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The effect of spironolactone on cardiovascular function and markers of fibrosis in people at increased risk of developing heart failure: the heart 'OMics' in AGEing (HOMAGE) randomized clinical trial

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Aims To investigate the effects of spironolactone on fibrosis and cardiac function in people at increased risk of developing heart failure.

Methods and results Randomized, open-label, blinded-endpoint trial comparing spironolactone (50 mg/day) or control for up to 9 months in people with, or at high risk of, coronary disease and raised plasma B-type natriuretic peptides. The

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primary endpoint was the interaction between baseline serum galectin-3 and changes in serum procollagen type-III N-terminal pro-peptide (PIIINP) in participants assigned to spironolactone or control. Procollagen type-I C-terminal pro-peptide (PICP) and collagen type-1 C-terminal telopeptide (CITP), reflecting synthesis and degradation of type-I collagen, were also measured. In 527 participants (median age 73 years, 26% women), changes in PIIINP were similar for spironolactone and control [mean difference (mdiff): -0.15; 95% confidence interval (CI) -0.44 to 0.15 μg/L; $P = 0.32$] but those receiving spironolactone had greater reductions in PICP (mdiff: -8.1; 95% CI -11.9 to -4.3 μg/L; $P < 0.0001$) and PICP/CITP ratio (mdiff: -2.9; 95% CI -4.3 to -1.5; <0.0001). No interactions with serum galectin were observed. Systolic blood pressure (mdiff: -10; 95% CI -13 to -7 mmHg; $P < 0.0001$), left atrial volume (mdiff: -1; 95% CI -2 to 0 mL/m^2; $P = 0.010$), and NT-proBNP (mdiff: -57; 95% CI -81 to -33 ng/L; $P < 0.0001$) were reduced in those assigned spironolactone.

Conclusions Galectin-3 did not identify greater reductions in serum concentrations of collagen biomarkers in response to spironolactone. However, spironolactone may influence type-I collagen metabolism. Whether spironolactone can delay or prevent progression to symptomatic heart failure should be investigated.

Keywords Spironolactone • Heart failure prevention • Fibrosis • Collagen markers

Introduction

Many people with cardiovascular disease will develop heart failure, leading to substantial disability, demands on health-services and mortality.[1,2] Early identification of cardiac dysfunction and therapeutic targeting of specific pathways of disease progression, such as myocardial and vascular fibrosis,[3–12] might delay or prevent the onset of heart failure.

Mineralo-corticoid receptor antagonists (MRA) improve cardiac structure, function and prognosis in patients with a reduced left ventricular ejection fraction (LVEF) and heart failure (HFrEF), and perhaps also when LVEF is preserved (HFpEF).[13–15]MRA also reduce serum markers of collagen synthesis in patients with a range of cardiovascular diseases, including HFrEF and HFpEF,[9,11] which might reflect favourable effects on fibrosis. Galectin-3 is a proposed marker of fibrotic activity[16,17] and, in experimental models, a mediator of aldosterone-induced fibrosis.[18] In the general population, higher plasma concentrations of galectin-3 predict the development of heart failure and, subsequently, a worse outcome.[19,20]

Accordingly, we investigated whether spironolactone had favourable effects on serum markers of collagen metabolism in people at increased risk of developing heart failure and whether the effect was greater in patients with higher serum concentrations of galectin-3.

Methods

Trial design and oversight

Heart 'OMics' in AGEing (HOMAGE) is a research consortium, based in Europe, investigating biomarkers for predicting incident heart failure in older people and bio-targets for interventions that might prevent it; assets include several large clinical databases and collections of biological material.[21,22] The HOMAGE clinical trial was a prospective, randomized, open-label, blinded-endpoint (PROBE) multi-centre trial, investigating the effects of MRA on markers of collagen metabolism and cardiovascular structure and function in people at increased risk of developing heart failure.[11] The protocol and statistical analysis plan are available at https://clinicaltrials.gov/ct2/show/NCT02556450. The trial was funded by the European Union 7th Framework Programme for Research and Technological Development (grant: 305507 http://www.homage-hf.eu). The sponsor was ACS Biomarkers (Amsterdam, The Netherlands). The European Drug Development Hub (EDDH) (Nancy, France) managed monitoring and data collection. The trial was approved by relevant ethics committees and regulatory bodies. Participants provided written, informed consent. An executive committee developed the protocol, oversaw trial conduct, and interpreted the results. A clinical endpoints committee adjudicated hospitalizations and deaths blind to assigned treatment. An independent data monitoring committee oversaw safety.

