PLOS ONE

RESEARCH ARTICLE

Effect of ACE-inhibition on coronary microvascular function and symptoms in normotensive women with microvascular angina: A randomized placebo-controlled trial

Marie Mide Michelsen☯*, Anna Bay Rask☯, Elena Suhrs, Kristoffer Flintholm Raft, Nis Høst, Eva Prescott

Department of Cardiology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

☯ These authors contributed equally to this work.
* marie.m.michelsen@gmail.com

Check for updates

OPEN ACCESS

Citation: Michelsen MM, Rask AB, Suhrs E, Raft KF, Høst N, Prescott E (2018) Effect of ACE-inhibition on coronary microvascular function and symptoms in normotensive women with microvascular angina: A randomized placebo-controlled trial. PLoS ONE 13(6): e0196962. https://doi.org/10.1371/journal.pone.0196962

Editor: Yoshihiro Fukumoto, Kurume University School of Medicine, JAPAN

Received: January 6, 2018

Accepted: April 23, 2018

Published: June 8, 2018

Copyright: © 2018 Michelsen et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All data are available in the main manuscript and supplementary files.

Funding: This work was supported by the Danish Heart foundation, Copenhagen University, Bispebjerg and Frederiksberg University Hospital. The grants described are for staffing and related expenses and not specifically for this study. No grant numbers allocated.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Objective

Studies have suggested a beneficial effect of angiotensin-converting enzyme (ACE) inhibition. To explore whether the ACE inhibitor ramipril has a direct effect on the microvasculature beyond the blood pressure (BP) lowering effect, we investigated whether ramipril improved coronary microvascular function in normotensive women with coronary microvascular dysfunction (CMD).

Methods

We included 63 normotensive women with angina, no epicardial stenosis>50% and CMD defined as a coronary flow velocity reserve (CFVR)<2.2 assessed by adenosine stress-echocardiography in a randomized double-blinded, superiority trial with 1:1 allocation to placebo or ramipril (maximum dose 10 mg depending on blood pressure) for 24±6 weeks. Primary outcome was CFVR. Secondary outcomes were left ventricular systolic and diastolic function and symptoms evaluated by Seattle Angina Questionnaire (clinicaltrials.gov, NCT02525081).

Results

Follow-up was available on 55 patients. BP remained unchanged during treatment in both groups. CFVR improved in both the ramipril ($p = 0.004$) and placebo group ($p = 0.026$) with no difference between groups ($p = 0.63$). Symptoms improved in both groups with no significant between-group differences. No changes were detected in parameters of systolic and diastolic function. No serious adverse reactions were reported.

Conclusions

In normotensive women with angina and CMD, treatment with ramipril had no significant effect on CFVR or symptoms compared with placebo. The effect of ACE inhibition previously reported may be mediated by blood pressure reduction.

Abbreviations: ACE, Angiotensin-converting enzyme; BP, Blood pressure; CAD, Coronary artery disease; CFV, Coronary flow velocity; CFVR, Coronary flow velocity reserve; CMD, Coronary microvascular dysfunction; eGFR, Estimated glomerular filtration rate; FEV1, Forced expiratory volume in 1 second; GLS, Global longitudinal strain; iPOWER, ImProve diagnOsis and treatment of Women with angina pEctoris and micRovessel disease; IQR, Interquartile range; LAI, Left atrium index; LV, Left ventricle; LVEF, Left ventricular ejection fraction; LVMI, Left ventricle mass index; SD, Standard deviation; TTDE, Transthoracic Doppler echocardiography; ΔGLS, Absolute increase of global longitudinal strain; ΔLVEF, Absolute increase of left ventricular ejection fraction.

Introduction

Angina pectoris in the absence of significant obstructive coronary artery disease (CAD) can be caused by coronary microvascular dysfunction (CMD). CMD causes insufficient coronary blood flow in situations with increased cardiac oxygen demand leading to transient ischemia and pain [1]. Up to 40% of patients with angina and no obstructive CAD have CMD [2–4], which is a predictor of poor cardiovascular prognosis [3]. However, evidence-based treatment strategies are lacking [5].

Vascular remodelling is suggested as a main pathogenic mechanism of CMD in the absence of obstructive CAD [1]. By a reduction in blood pressure, antihypertensive treatment should theoretically increase the blood flow reserve through a reduction in resting coronary flow [6]. However, evidence suggests that treatment with angiotensin-converting enzyme (ACE) inhibitor has an additional beneficial effect. Treatment has been associated with positive vascular changes beyond the antihypertensive effect and has been suggested to improve both non-endothelial and endothelial dependent CMD [7–11]. Concurrently, treatment with ACE-inhibition in patients with refractory microvascular angina has received a IIb recommendation in current guidelines [12]. However, results obtained from interventional studies on the non-endothelial aspect of CMD have been inconsistent [13–26] and whether the effect observed in some studies on coronary microvascular function could be indirectly mediated via treatment of hypertension is unclear.

In salt replete normotensive individuals without heart failure, effect of ACE inhibition on blood pressure is absent or modest [10,27–38]. Therefore, to explore whether the ACE-inhibitor ramipril had a beneficial effect on the coronary microvasculature beyond the blood pressure lowering effect, we designed a 6 months long double blind placebo-controlled study including only normotensive women. Furthermore, to study whether treatment with ACE inhibitor is indicated in patients with angina and normal blood pressure.

Methods

Study population

Normotensive women with angina pectoris, no significant obstructive CAD (<50% coronary artery stenosis) and a history of a coronary flow velocity reserve (CFVR)<2.2 assessed by transthoracic Doppler echocardiography (TTDE) with dipyridamole infusion in the iPOWER (ImProve diagnOsis and treatment of Women with angina pEctoris and micRovessel disease) cohort study were systematically invited [4,39,40]. Normal blood pressure was defined as a history of systolic blood pressure ≤150 mmHg and no current treatment for hypertension. Exclusion factors in the iPOWER cohort are described in detail in previous publications [4,39]. In short, participants had no previous history of myocardial infarction, valvular- or congenital heart disease, no severe pulmonary disease and a left ventricular ejection fraction (LVEF) above 45%. Further exclusion factors in this trial were atrial fibrillation, pacemaker, ACE inhibitor or angiotensin II-antagonist treatment, no angina symptoms within 6 months

and an estimated glomerular filtration rate (eGFR)<50 mL/min/1.73m^2. At study baseline examination (initial screening), participants with a baseline CFVR>2.5 indicating no CMD assessed by TTDE with adenosine stress or baseline systolic blood pressure >150 mmHg were excluded.

Study design

This study is a randomized placebo-controlled two-arm parallel, superiority trial with 1:1 allocation to treatment with the oral ACE inhibitor, ramipril, and an oral matching placebo, as add-on to usual treatment. After baseline measurements participants were randomized in blocks of 10 participants by the pharmacy (Glostrup Apotek, Copenhagen, Denmark). The allocation sequence was concealed in sealed opaque envelopes until the end of the study. Participants, health care providers and data collectors were blinded.

Project medication was up titrated at hospital visits to the highest dose possible according to blood pressure level and side effects following the algorithm depicted in Fig 1. In guidelines or clinical trials the maximum dose of ramipril for treatment of hypertension, heart failure and secondary prevention after myocardial infarction is 10 mg [41–43]. A trial overview is depicted in Fig 2. Kidney function was controlled at each visit by analysing blood samples for creatinine level and estimation of glomerular filtration rate (eGFR). A research assistant, who was not performing the primary endpoint measurements, was in charge of hospital visits and control of adverse events. The pre-specified primary endpoint was CFVR, which was performed by an operator, who had no other contact with the participants. The pre-specified secondary endpoints were the burden of symptoms and parameters of left ventricular systolic and diastolic function measured by TTDE. Examinations were performed at baseline and after 24±6 weeks. The project medication was discontinued 24 hours before the final measurements. After trial commencement no study changes were made.

