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Effect of liraglutide on body weight and microvascular function in non-diabetic overweight women with coronary microvascular dysfunction

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ABSTRACT

Background: Coronary microvascular dysfunction (CMD) is associated with adverse cardiovascular outcomes and CMD is a hallmark of type 2 diabetes. Liraglutide improves cardiovascular prognosis through partly unknown mechanisms. We hypothesized that treatment with liraglutide improves CMD and symptoms through weight loss, in non-diabetic overweight patients with angina and no obstructive coronary artery disease (CAD).

Methods: We included 33 non-diabetic overweight women (BMI > 25) with CMD (Coronary flow velocity reserve (CFVR) ≤2.5), angina symptoms and no obstructive CAD, in an open-label proof-of-concept study. The protocol included a control period of 5 weeks followed by an intervention period with liraglutide aiming at 3 mg daily for 12 weeks. Participants were investigated before and after the control period and again 1–2 weeks after last liraglutide dose. Primary outcomes were change in CFVR and change in angina symptoms measured by the Seattle Angina Questionnaire (SAQ) in the intervention period compared with the control period. (clinicaltrials.gov, NCT02602600, and ethically approved).

Results: Twenty-nine participants completed the study. Liraglutide treatment led to a significant weight loss (mean 6.03 kg (95%CI: 5.22;6.84)) and decrease in systolic blood pressure (mean 10.95 mm Hg (95%CI: 4.60;17.30)). Baseline median CFVR was 2.30 (IQR 1.91;2.51) and remained unchanged after liraglutide treatment (mean change 0.07 (95%CI: −0.07;0.21)). There were no effects on symptoms measured by SAQ or parameters of left ventricular systolic as well as diastolic function.

Conclusions: Treatment with liraglutide led to significant weight loss and lowering of blood pressure with no concomitant symptoms alleviation during treatment and no improvement in coronary microvascular function.

1. Introduction

Coronary microvascular dysfunction (CMD) and microvascular angina are associated with impaired prognosis in terms of adverse cardiovascular outcomes and continued symptoms, but no evidence-based treatment exists. Microvascular dysfunction is a hallmark of diabetes [1], and treatment with the GLP-1 receptor agonist, liraglutide, improves cardiovascular prognosis in patients with type 2 diabetes through partly unknown mechanisms [2]. Treatment with liraglutide leads to weight loss which per se has previously been associated with improvement in coronary microvascular function [3–7]. We speculated that the cardiovascular effect of liraglutide may be mediated through weight loss and improvement of coronary microvascular function and we tested this hypothesis in a study of overweight women with angina and CMD but without obstructive coronary artery disease (CAD).

2. Methods

2.1. Study design

The study was an open-label study. Participants were recruited for a 4–6 weeks observational period, followed by an interventional period with liraglutide treatment for 11–13 weeks (Fig. 1). The observational period provided an estimate of the variance of repeated measurements of coronary microvascular function and served as control for possible

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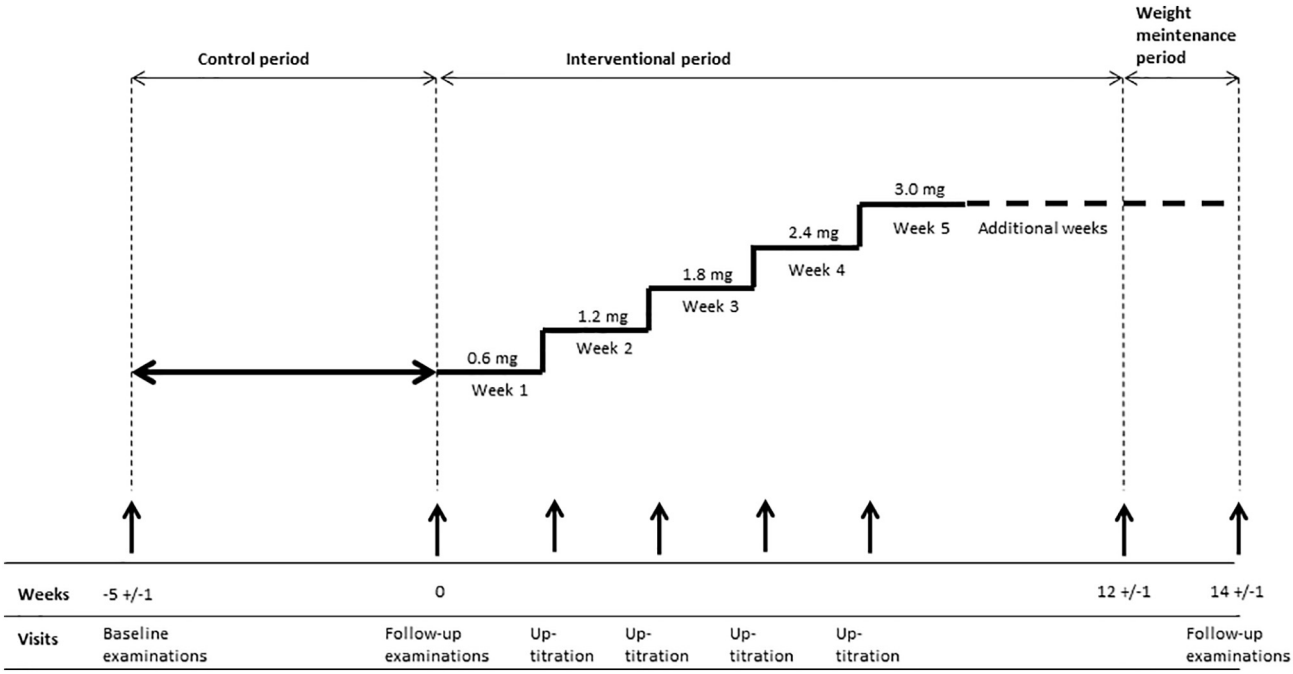