Trial population

People of either sex, aged ≥65 years (amended to ≥60 years) at increased risk of developing heart failure because they had, or were at high risk of, coronary disease were screened at hospital clinical research clinics. Those with a plasma amino-terminal pro-B-type natriuretic peptide (NT-proBNP) of 125–1000 ng/L or BNP 35–280 ng/L were eligible for randomization provided none of the exclusion criteria were met. This 'window' excluded people at low risk of developing heart failure and those with advanced disease requiring further investigation and treatment. The main exclusion criteria (Supplementary material online, *Table S1*) were an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2,[23] serum potassium >5.0 mmol/L, LVEF <45%, atrial fibrillation, a diagnosis of heart failure, or treatment with loop diuretics. Background therapy could include any conventional treatment except loop diuretics, MRA, or other potassium-sparing diuretics. Other treatments for concomitant conditions, such as hypertension, diabetes mellitus, or coronary disease, were permitted.

Aims and endpoints

Serum concentration of procollagen type-III N-terminal pro-peptide (PIIINP), thought to reflect type-III collagen synthesis, was chosen as the marker of response, based on a landmark trial of spironolactone for HFrEF.[9] Serum galectin-3 was chosen as a marker of fibrotic activity.[16,17] The primary endpoint was the interaction between changes in PIIINP from baseline to final visit and baseline galectin-3.

Secondary aims were to investigate the effects of spironolactone on other serum markers of collagen metabolism, on cardiac structure and function (assessed by echocardiography and NT-proBNP), and on exercise capacity. Specific secondary endpoints included changes in serum markers of type-I collagen synthesis (procollagen type-I C-terminal propeptide; PICP) and degradation (collagen type-1 C-terminal telopeptide; CITP), galectin-3 and NT-proBNP; echocardiographic left atrial volume and left ventricular mass; Doppler measures of cardiac function; tricuspid

annular plane systolic excursion (TAPSE) and an incremental shuttle walk-test.[24] Safety endpoints included incidences of serum potassium >5.5 or <3.5 mmol/L, decline in eGFR by >20%, and a clinical composite of heart failure or atrial fibrillation, non-fatal myocardial infarction or stroke or cardiovascular death.[11]

Laboratory assays

Blind to clinical data and randomization, PIIINP and CITP were measured by radio-immunoassay (Orion Diagnostica®), PICP by enzyme immune-assay (METRA; Quidel Corporation®) and matrix metalloproteinase-1 (MMP-1) by an amplified luminescent proximity homogenous assay (ALPHA-LISA) (PerkinElmer®). Galectin-3 was measured by enzyme-linked immunosorbent assay (ELISA) (BG Medicine®) and NT-proBNP, high-sensitivity troponin T (hsTnT) and growth differentiation factor 15 (GDF-15) by electro-chemi-luminescence (ELECSYS® 2010 analyser; Roche Diagnostics, Mannheim, Germany). All intra-assay variations were <10%.

Echocardiography

Echocardiograms were recorded, de-identified and transferred to a core laboratory (University Hospital of Nancy). Blind to treatment allocation, a single experienced echocardiographer (Erwan Bozec) measured variables using dedicated software (Echo PAC, GE Healthcare). Measurements were repeated at least 2 months later, blind to the first measurement. All recordings with suboptimal images and/or with differences >10% were reviewed by a senior cardiologist (Nicolas Girerd) to exclude measurement error.

Randomization and blinding

Participants were randomized by a co-ordinating centre (Leuven) using statistical software (SAS 9.4), web-based management system and random, permuted blocks, stratified by site. Spironolactone was initiated at 25 mg/day. Doses could be increased up to 50 mg/day or reduced to 25 mg every other day or stopped with or without re-initiation according to serum potassium and renal function (Supplementary material online, *Table S2*). Those assigned to the control group received no additional treatment. All core-laboratory staff and the clinical endpoints committee were blind to treatment allocation, but investigators were not.

Follow-up

After randomization, follow-up visits were planned after 1 week and at 1, 2, 3, 6, and 9 months, to assess serum potassium, renal function, and blood pressure. At the 1-month and final visits, baseline assessments were repeated, although echocardiography was not mandated at 1 month. The final visit was planned to occur at 9 months. Due to slow recruitment, enrolment was extended but due to the fixed funding-duration, the final visit occurred between 3 and 8 months for some participants.