Examinations

Assessment included clinical and demographic data. Trained health professionals interviewed participants regarding cardiovascular risk factors including age, body mass index, diabetes, hypertension, and hyperlipidaemia, smoking and family history of cardiovascular disease. Blood pressure and heart rate measures were obtained at rest as the mean of 3 measurements obtained with 3-minute intervals.

Seattle Angina Questionnaire was used to evaluate the burden of symptoms, which were 5 dimensions of functional status: physical limitation, angina stability, angina frequency, treatment satisfaction and disease perception. Participants could score between 0 and 100, and a high score equalled a higher functional status [44].

Echocardiographic examination

Participants underwent a standard resting transthoracic echocardiography (TTE) 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, who was blinded to all participant data, performed all image acquisition and analyses at Bispebjerg University Hospital (Copenhagen, Denmark). Before examinations, participants were instructed to be abstinent from caffeine and food containing a significant amount of methylexanthine (coffee, tea, chocolate, cola and banana) for 24 hours. Medication containing dipyridamole was paused for 48 hours, anti-ischemic agents (long-lasting nitroglycerine, beta-blockers, calcium antagonist, ivabradine etc.), anti-hypertensive medication including project medication and diuretics for

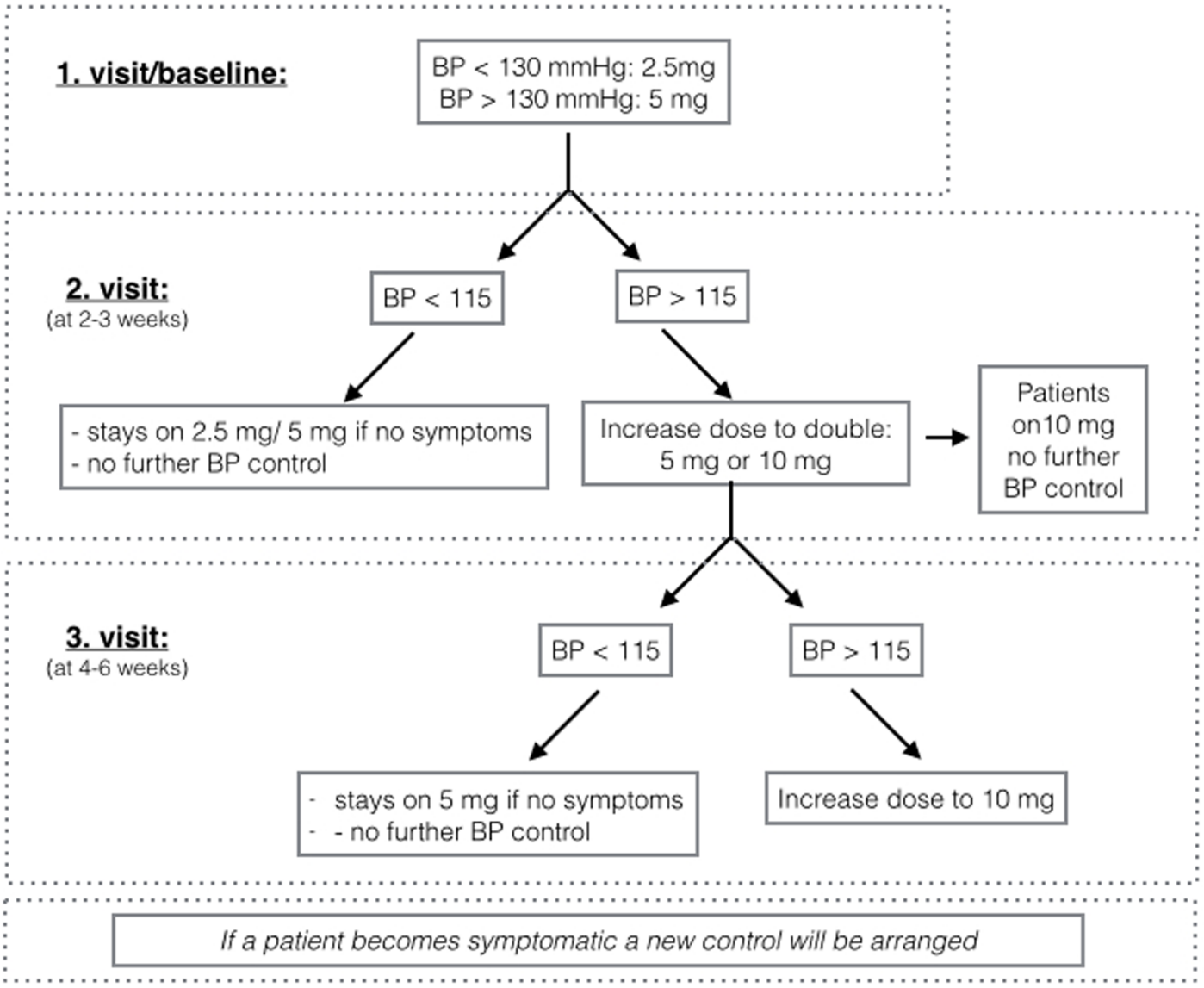

Fig 1. Up titration of project medication. Blood pressure (BP) will be controlled after each visit. If possible treatment dose (per day) is increased. If patients have adverse reactions, project medication dose will be reduced. The least acceptable dose for staying in the study is 2.5 mg per day.

https://doi.org/10.1371/journal.pone.0196962.g001

24 hours and short-lasting nitroglycerine one hour before the examination. Before the examination, abstinence of the abovementioned food and medication was confirmed.

Examination at rest: LVEF, GLS and parameters of diastolic function. We acquired 2-dimensional images of the left ventricle in apical long axis, 2- and 4-chamber views at frame rates between 60–90 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. The left ventricular endocardial border was traced in all three views, and the automatically created region of interest was manually adjusted until tracking was considered optimal. Segments were discarded if tracking was persistently poor. Subsequently, deformation parameters were automatically obtained for all accepted left ventricular segments and GLS was calculated as the

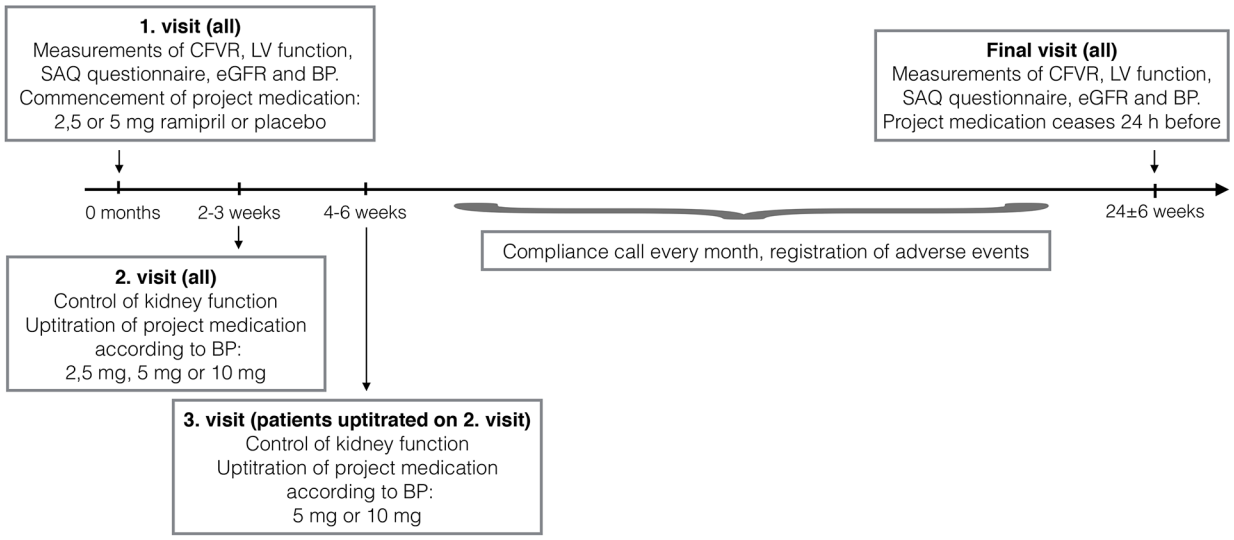

Fig 2. General study outline. CFVR: Coronary flow reserve velocity, LV: Left ventricular, SAQ: Seattle Angina Questionnaire, eGFR: Estimated glomerular filtration rate, BP: blood pressure.

https://doi.org/10.1371/journal.pone.0196962.g002

average of these [45]. We have previously reported good inter-analyser reproducibility for GLS [39]. In a sub-analysis testing robustness of results, 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 (LVMI), and left atrium volume index (LAVI) by the 'Volume Method of Discs' were performed and calculated according to European and American recommendations [46,47]. Echocardiographic parameters of diastolic function including the early (E) and late (A) mitral inflow velocities, tissue Doppler early and late diastolic velocities in the lateral mitral annulus (e' and a') and the E/A and e'/a'-ratios were used as surrogates of myocardial relaxation and left ventricular compliance, the deceleration time as a surrogate of early left ventricular stiffness and, E/e' as a surrogate estimate of left ventricular filling pressures [47]. All measures were averaged over 3 heart cycles. In case of fusion of the E and A or e' and a' waves, measurements were registered as missing.