Fig. 1. Illustration of study design, including a control period followed by an interventional period with weekly uptitration in liraglutide dose. Final outcome measurements were performed after a weight maintenance period of 1-2 weeks after last liraglutide dose.

behavioural changes among the individuals upon inclusion in a trial. The study was investigator initiated and had no industry sponsorship.

2.2. Study population

Patients were recruited from the iPOWER (ImProve diagnOsis and treatment of Women with angina pEctoris and micRovessel disease) study cohort. Briefly, the iPOWER study comprises women with angina like symptoms and no obstructive CAD defined as <50% coronary artery stenosis assessed by invasive CAG in eastern Denmark. Inclusion and exclusion criteria in the iPOWER study have been described in detail previously [8,9]. Participants had no history of myocardial infarction, valvular- or congenital heart disease. Participants with systolic heart failure (left ventricular ejection fraction, LVEF <45%) or severe pulmonary disease (FEV_1 <50% or uncontrolled asthma) were excluded. Further exclusion criteria in the present trial were diabetes mellitus, moderate/severe renal disease (eGFR <30), hepatic co-morbidity, atrial fibrillation, chronic or previous acute pancreatitis, inflammatory bowel disease, history of thyroid adenoma/carcinoma or participation in other clinical trial if relevant for the present study (Fig. 2).

Participants from the iPOWER cohort with CMD (defined as coronary flow velocity reserve, CFVR, ≤2.5) were invited to participate. CFVR was re-assessed at study baseline examination and women with CFVR >2.5, were excluded.

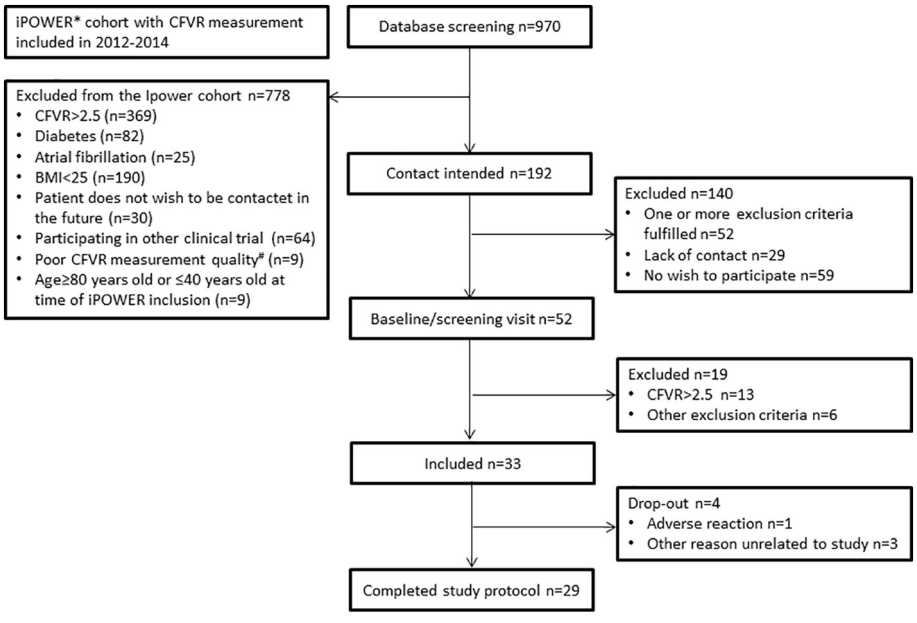

Fig. 2. Inclusion flow chart.