Statistical analysis

Sample-size was based on a test for interaction by analysis of variance[25] and required 800 participants to detect an interaction term of 0.79 µg/L between PIIINP and median galectin-3 with a two-sided significance of 5% and 90% power, given a residual standard deviation for PIIINP of 1.73 µg/L.[26] Analyses (Stata® version 15.1) used the intention-to-treat principle. Baseline characteristics were summarized for categorical variables using frequencies and percentages, and for continuous variables using median and interquartile range (IQR, defined as the 1st and 3rd quartiles). Analysis of covariance (ANCOVA) was used for the primary endpoint. A linear regression model was fitted, including variables to indicate treatment group, galectin-3 above or below median, and baseline PIIINP. An interaction term was included to evaluate the effect of spironolactone when galectin-3 was above median. Residual analysis was used to examine the fit of the model to the assumptions of linear regression with data transformed as required. Secondary endpoints were analysed using ANCOVA for continuous data or multi-variable logistic regression for composite clinical events. No adjustments were made for multiple comparisons to allow for type-1 error in view of the exploratory nature of this proof-of-concept trial.

Results

Between January 2016 and June 2018, 877 patients were consented, of whom 561 were eligible and 527 were randomized (*Figure 1*—Consort Diagram; Supplementary material online, *Table S3*). The main reason for exclusion was NT-proBNP <125 ng/L or BNP <35 ng/L. Baseline characteristics of those assigned to spironolactone or control were similar (*Table 1*). Median age was 73 (IQR 68–78) years, 26% were women, 72% had coronary disease, 42% had diabetes, and 22% had an eGFR <60 mL/min/1.73 m^2. Most participants (78%) had a history of hypertension, were overweight or obese and, despite receiving two or more anti-hypertensive medications, 50% had a systolic blood pressure >140 mmHg. Participants reported breathlessness at moderate levels of exertion (*Table 1*) and the shuttle walk-test (*Table 2*) was mildly impaired (50; IQR 32–70 completed shuttles) compared with published normal values (mean ± SD for general population age ≥70 years is 63 ± 19 shuttles).[24] The rise in heart rate with exercise was modest (many participants were receiving beta-blockers) but systolic blood pressure rose from 140 (IQR 127–155) mmHg to 165 (IQR 144–187) mmHg. Left ventricular volumes, ejection fraction, and mass were normal but left atrial volume [31 (IQR 26–37) mL/m^2], plasma NT-proBNP (median 214; IQR 137–356 ng/L),[27] and serum galectin-3 (median 16.1; IQR 13.5–19.7 µg/L) were increased[19] (*Table 2*). Serum concentrations of markers of collagen metabolism were not greater than published normal values but this could reflect differences amongst assays[11,28] (*Table 2*). Participants with a serum galectin-3 above median had higher serum CITP and NT-proBNP but similar PIIINP and PICP.

Follow-up

Data were acquired on 506 (96%) patients at the final visit; 345 (68%) had >250 days of follow-up (Supplementary material online, *Figure S1*). At the 1-month visit, of those assigned to spironolactone, 99 were prescribed 50 mg/day, 118 were prescribed 25 mg/day, 26 were prescribed 25 mg every other day, 10 were not prescribed spironolactone, and information on dose was missing for 12. No participant assigned to the control group was prescribed an MRA. During the trial, serum potassium exceeded 5.5 mmol/L in seven participants assigned to spironolactone but only one developed a value >6.0 mmol/L. Two participants in each group died. Fourteen clinical composite endpoints occurred in 11 participants assigned to placebo and 12 endpoints in nine participants assigned to spironolactone ($P = 0.50$) (Supplementary material online, *Table S4*).