Left ventricular filling pressure was categorized as normal or high by a modified algorithm from the recommendations of the American and European Societies of Echocardiography concerning patients with preserved left ventricular ejection fraction (LVEF), i.e. normal filling pressure if E/e'<8 or E/e' 8–12 and LAVI<34 mL/m2, and high filling pressure if E/e' 8–12 and LAVI>34 or E/e'>12, respectively [47].

All parameters were reviewed for outliers by histogram plots and pre-specified cut-offs.

Adenosine stress examination: CFVR and left ventricular contractile reserve. TTDE of the left anterior descending artery during rest and adenosine infusion (0.84 mg/kg) over 6 minutes to obtain coronary flow velocities (CFV) at baseline and at maximal hyperaemia using a 2.7–8 MHz transducer (GE Vivid 6S probe) was performed as previously described [4,40]. The primary endpoint, CFVR, was calculated as the ratio between peak diastolic CFV during hyperaemia and during rest. Two experts, blinded to participant data with no participant contact, analysed every CFVR examination independently. The first reading was used, except for estimates that differed by >0.2, in which case the two investigators reanalysed the CFVR examination and reached agreement. In our previous validation study with repeated TTDE CFVR

examinations in 10 young, healthy individuals by the same observer, we found an intraclass correlation coefficient (CI) of 0.97 (0.92; 1.00) and limits of agreement (2xSD [CI]) was 0.44 (0.21; 0.68). The test-retest properties were similar in a patient population of 10 women with angina and no obstructive CAD where we found an intraclass correlation coefficient (CI) of 0.90 (0.78; 1.02) and limits of agreement (2xSD [CI]) was 0.48 (0.22; 0.74) [48]. In a sample of 10 participants from the iPOWER study, CFVR readings for the 2 observers were highly reproducible [39]. Two-dimensional images of the left ventricle in apical long axis, 2- and 4-chamber views were acquired at hyperaemia. Strain measurements were analysed as described above. The left ventricular contractile reserve was assessed as an increase of GLS (ΔGLS) from rest to peak hyperaemia.

Compliance

A research assistant, who was not in charge of the primary endpoint, performed compliance telephone calls each month. Furthermore, we had the following information regarding compliance:

- Participants completed a diary each day indicating whether the daily medication was taken.
- Participants returned excess containers with medication at the end of the study and tablets were counted.

Compliance was defined as the ratio of project medication actually taken and the calculated amount of medication prescribed.

Statistical analyses

Prior to study commencement, sample size was estimated to be 60 participants completing the study. This was based on the detection of a 0.3 change in CFVR (which we judged as clinically relevant), a power of 90%, and a two–sided significance level of 0.05. We assumed that the SD on mean difference in CFVR would be 0.4 in the ramipril group and 0.3 for the placebo group based on previous studies [48,49]. The intention was to include 72 patients due to expected drop out during the study.

Strict intention-to-treat analysis was not possible due to missing outcomes on participants who dropped out. A linear mixed model with unstructured covariance (PROC MIXED in SAS) was used to perform analysis of the primary (CFVR) and secondary endpoints, including baseline measures on participants who dropped out (analyses were adjusted for baseline value of the outcome studied) [50]. A supplementary analysis investigating the treatment effect on the primary outcome parameter, CFVR, adjusted for resting systolic blood pressure and resting heart rate was performed. Furthermore, a subanalysis stratifying on project medication dosage prescribed was performed. This was not pre-specified in the protocol. We also performed a per protocol analysis including only participants with follow-up completed.

Model control included assumptions of linearity, variance homogeneity, and Gaussian distribution of residuals.

Continuous variables with a Gaussian distribution were 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 SAS Enterprise Guide 7.1 (SAS Institute Inc., North Carolina, USA) and STATA/IC 13.1 (StataCorp LP, College Station, Texas, USA).

Ethical standard

This study was performed in accordance with the Helsinki Declaration and was approved by the Danish Regional Committee on Biomedical Research Ethics (H-3-2014-138) and the Danish Health Authority. Informed consent was obtained from all individual participants included in the study. The clinical trial is registered as ACIM at clinicaltrials.gov (NCT02525081). Flow mediated dilation (FMD) was acquired as part of the original protocol, but data was excluded from the main manuscript due to poor quality of examinations (only 39 patients had a full FMD dataset). Methodology and results are displayed in the Supplementary material (Tables A and B in S1 File). The article is presented according to the CONSORT statement. The authors report no conflicts of interest. All authors have approved the final article.

Results

Population

A total of 201 cohort participants met the inclusion and exclusion criteria. We included 63 women between the 29th of July 2015 and the 30th of November 2015. Last participant follow-up examination was performed the 28th of April 2016. Eight participants dropped out and final outcome measures were missing as a loss to follow-up: four in the placebo group due to unrelated illness, depression, discomfort and lack of contact and four in the ramipril group due to discomfort, cough and other unrelated illness (Fig 3). Mean age (SD) for the included participants and the participants who dropped out was 58.0 (12.3) and 57.7 (9.9), respectively, and baseline CFVR (SD) was 2.15 (0.32) and 2.11 (0.22), respectively ($p>0.05$ between included participants and participants that dropped out). Baseline data are displayed in Table 1.

Uptitration and adherence to project medication

Treatment interval (SD) was 138 (20) and 145 (15) days in the placebo and ramipril group, respectively. Approximately 40% of participants were prescribed low dose ramipril (2.5mg), 20% were prescribed middle dose (5mg) and 40% high dose ramipril (10mg) (Table 1).

One participant from the placebo group was terminated early after 50 days of treatment and was not fully up titrated due to discomfort. Further, one participant from the placebo and one from the ramipril group were not fully up titrated according to blood pressure due to presumed adverse reactions.

Counting medication left in project medication containers together with patient diary data, median compliance for patients in the placebo and ramipril group who completed the study was 97% (IQR 93–98%; min. 85%; max. 100%) and 99% (IQR 98–100%; min. 77%; max 100%), respectively.

Haemodynamics and GFR

Treatment with ramipril did not significantly reduce systolic or diastolic blood pressure compared with placebo ($p = 0.91$ and $p = 0.83$, respectively). Heart rate and eGFR did not change during treatment with ramipril compared with placebo ($p = 0.09$ and $p = 0.60$, respectively) (Table 2).