2.3. Intervention

Participants were treated with the GLP-1 analogue, liraglutide (Novo Nordisk A/S, Bagsvaerd, Denmark) aiming at a maximum dose of 3 mg administered once daily for 12 weeks. The liraglutide dose was titrated from 0.6 to 3 mg and increased by 0.6 mg on a weekly basis unless a longer up-titration period was necessary due to side effects. Concurrently, participants were encouraged to increase daily physical activity and reduce caloric intake by 500 kcal/day, as has been the standard in previous liraglutide trials aiming at loss of body weight [10,11]. We aimed at a weight loss of 5% and body weight was measured at each up-titration visit to monitor responses to treatment. CFVR measurements are highly dependent on heart rate [12]. During induction of a catabolic state through fasting, an increase in heart rate and resting metabolic state is seen [13]. Further, liraglutide causes increase in heart rate [14]. To avoid influence of physiological changes during fasting and weight loss at the time of final outcome measurements, the participants went through a 1–2 weeks weight maintenance period without liraglutide before the final assessment of CFVR. This design was adapted from a previous weight loss study, showing effect on coronary microvascular function after 12 weeks of intervention [15,16].

2.4. Endpoints

Primary endpoints were change in coronary microvascular function, measured as CFVR, and change in burden of angina symptoms. Secondary endpoints included changes in cardiac function, hemodynamic parameters, body weight, body composition, biochemistry and change in peripheral endothelial function (see Appendix A).

2.5. Examinations

Assessment included clinical and demographic data (age, body mass index (BMI), hypertension, smoking, medication and family history of cardiovascular disease). Blood pressure and heart rate measures were obtained after 5 min rest. Fasting blood samples were analysed (haemoglobin, HbA1c, creatinine level, eGFR and cholesterol) and a urine sample was analysed for albumin creatinine ratio.

Burden of symptoms was measured by the Seattle Angina Questionnaire, which evaluates 5 dimensions of functional status: physical limitation, angina stability, angina frequency, treatment satisfaction and disease perception. Completion of the questionnaire was done at baseline and after each period. A score between 0 and 100 was calculated for each dimension of the questionnaire, and a high score equalled a higher functional status [17].

2.5.1. Echocardiographic examination

Participants underwent a standard resting transthoracic echocardiography using GE Healthcare Vivid E9 cardiovascular ultrasound system (GE Healthcare, Horten, Norway) with a 1.3–4.0 MHz transducer (GE Vivid 5S probe). Images were stored for off-line analysis (GE EchoPAC v.112, Norway). The same experienced echocardiographer performed all image acquisitions.

2.5.1.1. LVEF, GLS and parameters of diastolic function. We acquired 2-dimensional images of the left ventricle (LV) in apical long axis, 2- and 4-chamber views at frame rates between 60 and 90 frames/s adjusted as close to the patient's heart rate as possible. Global longitudinal strain (GLS) was measured using software for speckle tracking analysis (Q-analysis, GE EchoPAC v.112, Norway). Aortic valve closure was defined in tissue Doppler M-mode. GLS was calculated as the average of all accepted segmental values of peak systolic strain. [18] Only 3 discarded segments were permitted.

LVEF was analysed as a semi-automated biplane calculation (Auto-EF tool, GE EchoPAC v.112, Norway). Measurements of left ventricular internal dimensions, left ventricle mass index and left atrium volume index by the Volume Method of Discs were performed and calculated according to European and American recommendations. [19,20] Echocardiographic parameters of diastolic function were assessed at rest. Mitral inflow velocities, the E/A ratio, and tissue Doppler of an average of late diastolic velocities in the mitral annulus, e′, were used as surrogate markers of diastolic relaxation and left ventricular compliance; deceleration time as a surrogate of early left ventricular stiffness; and E/e′ as a surrogate estimate of left ventricular filling pressures [21].