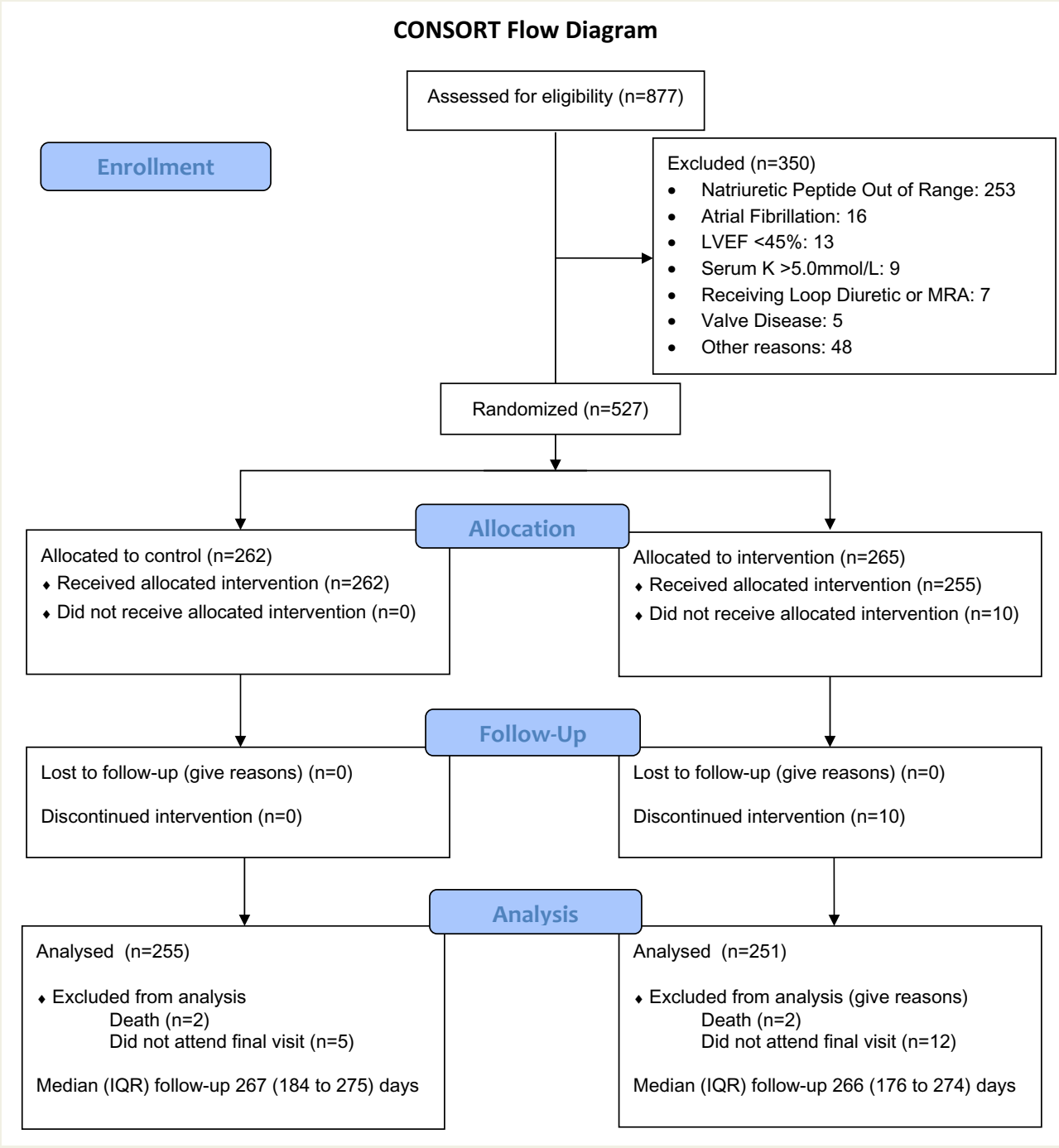


Figure 1 Consort diagram showing patient disposition, including screening, randomization, and follow-up.

Primary endpoint and other markers of fibrosis

Serum PIIINP changed little during follow-up, with a mean difference between groups from baseline to the final visit of -0.15 μg/L (95% CI -0.44 to 0.15; $P = 0.323$); there was no interaction ($P = 0.947$) with galectin-3 (*Figure 2*; *Table 3*). However, in those assigned to spironolactone, there was a greater decline in serum PICP (mean difference -8.1 μg/L; 95% CI -11.9 to -4.3; $P < 0.001$) and increase in CITP and consequently, a greater decline in PICP/CITP ratio (mean difference -2.9; 95% CI -4.3 to -1.5; $P < 0.001$) but, again, no interaction with