Primary endpoint: Effect on CFVR

CFVR improved for both participants receiving ramipril ($p = 0.004$) and placebo ($p = 0.026$) with no significant difference in response between the two groups ($p = 0.63$) (Table 3). Multivariable adjustment with resting systolic blood pressure and resting heart rate did not change results. In a sub-analysis stratifying on final dose of project medication, results obtained for

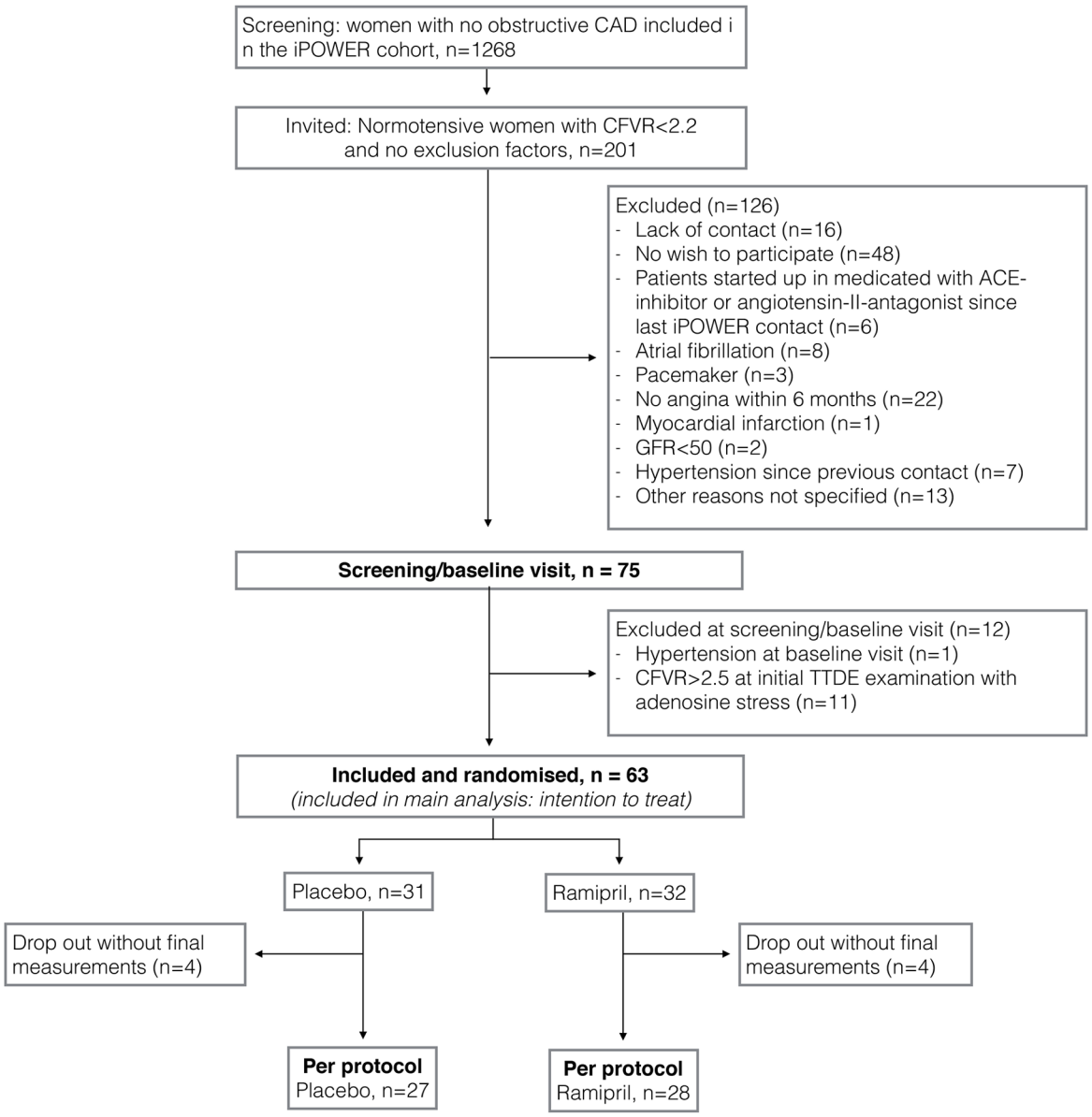

Fig 3. Participant flow-chart. 3 CAD: Coronary artery disease. CFVR: Coronary flow velocity reserve, ACE: Angiotensin-converting enzyme, GFR: Glomerular filtration rate.

https://doi.org/10.1371/journal.pone.0196962.g003

participants receiving middle to high dose ramipril (n = 32) were similar to the overall results with no between-group differences (p-interaction = 0.21).

Results from per protocol analysis were similar to the intention to treat analysis.

Secondary endpoints: Effect on other echocardiographic measures and burden of symptoms

Outcome measures are displayed in Table 3. There was no effect of treatment with ramipril on parameters of left ventricular systolic or diastolic function assessed by echocardiography compared with placebo.

Table 1. Baseline characteristics.

	Placebo (n = 31)	Ramipril (n = 32)
Age (years), mean (SD)	57.3 (12.5)	58.6 (11.6)
Body Mass Index, mean (SD)	25.3 (5.4)	27.3 (4.9)
Hypertension, n (%)	0 (0)	0 (0)
Diabetes Mellitus, n (%)	1 (3)	3 (9)
Smoking (current), n (%)	7 (23)	8 (25)
Hypercholesterolaemia, n (%)	12 (39)	19 (59)
Family history of CAD, n (%)	23 (74)	21 (66)
Heart rate (beats/min), mean (SD)	66.2 (10.1)	65.9 (11.3)
Systolic blood pressure (mmHg), mean (SD)	127.4 (11.0)	124.1 (11.0)
Diastolic blood pressure (mmHg), mean (SD)	70.2 (7.7)	67.2 (7.9)
Subclinical atherosclerosis (>0, <50% stenosis at CAG), n (%)	4 (13)	13 (42)
Medication		
Acetylsalicylic acid, n (%)	4 (13)	12 (38)
Beta-receptor blockers, n (%)	5 (16)	4 (13)
Statin, n (%)	8 (26)	11 (34)
Calcium antagonist, n (%)	1 (3)	3 (9)
Diuretic, n (%)	3 (10)	5 (16)
Project relevant information		
Dosage*		
2.5 mg, n (%)	11 (41)	12 (43)
5 mg, n (%)	4 (15)	6 (21)
10 mg, n (%)	12 (44)	10 (36)
Treatment interval (days)*, mean (SD)	138 (20)	145 (15)
Echocardiographic parameters		
CFVR, mean (SD)	2.26 (0.26)	2.03 (0.31)
GLS at rest (%), mean (SD)	-20.4 (2.5)	-21.0 (2.0)
GLS at hyperaemia (%), mean (SD) †	-23.1 (2.6)	-23.5 (2.3)
ΔGLS (%), mean (SD) †	-2.9 (2.3)	-2.5 (2.3)
LVEF (%), mean (SD)	52.6 (4.2)	52.0 (4.7)
LVMI (mg/m^2), mean (SD)	70.1 (9.8)	72.7 (14.7)
LAI (mL/m^2), mean (SD)	26.0 (4.5)	28.0 (8.9)
Deceleration time (ms), mean (SD) †	186.0 (27.9)	198.2 (39.2)
E/A ratio, mean (SD) †	1.02 (0.25)	1.04 (0.32)
e' (cm/s), mean (SD) †	10.21 (2.85)	10.09 (2.57)
E/e' ratio, mean (SD) †	6.60 (1.53)	7.03 (1.30)
Burden of symptoms: score by Seattle Angina Questionnaire ‡		
Physical limitation, mean (SD)	75.79 (17.46)	74.87 (17.69)
Angina stability, mean (SD)	49.66 (29.58)	51.61 (28.18)
Angina frequency, mean (SD)	65.52 (23.24)	78.44 (20.18)
Treatment satisfaction, mean (SD)	69.79 (23.59)	69.15 (5.71)
Perception/quality of life, mean (SD)	54.17 (19.61)	59.01 (23.52)

CFVR: Coronary flow velocity reserve, GLS: Global longitudinal strain, LVEF: Left ventricular ejection fraction, LVMI: Left ventricular mass index, LAI: Left atrium volume (method of discs) index,

*Only including patients that completed the study.

†A maximum of 6 observations missing (balanced).

‡ A maximum of 8 responses missing (balanced).

https://doi.org/10.1371/journal.pone.0196962.t001

Table 2. Effect on hemodynamic variables and eGFR.