2.5.1.2. Adenosine stress examination: CFVR and LV contractile reserve. Coronary flow velocities (CFV) were measured by transthoracic Doppler echocardiography (TTDE) of the left anterior descending artery (LAD) during rest and adenosine infusion (0.14 mg/kg) over 6 min using a 2.7–8 MHz transducer (GE Vivid 6S probe) as previously described. [9,22] The primary endpoint, CFVR, was calculated as the ratio of peak diastolic CFV during adenosine induced hyperemia and rest. Two experts, blinded to participant data, analysed every CFVR examination independently. The first reading was used, except for estimates that differed by >0.2, in which case the 2 analysers reanalysed the CFVR examination and reached agreement. We have previously reported good inter-analyser and intra-observer reproducibility of CFVR [23]. Two-dimensional images of the LV in apical long axis, 2- and 4-chamber views were acquired at hyperaemia. Strain and LVEF measurements were performed as described above. Contractile reserve parameters included absolute increases of GLS (ΔGLS) and LVEF (ΔLVEF) from rest to peak hyperaemia.

Before examinations, participants were instructed to be abstinent from caffeine and food containing significant amount of methylexanthine (coffee, tea, chocolate, cola and banana) and tobacco for 24 h. Medication containing dipyridamole was paused for 48 h, anti-ischemic agents (long-lasting nitroglycerine, beta-blockers, calcium antagonist, ivabradine etc.), anti-hypertensive medication and diuretics for 24 h and short-lasting nitroglycerine for one hour before the examination.

2.5.2. Flow mediated dilation

The flow mediated dilation (FMD) method was found to be an imprecise marker of vascular endothelial function, confirming results from previous work done by our study group [24]. For a detailed description of the method and results, please see Appendix A.

2.5.3. Weight and body composition

Waist circumference was measured halfway between the lower rib and the iliac crest. To estimate body fat mass and fat free mass (FFM) a whole-body dual X-ray absorptiometry scan (GE Lunar iDXA Forma Version 15, GE Lunar Corp, Madison, WI, USA) was performed.

2.6. Compliance

Compliance was evaluated at each up-titration visit and thereafter by regular compliance telephone calls. Injection pens were returned, and any residue was evaluated against expected residue. If <70% of planned doses were taken participants would be withdrawn from the study.

2.7. Statistical analyses

Sample size was estimated prior to study commencement for the two primary outcome variables: 33 participants should be included based on the ability to detect a 0.3 change in CFVR with a power of 90% and a two-sided significance level of 0.05, including an anticipated 20% dropout rate. Strict intention-to-treat analysis was not possible due to missing outcome data on participants who dropped out.

An unstructured linear mixed model (xtmixed in stata) with splines was used to analyse change in slope for outcome measurements in the interventional period compared with the control period (Table 2). Multivariable adjustment with baseline CFVR measurements, change in resting systolic blood pressure and resting heart rate was performed on CFVR. Model control was performed and assumptions of linearity, variance homogeneity, and Gaussian distribution of residuals were tested.

Continuous variables with a Gaussian distribution are expressed as mean ± standard deviation (SD), and continuous variables with a non-Gaussian distribution as median ± interquartile range (IQR). A two-sided *p*-value below 0.05 was considered significant. All analyses were performed using STATA/IC 13.1 (StataCorp LP, College Station, Texas, USA).

2.8. Approvals

This study was performed in accordance with the Helsinki Declaration and was approved by the Danish Regional Committee on Biomedical Research Ethics (H-15010282) and the Danish Health Authority and registered at clinicaltrials.gov (NCT02602600). All participants gave written informed consent, after receiving oral and written information about the study.

3. Results

3.1. Population

We included 33 women between November 19th 2015 and December 13th 2016 and 29 completed the study. Last participant follow-up examination was performed April 18th 2017. Four participants dropped out and final outcome measures were missing: one due to side-effects, three due to reasons unrelated to the study (Fig. 2). Median (IQR) age for the included participants was 67 (62;72) years and baseline median CFVR (IQR) was 2.30 (1.91;2.51). Baseline mean (SD) body weight was 88 (15) kg, median (IQR) BMI was 31 (28;34) and mean (SD) waist circumference was 106.5 (13) cm. Baseline data are displayed in Table 1. The participants completing the study and the four that dropped out did not differ in age, baseline CFVR or BMI (data not shown).

Approximately 60% of participants had a family history of ischemic heart disease, 45% were ex-smokers and 12% were active smokers. Most participants were in NYHA class 2 and experienced weekly chest pain/discomfort. Approximately 40% were taking beta-blockers and statins, and around 20% were taking aspirin, calcium antagonists, angiotensin-converting enzyme inhibitors and angiotensin II antagonists (Table 1).

3.2. Up titration and adherence to project medication

Mean treatment duration was 12 weeks (range 11.3–14.1 weeks). Sixteen participants (55%) reached the target dose of 3 mg and mean

Table 1
Baseline characteristics.

