the unsuppressed water signal, multiplied by 100%[9].

The extracellular volume (ECV) as a measure of myocardial diffuse fibrosis was determined using native and post-contrast T1 mapping (5s(3s)3s and 4s(1s)3s(1s)2s modified Look-Locker inversion recovery (MOLLI) scheme, respectively), obtained in short-axis orientation at the mid-ventricular level. Post-contrast T1 mapping was acquired 20-25 minutes after contrast administration. Typical imaging parameters were: TE/TR 1.1/2.3 ms, FA 20°, FOV 350x300 mm^2, slice thickness 8 mm, acquired voxel size 2.1x2.1 mm^2,

SENSE factor 2. ECV and T1 relaxation times were obtained using [blinded], in the mid-ventricular septum, after manual correction for motion of the T1 images

Late gadolinium enhanced (LGE) MRI was acquired 15-20 minutes after contrast administration, with an ECG-triggered 3D whole-heart gradient-echo phase-sensitive inversion recovery sequence, with respiratory navigating, as previously described[10]. LGE MRI was assessed visually by an experienced radiologist and a clinical investigator.

Power calculation

The power calculation for LV diastolic and systolic function, based on previous data on MRI-derived LV function in type 2 diabetes[11], showed that a total of 25 patients in each group would be needed to detect a change upon liraglutide of approximately 15-20% and 10-20% in diastolic and systolic function parameters, respectively, with at least 90% power (α=0.05) and estimated drop-out rate of 10%. Post-hoc power calculation for the secondary endpoints demonstrated a power of at least 90% (α=0.05) to detect an absolute change upon liraglutide of 0.40% in myocardial triglyceride content, 2.5% in ECV and 2.5 m/s in aortic PWV.

Missing data

At baseline, the number of missing values in the liraglutide group was: n=1 for LV diastolic function, and in the placebo group: n=1 for LV diastolic function and myocardial tissue characteristics. For the assessment of the difference between baseline and follow-up, the number of missing values in the liraglutide group was: n=3 for E, A, E/A ratio, Ea and LV filling pressure (n=1 excluded because of mitral valve stenosis, n=2 missing due to a heart rate of ≥100 bpm with fusion of the E/A peak at follow-up), n=4 for Edec peak (n=1 missing due to a heart rate of 96 bpm with partial fusion of the E/A peak at follow-up), n=1 for myocardial triglyceride content (excluded due to insufficient quality), and in the placebo group: n=1 for LV diastolic function (excluded because of mitral valve stenosis) and peak ejection rate (missing due to imaging time constraints), n=1 for myocardial triglyceride content (excluded due to ischemic scarring of the septum) and native T1 (excluded due to insufficient quality), n=2 for post-contrast T1 and extracellular volume (missing due to imaging time constraints). In both the liraglutide and placebo group n=1 was missing for study drug compliance.

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