	Placebo (n = 31)		Ramipril (n = 32)		
	Estimated change at 2. visit (95% CI)	**Estimated change at final visit (95% CI)**	**Estimated change at 2. visit (95% CI)**	**Estimated change at final visit (95% CI)**	**p-value** *
Systolic BP (mmHg)	-3.4 (-7.2; 0.3)	-0.03 (-3.2; 3.1)	-4.1 (-7.7; -0.5)	0.47 (-2.6; 3.5)	0.91
Diastolic BP (mmHg)	3.7 (0.4; 7.1)	1.5 (-1.0; 4.1)	2.3 (-0.9; 5.6)	1.2 (-1.3; 3.7)	0.83
Heart rate (beats/min)	3.6 (0.9; 6.3)	2.2 (-0.6; 4.9)	-0.2 (-2.8; 2.4)	1.7 (-1.0; 4.4)	0.09
eGFR (mL/min/1.73m^2)	-2.2 (-4.2; -0.2)	-1.3 (-4.5; 1.9)	-2.6 (-4.6; -0.7)	-3.6 (-6.7; -0.4)	0.60

*p-value obtained by baseline adjusted repeated measure analysis (mixed model)—between group change.

2. Visit at 2–3 weeks after baseline visit. eGFR: estimated glomerular filtration rate.

BP: blood pressure

https://doi.org/10.1371/journal.pone.0196962.t002

Table 3. Effect of intervention.

	Placebo (n = 31)		Ramipril (n = 32)		
	Estimated Change (95% CI)	**p** *	**Estimated Change (95% CI)**	**p** *	**p** **
Echocardiographic parameters					
CFVR	0.26 (0.03; 0.48)	**0.026**	0.34 (0.11; 0.56)	**0.004**	0.63
GLS at rest (%) †	-0.03 (-0.85; 0. 79)	0.94	-0.24 (-1.07; 0.59)	0.56	0.71
GLS at hyperaemia (%) †	-0.03 (-0.91; 0.86)	0.95	-0.17 (-1.06; 0.72)	0.70	0.80
ΔGLS (%) †	-0.07 (-1.07; 0.93)	0.89	-0.03 (-1.04; 0.99)	0.96	0.94
LVEF (%)	0.93 (-1.14; 3.00)	0.37	0.56 (-1.47; 2.59)	0.59	0.79
LVMI (g/m^2)	0.76 (-5.11; 6.64)	0.80	0.76 (-5.01; 6.53)	0.79	1.00
LAI (mL/m^2)	1.44 (-0.89; 3.78)	0.22	1.49 (-0.80; 3.79)	0.20	0.97
Deceleration time (ms) †	-9.53 (-22.97; 3.90)	0.16	-18.19 (-31.83; -4.55)	**0.01**	0.31
E/A ratio †	-0.01 (-0.07; 0.05)	0.72	-0.04 (-0.10; 0.02)	0.10	0.47
e' (cm/s) †	0.14 (-0.41; 0.70)	0.61	-0.09 (-0.63; 0.46)	0.75	0.56
E/e' ratio †	-0.15 (-0.68; 0.38)	0.57	-0.22 (-0.75; 0.32)	0.42	0.86
Burden of symptoms: score by Seattle Angina Questionnaire ‡§					
Physical limitation	3.74 (-1.85; 9.33)	0.18	-0.36 (-5.99; 5.28)	0.90	0.31
Angina stability	15.39 (5.20; 25.58)	**0.004**	27.33 (17.37; 37.29)	<**0.001**	0.07
Angina frequency	5.59 (-2.67; 13.85)	0.18	11.95 (3.91; 19.99)	**0.004**	0.23
Treatment satisfaction	2.89 (-5.13; 10.91)	0.47	8.24 (0.79; 15.68)	**0.03**	0.27
Perception/quality of life	2.64 (-4.31; 9.58)	0.45	4.39 (-2.27; 11.06)	0.19	0.71

p-value obtained by baseline adjusted repeated measure analysis (mixed model).

p* within group change.

p** between group change.

CFVR: Coronary flow velocity reserve, GLS: Global longitudinal strain, LVEF: Left ventricular ejection fraction, LVMI: Left ventricular mass index, LAI: Left atrium volume (method of discs) index,

†A maximum of 9 observations missing (balanced).

‡ A maximum of 19 pre-and post treatment responses missing (balanced).

§A high score equals a higher functional status.

https://doi.org/10.1371/journal.pone.0196962.t003

Angina stability improved significantly in both the ramipril ($p<0.001$) and the placebo group ($p = 0.004$). There was an insignificant trend for a larger improvement of angina stability in participants treated with ramipril compared with placebo ($p = 0.07$). Angina frequency and treatment satisfaction was significantly higher after treatment with ramipril ($p = 0.004$ and $p = 0.03$, respectively), but not when compared with placebo ($p = 0.23$ and $p = 0.27$, respectively).

Results obtained by per protocol analyses were similar to the intention to treat analyses.

Adverse reactions

The proportion of participants experiencing an event categorized as either an adverse event, adverse reaction (common side effects) or serious adverse event was not significantly different between treatment groups: 20% vs. 18% ($p = 0.74$), 22% vs. 28% ($p = 0.33$) and 5% vs. 5% ($p = 0.80$), respectively. None of the participants experienced a serious adverse reaction.

Discussion

In this randomized double-blinded placebo-controlled trial of normotensive women with angina and CMD, we found no significant improvement of coronary microvascular function assessed by CFVR in participants treated with the ACE inhibitor, ramipril, compared with placebo. Further, no effect of treatment with ramipril on burden of symptoms or parameters of left ventricular diastolic and systolic function compared with placebo was detected.

CFVR increased in both groups. This could be a placebo effect and is also likely to be caused by regression towards the mean because participants were selected into the study based on a low CFVR at study entry. As expected, systolic and diastolic blood pressure did not change significantly for patients treated with ramipril. Based on previous studies in normotensive individuals with diabetes and microalbuminuria or nephropathies, we assumed no or only modest blood pressure reduction. In the Heart Outcomes Prevention Evaluation (HOPE) study, ramipril (10 mg per day) lowered blood pressure only modestly (by 3·3/1·4 mm Hg) in high-risk, mostly normotensive patients. However, most studies have found no change in blood pressure [31–38].

A few previous studies have addressed the effect of ACE inhibitor treatment on non-endothelium dependent CMD. In patients with angina and no obstructive CAD, two randomized placebo-controlled studies of ACE inhibition on coronary microvascular function using intracoronary Doppler flow measurements have been conducted. One study (n = 20) showed a significant effect of 10 mg enalapril during 2 months of treatment. Mean systolic blood pressure (SD) was 136 (±20). Drop out was 18% [13]. The other study (n = 61) did not show an effect of 80 mg quinapril over 4 months, but the trial reported an effect in a subanalysis, which was not pre-specified in the trial protocol, of patients with a baseline CFVR<2.5 (n = 32). Approximately 40% had hypertension and mean systolic blood pressure was 126 (±19). Drop out was 21% [14]. In a randomized study including patients with CAD (n = 29), no effect of 6 months of treatment with 40 mg quinapril was found on CFVR assessed by intracoronary Doppler in a non-stenotic target coronary artery compared with placebo. However, only 13 out of 29 randomized patients completed the study [21]. In a randomized comparator study including patients with diabetes (n = 24) CFVR assessed by TTDE improved in the group treated with ACE inhibitor, but the effect was not compared with placebo [17]. Other randomized studies with a comparator have investigated the effect of treatment with ACE inhibitor on coronary microvascular function using positron emission tomography (PET) in patients with hypertension. None of the studies were placebo-controlled and results were inconsistent: One study observed an improvement in coronary microvascular function in the group treated with ACE

inhibitor (n = 9) [15], whereas no effect (n = 10, 15) was detected in two of the studies [16,18]. Many small non-randomized studies (n<24) have been conducted using different methods for assessment of coronary microvascular function. In 2 out of 4 small studies including hypertensive patients, ACE inhibitor treatment improved coronary microvascular function [19,20,22,23]. Further, ACE inhibitors in combination with either a calcium antagonist or indapamide have been shown to be beneficial [24,25]. A small study including patients with diabetes also showed an effect [26].