N	33
Age, median(IQR)	67 (62;72)
CVD risk factors	
Family history of IHD, n (%)	20 (61)
Active smoker, n (%)	4 (12)
Previous smoker, n (%)	15 (45)
Symptoms	
NYHA 1, n (%)	11 (33)
NYHA 2, n (%)	19 (58)
NYHA 3, n (%)	3 (9)
Chest pain/discomfort daily, n (%)	5 (16)
Chest pain/discomfort weekly, n (%)	15 (47)
Chest pain/discomfort monthly, n (%)	12 (38)
Medication	
ASA, n (%)	9 (29)
Beta blocker, n (%)	12 (39)
Calcium antagonist, n (%)	6 (19)
ACE-Inhibitor, n (%)	7 (23)
Angiotensin-II antagonist, n (%)	6 (19)
Statin, n (%)	13 (42)

IHD ischemic Heart Disease, NYHA New York Heart Association.

treatment duration on target dose was 6.5 weeks. Maximum dosage reached was 2.4 mg in 3 patients, 1.8 mg in 9 and 1.2 mg in one patient. Compliance was good with a mean of 99.2% (min. 87.8%; max. 100%) of all doses taken as planned.

3.3. Effect on body composition, hemodynamic variables and laboratory parameters

There was no significant change in weight over the control period (mean 0.55 kg (CI −0.23;1.32)) whereas a mean weight loss of 6.03 kg (CI 5.22;6.84) was achieved after liraglutide treatment, corresponding to approximately 7% (max 15%, min 1%) of initial body weight. Ten participants achieved weight loss ≥7%. Weight loss after liraglutide treatment led to a mean loss of total body fat mass of 4.16 kg (CI 3.37;4.94) and a mean loss of total body fat-free mass of 0.99 kg (CI 0.61;1.37). Systolic and diastolic blood pressure decreased significantly after treatment while heart rate remained unchanged. Liraglutide led to a significant mean decrease in HbA1c of 1.54 mmol/mol (CI 0.84;2.25), in total cholesterol of 0.36 mmol/L (CI 0.16;0.56) and in LDL-cholesterol of 0.24 mmol/L (CI 0.08;0.41) (Table 2).

3.4. Effect on cardiac function

Despite a significant weight loss and improvement in blood pressure, the primary outcome measure, CFVR, did not change significantly after liraglutide treatment. Mean change in the control period was 0.11 (SD 0.44) and mean change in the intervention period was 0.07 (SD 0.34). Multivariable adjustment including age, baseline CFVR, changes in systolic blood pressure and baseline resting heart rate had no impact on change in CFVR. Change in CFVR remained insignificant when the six participants that did not achieve weight loss >5% were excluded from the analysis. There was no effect of treatment with liraglutide on parameters of LV systolic and diastolic function assessed by TTE (Table 2).

3.5. Effect on symptoms

Physical limitation score, angina stability score, angina frequency score and treatment satisfaction score improved significantly after treatment with liraglutide ($p < 0.05$) whereas disease perception score increased significantly in the control period but not after treatment with liraglutide. Only the physical limitation score improved significantly after treatment compared with the control period (Table 2).

3.6. Side effects

The proportion of participants experiencing an event categorised as either an adverse event or adverse reaction (common side effect) was 59% and 83% respectively. None of the participants experienced a serious adverse event. The most common adverse reactions are presented in Appendix B.

4. Discussion

For this study of overweight non-diabetic women with angina symptoms and CMD, we hypothesized that liraglutide would have an effect on cardiac function mediated through direct actions on the heart or via indirect effects on cardiovascular risk factors through weight loss. However, although liraglutide treatment led to significant weight loss, reduction in blood pressure and had beneficial effects on serum lipids, there was no concomitant improvement in coronary microvascular function or symptom alleviation 1–2 weeks after the termination of liraglutide therapy. Because participants were not on liraglutide at the final CFVR measurement, we may have missed a direct effect of liraglutide treatment on coronary microvascular function.

4.1. Coronary microvascular function

Obesity and type 2 diabetes are associated with coronary microvascular dysfunction [4,9,25] and low CFVR as a measure of CMD is associated with adverse outcomes in patients with and without diabetes [26,27]. CMD reflects an impaired vasodilator reserve of the small coronary arteries in response to increased oxygen demand. CMD can occur in the context of a specific cardiac or systemic disease or independently of any other known disease [28]. It has been hypothesized that comorbidities known to be associated with CMD (obesity, diabetes, hypertension) lead to systemic inflammation and coronary endothelial inflammation causing CMD. Indeed, low-grade inflammation has been associated with CMD in several studies [25,29–31]. Various methods exist for assessment of CMD and we used TTDSE to measure increase in flow in response to pharmacological stress, which is an easily accessible method that has been validated in previous studies. Both endothelial independent- and dependent pathways may lead to CMD, and by using adenosine to induce hyperaemia, we examined the primarily endothelial independent aspect of microvascular function [32].