Table 1 Patient characteristics at baseline

	Control N = 262		Spironolactone N = 265	
Demographics and lifestyle	**Median**	**Quartiles 1 & 3**	**Median**	**Quartiles 1 & 3**
Age (years)	73	(68 to 78)	73	(69 to 79)
	N =	%	*N* =	%
Women	75	(29)	60	(23)
Current smoker	20	(8)	24	(9)
Prior medical history, *N* (%)	*N* =	%	*N* =	%
Hypertension	199	(76.0)	214	(80.8)
Diabetes mellitus	109	(41.6)	110	(41.5)
Coronary artery disease	190	(72.5)	190	(71.7)
Myocardial infarction	103	(39.3)	113	(42.6)
Percutaneous coronary intervention	138	(52.7)	129	(48.7)
Coronary artery bypass graft	69	(26.3)	67	(25.3)
Stroke/transient ischaemic attack	13	(5.0)	15	(5.7)
Medications	*N* =	%	*N* =	%
Thiazide diuretics	41	(15.6)	46	(17.4)
ACE inhibitors	140	(53.4)	135	(50.9)
Angiotensin receptor blockers	71	(27.1)	74	(27.9)
Beta-blockers	180	(68.7)	186	(70.2)
Calcium channel blockers	51	(19.5)	59	(22.3)
Lipid-lowering therapy	217	(82.8)	218	(82.3)
Aspirin	181	(69.1)	195	(73.6)
Any antiplatelet (including aspirin)	205	(78.2)	209	(78.9)
New York Heart Association Class	*N* =	%	*N* =	%
I	219	(83.6)	223	(84.2)
II	35	(13.4)	33	(12.5)
III	5	(1.9)	5	(1.9)
Patients self-rated symptoms	Median	Quartiles 1 & 3	Median	Quartiles 1 & 3
Breathless on moderate exertion	5	(2 to 6)	5	(2 to 6)
Ankle swelling	0	(0 to 2)	0	(0 to 2)
EQ5D VAS	80	(70 to 90)	76	(66 to 85)
Physical examination	Median	Quartiles 1 & 3	Median	Quartiles 1 & 3
Weight, kg	81.7	(71.0 to 91.0)	82.0	(73.0 to 92.3)
BMI, kg/m^2	27.6	(25.3 to 31.4)	28.4	(25.4 to 31.7)
Heart rate, b.p.m.	61	(54 to 67)	60	(55 to 67)
Systolic blood pressure, mmHg	140	(128 to 155)	140	(126 to 155)
Diastolic blood pressure, mmHg	77	(71 to 84)	78	(71 to 85)
Systolic blood pressure category	*N* =	%	*N* =	%
140–159 mmHg	87	(33.2)	92	(34.7)
≥160 mmHg	45	(17.2)	45	(17.0)

BMI, body mass index; VAS, visual analogue score.

galectin-3 was observed (*Figure 2*; *Table 3*). Serum MMP-1 did not change. Serum galectin-3 increased at both time points for those assigned to spironolactone (*Table 2*).

Secondary endpoints

Systolic blood pressure (mean difference -10 mmHg; 95% CI -13 to -7; $P < 0.001$) and plasma NT-proBNP (mean difference -41 ng/L; 95% CI -75 to -11; $P = 0.009$) were lower at the final visit in those assigned to spironolactone but symptoms, exercise capacity, haemoglobin, troponin, and GDF-15 were no different (*Figure 3*; *Tables 4 and 5*). Serum concentrations of sodium fell, and serum potassium, urea and creatinine rose on spironolactone. Small reductions in QRS duration ($P = 0.003$), left atrial volume index ($P = 0.010$), left ventricular mass index ($P = 0.079$) and early mitral flow (E-wave) velocity ($P < 0.001$), and increases in LVEF ($P = 0.022$) were observed in those assigned to spironolactone (*Table 5*) but the ratio of *E* to early