Whether the effect of ACE-inhibition on coronary microvascular function in some studies is indirectly mediated via treatment of hypertension with no direct effect on the microvasculature is uncertain. In support of this, a study found that blood pressure at follow-up after ACE inhibitor treatment could predict change in CFVR [14]. To our knowledge, no previous studies investigating coronary microvascular function have focused exclusively on normotensive patients and contrary to other studies we did not detect an effect compared with placebo. Therefore, this trial suggests that the effect of ACE inhibition on coronary microvascular function previously reported could be mediated by blood pressure reduction and that treatment with ACE inhibition per se does not improve microvascular function.

Studies in patients with angina and no CAD, but a positive stress test or microvascular angina (CFVR<3) have shown that patients treated with a combination of atorvastatin and ramipril or quinapril alone, respectively, significantly reduced burden of symptoms compared with placebo [13,14,51]. In another study of patients with angina, a positive stress test and no obstructive CAD, burden of symptoms were reduced after treatment with enalapril, but it is not mentioned whether this reduction is significant when comparing with the placebo group [13]. In this present study we detected an improvement in the burden of symptoms with ACE inhibitor treatment, which was not significant when comparing with placebo treatment.

A meta-analysis (39 arms, 1068 patients) has shown that ACE inhibitor treatment reduced estimated left ventricular mass by a mean of 10% (95% CI 8%; 12%) [52]. However, the studies included were on patients with hypertension and a more abnormal baseline echocardiography. Similar to our trial, a study of normotensive patients (n = 46) found no change in LVMI assessed by TTDE [53]. Furthermore, most participants in this present trial had echocardiographic parameters of left ventricular diastolic function and LVMI within reference range.

Strengths and limitations

The study is a randomized placebo-controlled trial. The participants were included systematically, and the study is therefore generalizable for normotensive women with CMD and angina and no obstructive CAD. The primary outcome measure, TTDE CFVR, showed good reproducibility in both healthy individuals and women with angina an no obstructive CAD in our clinic, and the method for CFVR measurements is accessible and highly feasible [39,40,48]. Differences in resting baseline flow will be reflected in CFVR, i.e. an impaired CFVR may be due to a high baseline flow. Therefore, part of the variability in the outcome measure can be due to changes in baseline flow during the trial. However, none of the participants developed disease states known to influence resting blood flow during the trial such as diabetes or hypertension and furthermore we found no significant difference between changes in hemodynamic variables, which are also known to influence resting blood flow, in the group randomized to ACE inhibitors compared with the placebo group.

We intended to include 72 patients to obtain 60 patients completing the study based on a sample size calculation with 90% power. However, only 63 patients could be included from the cohort due to a lower inclusion rate than expected, but 55 patients completed the study with both baseline and follow up measurements (lacking only 5 patients from the original power

calculation). The SDs on mean CFVR change obtained in this study were larger than hypothesized probably due to a long treatment interval and a divergent treatment response in the ramipril group (SD 0.4 and 0.7 in the placebo- and ramipril group, respectively). This implies that the change in CFVR needs to be more than 0.4 greater in the ramipril group than the placebo group to detect a significant treatment effect (power set at 80%). Thus, we cannot exclude that we have overlooked a small but clinically relevant effect of the intervention. The two previous studies of ACE-inhibition in patients with no obstructive CAD detected an improvement in CFVR of 0.19 and 1.02 in the intervention group compared with the placebo group, respectively [13,14]. A 13% drop out in this study meets the less than 20% drop out trial quality criteria.

It could be speculated that a higher dosage might have been more effective in yielding persistent coronary microvascular changes. In this present trial the dose of ramipril ranged from 2.5 to 10.0 mg. Due to lower blood pressure at baseline (<115 mmHg) approximately 40% of patients ended up on low dose ramipril, whereas 20% were on medium dose ramipril and 40% on high dose. This was according to the algorithm depicted in Fig 1. However, the maximum dose in the studies investigating the effect of ACE-inhibitor on CMD varies and there seems to be no trend of greater effect on coronary microvascular function with higher dosage. In addition, in the present study no effect of ramipril was detected in subgroups when stratifying by achieved dosage. However, this study was not powered sufficiently to detect effect in subgroups nor was this analysis pre-specified.

Treatment interval was set to 24±6 weeks. Some previous studies looking at coronary microvascular function as a main endpoint found an effect of treatment with ACE-inhibitors between 2–4 months [13,14]. However, the effect seen in these studies may be due to the blood pressure lowering effect of ACE inhibitors. It is possible that a longer treatment period is necessary to result in structural remodelling leading to changes in microvascular function beyond the blood pressure effect.

Conclusion

Treatment with ACE inhibitor had no significant effect on coronary microvascular function, echocardiographic parameters of systolic and diastolic function or burden of symptoms compared with placebo in normotensive women with angina and CMD. Thus, current guideline recommendations of ACE inhibition in treatment of patients with microvascular angina do not extend to normotensive patients.

Perspectives

Evidence-based treatment options for patients with angina pectoris are lacking. We investigated the efficacy of treatment with the ACE inhibitor ramipril on coronary microvascular function in normotensive patients with CMD and found no effect compared with placebo, suggesting that the effect previously detected with ACE-inhibition could be mediated through blood pressure reduction. This signifies that treatment with ACE inhibitor might only be indicated for the part of angina patients with hypertension. This could be a step towards a more individualized approach in treating CMD.

Supporting information

S1 File. Supplementary.
(DOCX)

S2 File. CONSORT 2010 checklist.
(PDF)

S3 File. Protocol in Danish.
(DOCX)

S4 File. Protocol in English.
(DOCX)

S5 File. Data file—Inclusion status.
(XLSX)

S6 File. Data file—Baseline characteristics.
(XLS)

S7 File. Data file—Echocardiography data.
(XLSX)

S8 File. Data file—Echocardiography, strain data.
(XLSX)

S9 File. Data file—FMD data.
(XLSX)

S10 File. Data file—Burden of symptoms.
(XLSX)

S11 File. Data file—Adverse events.
(XLS)

Acknowledgments

We thank the Department of Cardiology at Bispebjerg Hospital and all participating women for their willingness to contribute to this research. We further thank The Danish Heart Foundation, the University of Copenhagen and Bispebjerg/Frederiksberg University Hospital who funded this study.

Author Contributions

Conceptualization: Marie Mide Michelsen, Anna Bay Rask, Nis Høst, Eva Prescott.

Data curation: Marie Mide Michelsen, Anna Bay Rask, Elena Suhrs, Kristoffer Flintholm Raft, Eva Prescott.

Formal analysis: Marie Mide Michelsen, Anna Bay Rask, Elena Suhrs, Kristoffer Flintholm Raft, Nis Høst, Eva Prescott.

Funding acquisition: Marie Mide Michelsen, Nis Høst, Eva Prescott.

Investigation: Marie Mide Michelsen, Anna Bay Rask, Elena Suhrs, Eva Prescott.

Methodology: Marie Mide Michelsen, Anna Bay Rask, Elena Suhrs, Kristoffer Flintholm Raft, Nis Høst, Eva Prescott.

Project administration: Marie Mide Michelsen, Kristoffer Flintholm Raft, Eva Prescott.

Resources: Marie Mide Michelsen, Eva Prescott.

Software: Marie Mide Michelsen, Kristoffer Flintholm Raft, Eva Prescott.

Supervision: Nis Høst, Eva Prescott.

Validation: Marie Mide Michelsen, Elena Suhrs, Kristoffer Flintholm Raft, Eva Prescott.

Visualization: Marie Mide Michelsen, Eva Prescott.

Writing – original draft: Marie Mide Michelsen.