In the LEADER study prolonged treatment with the GLP-1 agonist liraglutide resulted in reduced cardiovascular morbidity and mortality in patients with type 2 diabetes at high risk of cardiovascular events [2]. The mechanisms behind these effects are not well understood. It has been speculated that slower progression of atherosclerosis may be an explanation due to delayed separation of Kaplan-Meier survival curves (>12 months for CV death and >18 months for all-cause deaths and hospitalization for heart failure) [2,33]. In support of this hypothesis, it has been demonstrated that liraglutide therapy leads to reduction in carotid intima media thickness (cIMT), which is a known marker of atherosclerosis, after a few months treatment [34]. CMD may be observed prior to development of macrovascular atherosclerosis [35,36], thus investigating the effect of GLP-1 in CMD is highly relevant. Endothelial cells and vascular smooth muscle cells seem to be targets for GLP-1 action [37]. Inflammation of the vascular wall may precipitate and accelerate atherosclerosis, and an anti-inflammatory effect of GLP-1 [38–40], and an improvement of peripheral endothelial function [41,42] in patients with diabetes has been observed. The effect of GLP-1 on myocardial function has previously been investigated [7,43,44] but only few studies have examined the effect on the coronary microcirculation. An open study examined the effect of exenatide for 12 weeks in patients with newly diagnosed type 2 diabetes compared with a control group and found improved microvascular function measured by TTDE CFVR [45]. In a cross-over study of 20 patients with type 2 diabetes, 10 weeks of treatment with liraglutide 1.2 mg/day led to a small

Table 2
Effect on outcome parameters.