Table 2 Investigations at baseline

Baseline investigations	Control N = 262		Spironolactone N = 265	
Shuttle walk-test	**Median**	**Quartiles 1 & 3**	**Median**	**Quartiles 1 & 3**
Number of shuttles completed	50	(32 to 70)	48	(33 to 65)
Breathlessness score after exercise	5	(3 to 7)	5	(3 to 7)
Post-exercise heart rate, b.p.m.	76	(64 to 93)	76	(64 to 90)
Post-exercise, systolic BP, mmHg	165	(144 to 187)	165	(144 to 188)
Electrocardiogram and echocardiography				
QRS duration (ms)	92	(84 to 102)	92	(84 to 108)
LV end-diastolic volume index (mL/m^2)	42	(36 to 49)	41	(35 to 48)
LV ejection fraction (%)	63	(57 to 66)	63	(59 to 67)
Left ventricular mass index (g/m^2)	95	(81 to 113)	94	(81 to 111)
Men	97	(79 to 103)	94	(82 to 111)
Women	88	(79 to 103)	96	(81 to 109)
Left atrial volume index (mL/m^2)	31	(26 to 37)	30	(26 to 35)
Early mitral flow velocity (E) m/s	0.7	(0.6 to 0.8)	0.7	(0.6 to 0.8)
Late (atrial) mitral flow velocity (A) m/s	0.8	(0.7 to 0.9)	0.8	(0.7 to 0.9)
E/A ratio	0.8	(0.7 to 1.0)	0.8	(0.7 to 1.0)
E/ early diastolic tissue velocity (e') ratio	9.4	(7.5 to 11.8)	9.2	(7.6 to 11.3)
Tricuspid annular plane systolic excursion	22.4	(16.7 to 26.6)	21.7	(17.5 to 26.2)
Blood tests				
Haemoglobin, g/dL	14.1	(13.2 to 14.9)	14.0	(13.1 to 14.9)
Sodium, mmol/L	139	(138 to 141)	140	(138 to 141)
Potassium, mmol/L	4.3	(4.1 to 4.6)	4.3	(4.0 to 4.6)
Creatinine, µmol/L	84	(74 to 98)	84	(71 to 100)
Galectin-3, µg/L	16.1	(13.2 to 19.7)	16.0	(13.8 to 19.7)
High-sensitivity troponin-T, ng/L	12.9	(9.2 to 18.5)	12.7	(8.4 to 17.6)
Growth differentiation factor, 15 ng/L	1,421	(1,011 to 1,954)	1,467	(1,076 to 2,207)
Collagen markers and NT-proBNP				
PIIINP, µg/L	4.0	(3.0 to 5.2)	3.9	(3.2 to 4.9)
PICP, µg/L	80.9	(67.2 to 97.6)	79.1	(63.2 to 97.0)
CITP, µg/L	3.8	(2.9 to 5.1)	3.7	(2.8 to 4.8)
MMP-1, µg/L	10.2	(6.7 to 15.3)	10.2	(6.7 to 17.1)
PICP/CITP, ratio	20.7	(15.6 to 28.9)	21.7	(16.2 to 28.3)
NT-proBNP, ng/L (core lab)	217	(134 to 334)	206	(135 to 368)
Galectin-3 below median				
PIIINP, µg/L	3.8	(3.0 to 5.1)	3.7	(3.1 to 4.6)
PICP, µg/L	79.2	(66.5 to 95.6)	80.4	(65.4 to 94.8)
CITP, µg/L	3.4	(2.7 to 4.2)	3.2	(2.7 to 4.3)
MMP-1, µg/L	10.2	(6.5 to 14.0)	9.1	(6.7 to 15.5)
PICP/CITP ratio	22.0	(17.5 to 30.3)	23.6	(17.3 to 29.8)
NT-proBNP, ng/L (core lab)	195	(119 to 292)	172	(132 to 296)
Galectin-3 above median				
PIIINP, µg/L	4.1	(3.1 to 5.3)	4.0	(3.2 to 4.9)
PICP, µg/L	81.2	(69.9 to 98.7)	78.4	(63.1 to 103.2)
CITP, µg/L	4.1	(3.1 to 5.6)	4.1	(3.0 to 5.3)
MMP-1, µg/L	10.2	(6.7 to 15.7)	11.3	(6.7 to 18.8)
PICP/CITP, ratio	19.6	(14.7 to 25.5)	19.6	(14.7 to 26.6)
NT-proBNP, ng/L (core lab)	239	(145 to 374)	263	(140 to 454)

BP, blood pressure; CITP, collagen type-1 C-terminal telopeptide; LV, left ventricular; MMP-I, matrix metalloproteinase-1; NT-proBNP, amino-terminal B-type natriuretic peptide; PICP, procollagen type-I C-terminal pro-peptide; PIIINP, procollagen type-III N-terminal pro-peptide.