Writing – review & editing: Marie Mide Michelsen, Anna Bay Rask, Elena Suhrs, Kristoffer Flintholm Raft, Nis Høst, Eva Prescott.

References

1. Camici PG, Crea F. Coronary Microvascular Dysfunction. N Engl J Med. 2007 Feb; 356(8):830–40. https://doi.org/10.1056/NEJMra061889 PMID: 17314342
2. Reis SE, Holubkov R, Conrad Smith AJ, Kelsey SF, Sharaf BL, Reichek N, et al. Coronary microvascular dysfunction is highly prevalent in women with chest pain in the absence of coronary artery disease: results from the NHLBI WISE study. Am Heart J. 2001 May; 141(5):735–41. PMID: 11320360
3. Sicari R, Rigo F, Cortigiani L, Gherardi S, Galderisi M, Picano E. Additive prognostic value of coronary flow reserve in patients with chest pain syndrome and normal or near-normal coronary arteries. Am J Cardiol. 2009 Mar; 103(5):626–31. https://doi.org/10.1016/j.amjcard.2008.10.033 PMID: 19231324
4. Mygind ND, Michelsen MM, Pena A, Frestad D, Dose N, Aziz A, et al. Coronary Microvascular Function and Cardiovascular Risk Factors in Women With Angina Pectoris and No Obstructive Coronary Artery Disease: The iPOWER Study. J Am Heart Assoc. 2016; 5(3).
5. Marinescu MA, Löffler AI, Ouellette M, Smith L, Kramer CM, Bourque JM. Coronary microvascular dysfunction, microvascular angina, and treatment strategies. JACC Cardiovasc Imaging. 2015 Feb; 8 (2):210–20. https://doi.org/10.1016/j.jcmg.2014.12.008 PMID: 25677893
6. Duncker DJ, Koller A, Merkus D, Canty JM. Regulation of Coronary Blood Flow in Health and Ischemic Heart Disease. Prog Cardiovasc Dis. 2015 Mar; 57(5):409–22. https://doi.org/10.1016/j.pcad.2014.12.002 PMID: 25475073
7. Heeneman S, Sluimer JC, Daemen MJAP. Angiotensin-converting enzyme and vascular remodeling. Circ Res. 2007 Aug; 101(5):441–54. https://doi.org/10.1161/CIRCRESAHA.107.148338 PMID: 17761934
8. Manolis AJ, Iraklianou S, Pittaras A, Zaris M, Tsioufis K, Psaltiras G, et al. Arterial compliance changes in diabetic normotensive patients after angiotensin-converting enzyme inhibition therapy. Am J Hypertens. 2005 Jan; 18(1):18–22. https://doi.org/10.1016/j.amjhyper.2004.08.014 PMID: 15691612
9. Benetos A, Albaladejo P, Levy BI, Safar ME. Acute and long-term effects of angiotensin converting enzyme inhibition on larger arteries and cardiac hypertrophy: mechanical and structural parameters. J Int Soc Hypertens. 1994 Jul; 12(4):S21–29.
10. Sleight P, Yusuf S, Pogue J, Tsuyuki R, Diaz R, Probstfield J. Blood-pressure reduction and cardiovascular risk in HOPE study. Lancet (London, England). 2001 Dec; 358(9299):2130–1.
11. Suhrs HE, Michelsen MM, Prescott E. Treatment strategies in coronary microvascular dysfunction: a systematic review of interventional studies. Microcirculation. 2017 Nov 11.
12. Task Force Members, Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. Eur Heart J. 2013 Oct; 34 (38):2949–3003. https://doi.org/10.1093/eurheartj/eht296 PMID: 23996286
13. Chen J-W, Hsu N-W, Wu T-C, Lin S-J, Chang M-S. Long-term angiotensin-converting enzyme inhibition reduces plasma asymmetric dimethylarginine and improves endothelial nitric oxide bioavailability and coronary microvascular function in patients with syndrome X. Am J Cardiol. 2002 Nov; 90(9):974–82. PMID: 12398965
14. Pauly DF, Johnson BD, Anderson RD, Handberg EM, Smith KM, Cooper-DeHoff RM, et al. In women with symptoms of cardiac ischemia, nonobstructive coronary arteries, and microvascular dysfunction, angiotensin-converting enzyme inhibition is associated with improved microvascular function: A double-blind randomized study from the National Heart, Lung and Blood Institute Women's Ischemia Syndrome Evaluation (WISE). Am Heart J. 2011 Oct; 162(4):678–84. https://doi.org/10.1016/j.ahj.2011.07.011 PMID: 21982660
15. Akinboboye OO, Chou R-L, Bergmann SR. Augmentation of myocardial blood flow in hypertensive heart disease by angiotensin antagonists: a comparison of lisinopril and losartan. J Am Coll Cardiol. 2002 Aug; 40(4):703–9. PMID: 12204500

16. Buus NH, Bøttcher M, Jørgensen CG, Christensen KL, Thygesen K, Nielsen TT, et al. Myocardial perfusion during long-term angiotensin-converting enzyme inhibition or beta-blockade in patients with essential hypertension. Hypertens (Dallas, Tex 1979). 2004 Oct; 44(4):465–70.
17. Kawata T, Daimon M, Hasegawa R, Teramoto K, Toyoda T, Sekine T, et al. Effect on coronary flow velocity reserve in patients with type 2 diabetes mellitus: comparison between angiotensin-converting enzyme inhibitor and angiotensin II type 1 receptor antagonist. Am Heart J. 2006 Apr; 151(4):798.e9–15.
18. Parodi O, Neglia D, Palombo C, Sambuceti G, Giorgetti A, Marabotti C, et al. Comparative effects of enalapril and verapamil on myocardial blood flow in systemic hypertension. Circulation. 1997 Aug; 96 (3):864–73. PMID: 9264494
19. Masuda D, Nohara R, Tamaki N, Hosokawa R, Inada H, Hikai T, et al. Evaluation of coronary blood flow reserve by 13N-NH3 positron emission computed tomography (PET) with dipyridamole in the treatment of hypertension with the ACE inhibitor (Cilazapril). Ann Nucl Med. 2000 Oct; 14(5):353–60. PMID: 11108164
20. Stamatelopoulos K, Bramos D, Manios E, Alexaki E, Kaladaridou A, Georgiopoulos G, et al. Pleiotropic effects of the acute and chronic inhibition of the renin-angiotensin system in hypertensives. J Hum Hypertens. 2014 Jun; 28(6):378–83. https://doi.org/10.1038/jhh.2013.125 PMID: 24284385
21. Schlaifer JD, Wargovich TJ, O'Neill B, Mancini GB, Haber HE, Pitt B, et al. Effects of quinapril on coronary blood flow in coronary artery disease patients with endothelial dysfunction. TREND Investigators. Trial on Reversing Endothelial Dysfunction. Am J Cardiol. 1997 Dec; 80(12):1594–7. PMID: 9416943
22. Schwartzkopff B, Brehm M, Mundhenke M, Strauer BE. Repair of coronary arterioles after treatment with perindopril in hypertensive heart disease. Hypertension. 2000 Aug; 36(2):220–5. PMID: 10948081
23. Motz W, Strauer BE. Improvement of coronary flow reserve after long-term therapy with enalapril. Hypertension. 1996 May; 27(5):1031–8. PMID: 8621193
24. Neglia D, Fommei E, Varela-Carver A, Mancini M, Ghione S, Lombardi M, et al. Perindopril and indapamide reverse coronary microvascular remodelling and improve flow in arterial hypertension. J Hypertens. 2011 Feb; 29(2):364–72. https://doi.org/10.1097/HJH.0b013e328340a08e PMID: 21045728
25. Engholm M, Mulvany MJ, Eftekhari A, Mathiassen ON, Buus NH, Christensen KL. Positive effects of aggressive vasodilator treatment of well-treated essential hypertensive patients. J Hum Hypertens. 2016 Nov; 30(11):690–6. https://doi.org/10.1038/jhh.2016.13 PMID: 26961172
26. Kawata T, Daimon M, Hasegawa R, Teramoto K, Toyoda T, Sekine T, et al. Effect of angiotensin-converting enzyme inhibitor on serum asymmetric dimethylarginine and coronary circulation in patients with type 2 diabetes mellitus. Int J Cardiol. 2009 Feb; 132(2):286–8. https://doi.org/10.1016/j.ijcard.2007.08.066 PMID: 18083252
27. Bakris GL, Slataper R, Vicknair N, Sadler R. ACE inhibitor mediated reductions in renal size and microalbuminuria in normotensive, diabetic subjects. J Diabetes Complications. 1994 Mar; 8(1):2–6. PMID: 8167383
28. Marre M, Chatellier G, Leblanc H, Guyene TT, Menard J, Passa P. Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. BMJ. 1988 Oct; 297(6656):1092–5. PMID: 2848604
29. Parving HH, Hommel E, Damkjaer Nielsen M, Giese J. Effect of captopril on blood pressure and kidney function in normotensive insulin dependent diabetics with nephropathy. BMJ. 1989 Aug; 299 (6698):533–6. PMID: 2507061
30. Elliott HL, Macdonald NJ, Meredith PA, Reid JL. Dose responses and pharmacokinetics for the angiotensin converting enzyme inhibitor quinapril. Clin Pharmacol Ther. 1992 Mar; 51(3):260–5. PMID: 1312002
31. Ravid M, Brosh D, Levi Z, Bar-Dayan Y, Ravid D, Rachmani R. Use of enalapril to attenuate decline in renal function in normotensive, normoalbuminuric patients with type 2 diabetes mellitus. A randomized, controlled trial. Ann Intern Med. 1998 Jun; 128(12 Pt 1):982–8. PMID: 9625684
32. Mehlsen J, Jeppesen P, Erlandsen M, Poulsen PL, Bek T. Lack of effect of short-term treatment with amlodipine and lisinopril on retinal autoregulation in normotensive patients with type 1 diabetes and mild diabetic retinopathy. Acta Ophthalmol. 2011 Dec; 89(8):764–8. https://doi.org/10.1111/j.1755-3768.2009.01847.x PMID: 20346089
33. Kvetny J, Gregersen G, Pedersen RS. Randomized placebo-controlled trial of perindopril in normotensive, normoalbuminuric patients with type 1 diabetes mellitus. QJM Mon J Assoc Physicians. 2001 Feb; 94(2):89–94.
34. Naqvi SAJ, Ahsan S, Fawwad A, Basit A, Shera AS. Effect of angiotensin converting enzyme inhibitor on glomerular hyperfiltration in patients with type 1 diabetes. Pakistan J Med Sci. 2016 Jun; 32(3):559–64.