	Baseline N = 33		Control period N = 33			Intervention period N = 29			
	Mean/median	SD/IQR	Delta[#]	CI	P	Delta[#]	CI	*p*	*p*[*]
Effect on hemodynamic parameters and body weight									
Weight (kg)	88.0	15.0	0.55	−0.23;1.32	0.17	−6.03	−6.84;−5.22	<0.001	<0.001
Waist circumference (cm)	106.5	13	−1.0	−2.35;0.35	0.15	−7.1	−8.50;−5.66	<0.001	<0.001
BMI	31	28;34	0.2	−0.08;0.47	0.16	−2.2	−2.50;−1.90	<0.001	<0.001
BP systolic (mm Hg)	146	21	0.36	−5.71;6.44	0.91	−10.95	−17.30;−4.60	<0.001	0.57
BP diastolic (mm Hg)	73	7	0.24	−2.38;2.86	0.86	−4.29	−7.03;−1.56	<0.001	0.47
Heart rate (beats/min)	62	10	−0.47	−2.86;1.93	0.70	−0.71	−3.16;1.75	0.57	0.69
Effect on body composition measured by DEXA scan									
Total fat-mass (kg)	40,90	9,35				−4.16	−4.94;−3.37	<0.001	
Total fat-free mass (kg)	43,79	6,15				−0.99	−1.37;−0.61	<0.001	
Effect on laboratory parameters									
Hgb (mmol/L)	8.30	0.56	−0.10	−0.26;0.06	0.23	−0.12	−0.29;−0.04	0.15	0.86
Creatinine (μmol/L)	72.58	20.64	−0.67	−3.35;2.02	0.63	1.83	−0.99;4.65	0.20	0.66
eGFR (mL/min)	74.18	17.25	0.24	−2.01;2.50	0.83	−1.79	−4.15;0.58	0.14	0.52
HbA1c (mmol/mol)	36.39	3.68	−0.33	−1.01;0.34	0.33	−1.54	−2.25;−0.84	<0.001	0.16
Total cholesterol (mmol/L)	5.08	1.15	0.12	−0.07;0.30	0.23	−0.36	−0.56;−0.16	<0.001	0.01
HDL (mmol/L)	1.75	0.43	0.05	−0.06;0.15	0.38	−0.09	−0.20;0.02	0.10	0.16
LDL (mmol/L)	2.81	1.11	0.07	−0.09;0.23	0.39	−0.24	−0.41;−0.08	<0.001	0.11
VLDL (mmol/L)	0.52	0.26	−0.01	−0.07;0.04	0.65	−0.02	−0.07;0.04	0.53	0.32
Triglycerides (mmol/L)	1.15	0.58	−0.01	−0.12;0.10	0.81	−0.07	−0.18;0.05	0.25	0.21
Urin alb/crea ratio ($\times 10^{-3}$)	20.79	28.89	−2.14	−12.02;7.74	0.67	−8.04	−18.43;2.36	0.13	0.48
Mean change in echocardiographic parameters in the control period and interventional period respectively									
CFVR	2.30	1.91;2.51	0.11	−0.02;0.25	0.10	0.07	−0.07;0.21	0.33	0.58
CFV at rest (m/s)	0.25	0.21;0.30	0.00	−0.01;0.02	0.60	−0.01	−0.02;0.01	0.53	0.61
CFV at hyperemia (m/s)	0.56	0.49;0.67	0.04	0.00;0.08	0.06	0.01	−0.04;0.05	0.73	0.32
LVEF rest (%)	62.6	2.7	0.14	−1.22;1.49	0.85	0.77	−0.62;2.16	0.28	0.92
LVEF stress (%)	63.5	4.0	0.95	−0.70;2.61	0.26	0.62	−1.11;2.34	0.48	0.88
GLS end systolic at rest (%)	22.06	1.73	−0.45	−1.18;0.28	0.23	0.14	−0.61;0.90	0.71	0.36
GLS end systolic at hyperemia (%)	22.83	3.11	0.10	−1.03;1.23	0.87	−0.04	−1.22;1.14	0.95	0.69
Delta GLS (%)	0.82	3.04	0.31	−0.77;1.40	0.58	0.03	−1.10;1.17	0.95	0.84
LVMI (mg/m^2)	68.16	12.65	−0.54	−3.74;2.66	0.74	0.94	−2.41;4.29	0.58	0.58
LAI (mL/m^2)	26.47	4.69	1.15	−0.29;2.59	0.12	−0.01	−1.51;1.50	0.99	0.11
Deceleration time (ms)	195.82	176.65;209.63	−10.80	−21.97;0.38	0.06	−1.66	−13.21;9.90	0.78	0.12
E/A ratio	0.95	0.23	−0.01	−0.07;0.06	0.86	0.04	−0.02;0.11	0.19	0.71
e′ (cm/s)	7.02	6.34;8.11	0.11	−0.25;0.47	0.54	0.21	−0.17;0.59	0.28	0.85
E/e′ ratio	9.80	7.60;11.62	−0.05	−0.72;0.61	0.87	−0.30	−0.99;0.40	0.40	0.72
Effect on angina symptoms measured by Seattle Angina Questionnaire									
Physical limitation score	71.96	22.5	−2.26	−6.34;1.83	0.28	7.78	3.41;12.12	<0.001	0.03
Angina stability score	58.75	28.71	−9.29	−20.61;2.03	0.11	26.60	14.85;38.35	<0.001	0.07
Angina frequency score	78.48	21.95	−1.38	−6.10;3.33	0.57	8.48	3.52;13.44	<0.001	0.19
Treatment satisfaction score	75.72	23.82	2.68	−5.12;10.47	0.50	9.18	1.08;17.28	0.03	0.63
Disease perception score	57.83	21.24	8.11	3.16;13.06	<0.001	1.82	−3.33;6.97	0.49	0.11

Baseline measurements and estimated effects after the control- and interventional period.
BMI body mass index, BP blood pressure, HDL high density lipoprotein, LDL low density lipoprotein, VLDL very low density lipoprotein, CFVR coronary flow velocity reserve, CFV coronary flow velocity, LVEF left ventricular ejection fraction, GLS global longitudinal strain. LVMI left ventricular myocardial index, LAI left atrial index.
[#] Change in the control period or interventional period.
[*] Compared with the control period.

insignificant improvement in coronary microcirculation measured by TTDE CFVR [7]. We found no improvement in CMD after liraglutide treatment in overweight women with lower CFVR at baseline than the above mentioned studies, when investigated after 12 weeks treatment, but 1–2 weeks after last injection of liraglutide. However, our patients did not have diabetes and were observed after termination of liraglutide treatment which may explain the discrepancy between these studies. Thus, again we examined an indirect effect of weight loss induced by liraglutide and may have missed a direct effect. However, in line with our study a recently published randomized, placebo-controlled study of 36 patients with heart failure and no diabetes found no effect of treatment with liraglutide for 24 weeks on myocardial flow reserve assessed by PET [44].