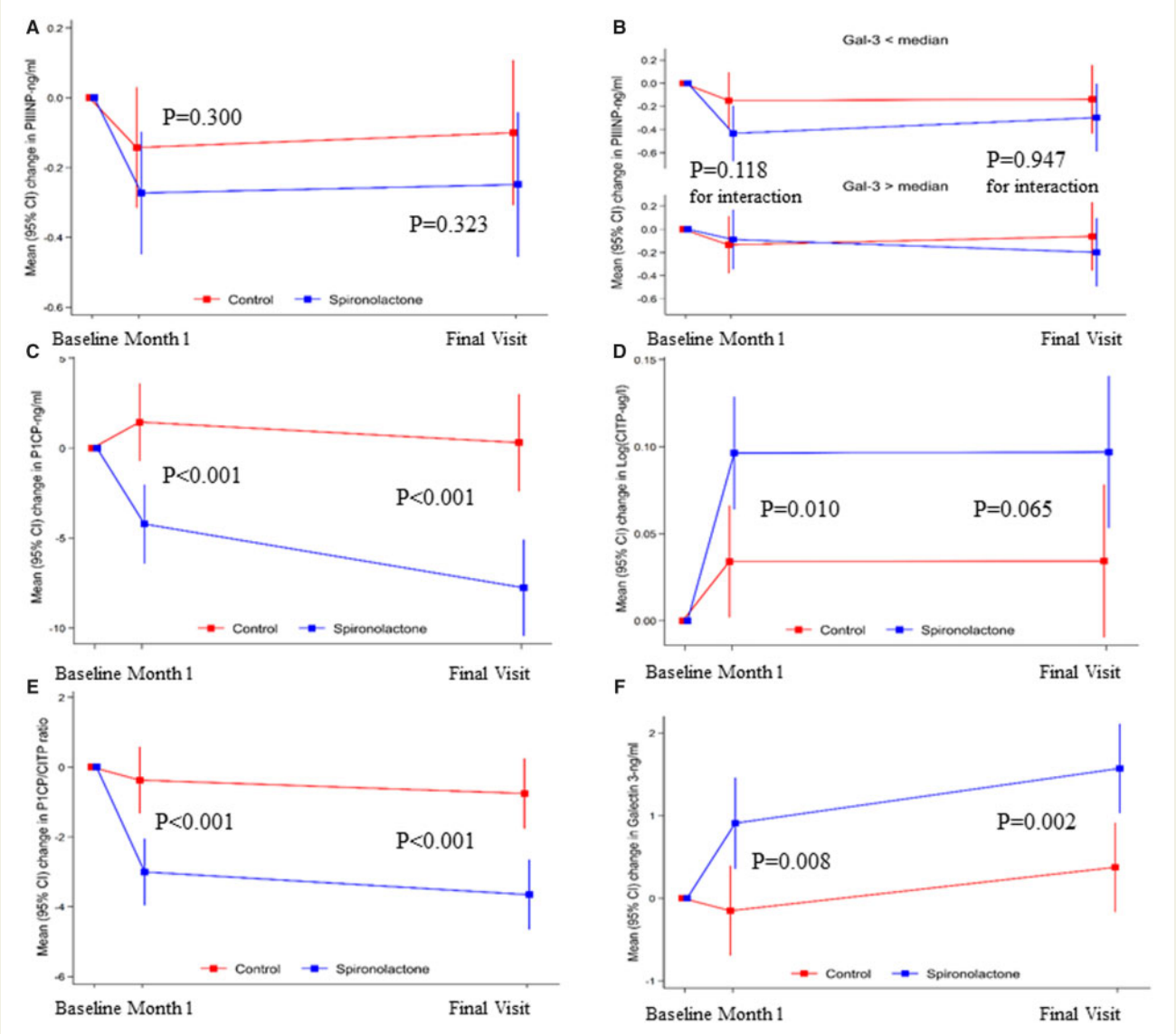


Figure 2 Changes from baseline to 1 month and final visits for serum concentrations of (*A*) procollagen type-III N-terminal pro-peptide (PIIINP); (*B*) procollagen type-III N-terminal pro-peptide for those with a baseline serum galectin above or below median; (*C*) procollagen type-I C-terminal pro-peptide (PICP); (*D*) collagen type-1 C-terminal telopeptide (CITP); (*E*) the ratio of procollagen type-I C-terminal pro-peptide to collagen type-1 C-terminal telopeptide; (*F*) Galectin-3. Data shown are mean change and standard deviation. *P*-values are for the comparison between intervention and control except for (*B*) where the *P*-values refer to the interaction between baseline serum galectin-3 and changes in plasma concentrations of procollagen type-III N-terminal pro-peptide. The interaction between baseline galectin-3 and change from baseline to final visit in procollagen type-III N-terminal pro-peptide was the primary endpoint of the trial ($P = 0.947$ for the interaction).

diastolic tissue velocity (e′) did not change. At 1 month, results were generally similar to those observed at the end of the trial.