35. Krysiak R, Okopień B. Different effects of perindopril and enalapril on monocyte cytokine release in coronary artery disease patients with normal blood pressure. Pharmacol reports PR. 2012; 64(6):1466–75.

36. Mathiesen ER, Hommel E, Giese J, Parving HH. Efficacy of captopril in postponing nephropathy in normotensive insulin dependent diabetic patients with microalbuminuria. BMJ. 1991 Jul; 303(6794):81–7. PMID: 1860008

37. Sano T, Kawamura T, Matsumae H, Sasaki H, Nakayama M, Hara T, et al. Effects of long-term enalapril treatment on persistent micro-albuminuria in well-controlled hypertensive and normotensive NIDDM patients. Diabetes Care. 1994 May; 17(5):420–4. PMID: 8062609

38. Ravid M, Savin H, Jutrin I, Bental T, Lang R, Lishner M. Long-term effect of ACE inhibition on development of nephropathy in diabetes mellitus type II. Kidney Int Suppl. 1994 Feb; 45:S161–164. PMID: 8158887

39. Prescott E, Abildstrøm SZ, Aziz A, Merz NB, Gustafsson I, Halcox J, et al. Improving diagnosis and treatment of women with angina pectoris and microvascular disease: the iPOWER study design and rationale. Am Heart J. 2014 Apr; 167(4):452–8. https://doi.org/10.1016/j.ahj.2014.01.003 PMID: 24655692

40. Michelsen MM, Pena A, Mygind ND, Frestad D, Gustafsson I, Hansen HS, et al. Coronary Flow Velocity Reserve Assessed by Transthoracic Doppler: The iPOWER Study: Factors Influencing Feasibility and Quality. J Am Soc Echocardiogr. 2016 Jul; 29(7):709–16. https://doi.org/10.1016/j.echo.2016.02.011 PMID: 27038514

41. Weber MA, Schiffrin EL, White WB, Mann S, Lindholm LH, Kenerson JG, et al. Clinical Practice Guidelines for the Management of Hypertension in the Community: A Statement by the American Society of Hypertension and the International Society of Hypertension. J Clin Hypertens. 2014 Jan; 16(1):14–26.

42. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, et al. 2013 ACCF/AHA Guideline for the Management of Heart Failure. J Am Coll Cardiol. 2013 Oct; 62(16):e147–239. https://doi.org/10.1016/j.jacc.2013.05.019 PMID: 23747642

43. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med. 2000 Jan; 342(3):145–53. https://doi.org/10.1056/NEJM200001203420301 PMID: 10639539

44. Spertus JA, Winder JA, Dewhurst TA, Deyo RA, Prodzinski J, McDonell M, et al. Development and evaluation of the Seattle Angina Questionnaire: a new functional status measure for coronary artery disease. J Am Coll Cardiol. 1995 Feb; 25(2):333–41. PMID: 7829785

45. Voigt J-U, Pedrizzetti G, Lysyansky P, Marwick TH, Houle H, Baumann R, et al. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. Eur Heart J Cardiovasc Imaging. 2015 Jan; 16(1):1–11. https://doi.org/10.1093/ehjci/jeu184 PMID: 25525063

46. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification. Eur J Echocardiogr J Work Gr Echocardiogr Eur Soc Cardiol. 2006 Mar; 7 (2):79–108.

47. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr J Work Gr Echocardiogr Eur Soc Cardiol. 2009 Mar; 10(2):165–93.

48. Michelsen MM, Mygind ND, Pena A, Olsen RH, Christensen TE, Ghotbi AA, et al. Transthoracic Doppler echocardiography compared with positron emission tomography for assessment of coronary microvascular dysfunction: The iPOWER study. Int J Cardiol. 2017 Feb; 228:435–43. https://doi.org/10.1016/j.ijcard.2016.11.004 PMID: 27870973

49. Faber R, Zander M, Pena A, Michelsen MM, Mygind ND, Prescott E. Effect of the glucagon-like peptide-1 analogue liraglutide on coronary microvascular function in patients with type 2 diabetes—a randomized, single-blinded, cross-over pilot study. Cardiovasc Diabetol. 2015; 14:41. https://doi.org/10.1186/s12933-015-0206-3 PMID: 25896352

50. Fitzmaurice Garrett M., Laird Nan M., Ware James H. Applied Longitudinal Analysis, 2nd Edition.

51. Pizzi C, Manfrini O, Fontana F, Bugiardini R. Angiotensin-converting enzyme inhibitors and 3-hydroxy-3-methylglutaryl coenzyme A reductase in cardiac Syndrome X: role of superoxide dismutase activity. Circulation. 2004 Jan; 109(1):53–8. https://doi.org/10.1161/01.CIR.0000100722.34034.E4 PMID: 14699004

52. Klingbeil AU, Schneider M, Martus P, Messerli FH, Schmieder RE. A meta-analysis of the effects of treatment on left ventricular mass in essential hypertension. Am J Med. 2003 Jul; 115(1):41–6. PMID: 12867233

53. Yu W-C, Lin Y-P, Lin I-F, Chuang S-Y, Chen C-H. Effect of Ramipril on Left Ventricular Mass in Normotensive Hemodialysis Patients. Am J Kidney Dis. 2006 Mar; 47(3):478–84. https://doi.org/10.1053/j.ajkd.2005.11.024 PMID: 16490627