CMD is associated with obesity [4,25,46] and previous studies have assessed the effect of weight loss on CFVR. Massive weight loss (37–50% of body weight) following gastric by-pass operation resulted in pronounced improvement in CFVR (49–56% increase in CFVR) [5,6,47]. A non-randomized study of a dietary intervention combined with exercise inducing a 10% weight loss in 40 obese women resulted in 22% increase in CFVR measured by TTDE [4]. In the CutIT trial [3] of overweight patients with ischemic heart disease, a mean weight loss of 10.6% after a low energy diet was associated with a 16% increased CFVR measured by TTDE. Thus, previous studies indicate that a weight loss of minimum 10% is associated with significant improvements in CFVR. However, in a study by Nielsen et al. there was no improvement in microvascular function after a modest weight loss in overweight patients with heart failure [44]. We achieved a mean weight loss of 7%, thus it is possible that a greater weight loss would have resulted in improvement in microvascular function.

We found no improvement in systolic or diastolic cardiac function after treatment with liraglutide. Small trials of prolonged treatment or infusion of GLP1 receptor agonists have given conflicting results regarding the effect on cardiac function [43,48,49]. Large randomized controlled clinical trials with albiglutide or liraglutide in patients with heart failure with or without diabetes have also failed to demonstrate

beneficial effects of prolonged treatment on cardiac function [50,51]. Thus, results are conflicting but the majority of evidence does not support an effect on cardiac function in patients without diabetes.

4.2. Angina symptoms

To our knowledge the effect of liraglutide on angina symptoms has not previously been examined. Our patient group had angina symptoms despite absence of macrovascular coronary stenosis. Microvascular dysfunction is the current explanation for angina in this patient group. Although some improvement was found in SAQ subscales following liraglutide treatment these findings were not significantly different from what was observed in the control period. Thus, neither weight loss nor improvement in lipids or reduction in blood pressure following liraglutide had any impact on patients' functional status. In terms of baseline symptom burden our study population was comparable with previous studies of stable angina with CAD [52].

4.3. Strengths and limitations

We achieved good compliance and drop-out rate was small. Overall, patients achieved the desired weight loss and exclusion of the few participants that did not reach 5% loss of body weight had no impact on change in CFVR. Median CFVR was 2.30 (IQR 1.91; 2.51) and it is possible that an effect would have been seen in patients with more severe CMD. The study was not powered for sub-group analyses; thus, we did not examine whether participants with the lowest baseline CFVR or participants with the highest weight loss had effect on CFVR after treatment. We found no significant effect on angina symptoms measured by SAQ. A possible drawback of the SAQ is that it evaluates chest pain/chest tightness/angina pectoris and many women with microvascular angina may experience angina equivalents such as dyspnoea, fatigue, indigestion and weakness. Therefore, we may not have been able to detect an effect on their symptoms.

We examined the effect of liraglutide on CMD measuring CFVR non-invasively by TTDE. This method has proven to be reproducible in previous studies and has advantages over PET since radiation is avoided [23,53]. As seen from the relatively narrow confidence intervals on mean change in CFVR it is unlikely that we have missed an effect because of inaccurate measurements.

An obvious limitation is that this study is not randomized and placebo-controlled. With the aim of weight loss, a randomized cross-over design was impossible. A parallel design was an option, but placebo medication could not be provided by the pharmaceutical company. However, we had a control period of 4–6 weeks prior to the interventional period, and by performing repeated measurements the sample size could be reduced by almost half. The study was considered a preliminary proof-of-concept study and in case of a positive result the plan was to follow up with a placebo-controlled randomized study.

Another limitation is that the participants were investigated 1–2 weeks after the last injection of liraglutide to avoid a catabolic state at the time of last CFVR examination, and therefore a possible direct effect of liraglutide on coronary microvascular function was not evaluated. Results indicate that 12 weeks treatment with high doses of liraglutide, with the aim of weight loss, had no indirect effect on microvascular function or cardiac function and there was no effect on angina symptoms during the treatment.

5. Conclusion

Treatment with liraglutide in patients with angina symptoms and impaired coronary microvascular function led to significant weight loss, improvement in blood lipids and lowering of blood pressure but had no effect on coronary microvascular function, angina symptoms or measures of left ventricular systolic and diastolic function. The present study does not support an indirect effect of liraglutide on coronary microvascular function. However, we cannot rule out a direct effect of liraglutide on coronary microvascular function.

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Conflicts of interest

The authors report no relationships that could be construed as a conflict of interest.

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