Discussion

In contrast to some previous reports, we did not show reductions in serum PIIINP after administration of spironolactone nor did we observe an interaction with baseline serum galectin-3.[11] However, we did observe a decline in serum PICP and a rise in CITP with spironolactone, suggesting, respectively, reduced synthesis and increased degradation of type-I collagen.[10,29–31] Changes in PICP and CITP were prominent within 1 month, persisted and were accompanied by reductions in left atrial volume and increases in LVEF in the longer term, indicating favourable effects on cardiac structure and function (*Take home figure*).

Type-I collagen comprises large-diameter fibres with a high propensity for cross-linking that make a substantial contribution to myocardial stiffness compared with the finer type-III collagen fibres.[10] Pathological myocardial fibrosis is characterized by an excess of

Table 3 Changes in Markers of Collagen Metabolism at 1 month and final visit for participants randomized to spironolactone or control

Serum Markers of Collagen Metabolism								
	One month				End of trial			
	Control	Spironolactone		*P*	Control	Spironolactone		*P*
n	257	259			255	251		
	Mean (SD) change		Mean difference (95% CI)		Mean (SD) change		Mean difference (95% CI)	
Galectin-3 μg/L	**−0.13 (3.9)**	**+0.89 (4.8)**	**+1.06 (0.28 to 1.84)**	**=0.008**	**+0.40 (4.4)**	**+1.55 (4.2)**	**+1.20 (0.43 to 1.96)**	**=0.002**
PIIINP μg/L	−0.22 (1.9)	−0.19 (1.5)	−0.13 (−0.38 to 0.12)	=0.300	−0.20 (2.0)	−0.15 (1.7)	−0.15 (−0.44 to 0.15)	0.323
<Bas.Galectin-3	−0.12 (1.8)	−0.30 (1.4)	−0.28 (−0.63 to 0.06)	Interaction	−0.12 (2.0)	−0.16 (1.9)	−0.16 (−0.57 to 0.26)	Interaction
>Bas.Galectin-3	−0.33 (1.9)	−0.07 (1.6)	+0.05 (−0.31 to 0.40)	=0.188	−0.27 (2.1)	−0.15 (1.5)	−0.14 (−0.56 to 0.28)	0.947
PICP μg/L	**1.2 (18.1)**	**−4.0 (19.0)**	**−5.7 (−8.8 to −2.6)**	**<0.001**	**−0.1 (21.5)**	**−7.3 (24.4)**	**−8.1 (−11.9 to −4.3)**	**<0.001**
<Bas.Galectin-3	2.4 (18.1)	−3.0 (18.8)	−6.1 (−10.4 to −1.8)	Interaction	−0.1 (20.1)	−5.1 (23.1)	−6.1 (−11.5 to −0.7)	Interaction
>Bas.Galectin-3	0.0 (18.2)	−5.2 (19.1)	−5.5 (−9.9 to −1.0)	=0.846	−0.2 (22.9)	−9.8 (25.7)	−10.3 (−15.7 to −4.9)	=0.280
CITP μg/L	**+0.03 (0.2)**	**+0.10 (0.3)**	**+0.06 (0.01 to 0.10)**	**=0.010**	+0.03 (0.3)	+0.10 (0.4)	+0.06 (−0.00 to 0.12)	=0.065
<Bas.Galectin-3	+0.01 (0.2)	+0.07 (0.3)	+0.05 (−0.01 to 0.11)	Interaction	0.00 (0.3)	+0.06 (0.4)	+0.04 (−0.04 to 0.13)	Interaction
>Bas.Galectin-3	+0.06 (0.2)	+0.13 (0.3)	+0.07 (0.01 to 0.13)	=0.723	+0.07 (0.4)	+0.15 (0.4)	+0.08 (−0.01 to 0.16)	=0.539
PICP/CITP ratio	**−0.3 (7.7)**	**−3.1 (9.7)**	**−2.6 (−4.0 to −1.3)**	**<0.001**	**−0.7 (8.3)**	**−3.7 (10.1)**	**−2.9 (−4.3 to −1.5)**	**<0.001**
<Bas.Galectin-3	+0.7 (8.4)	−2.8 (11.0)	−3.1 (−4.9 to −1.3)	Interaction	−0.3 (8.9)	−3.4 (11.6)	−2.4 (−4.4 to −0.4)	Interaction
>Bas.Galectin-3	−1.3 (6.7)	−3.5 (8.0)	−2.3 (−4.2 to −0.4)	=0.573	−1.0 (7.6)	−4.2 (8.3)	−3.5 (−5.5 to −1.5)	=0.442
MMP-1 μg/L	−0.01 (0.2)	0.00 (0.2)	0.00 (−0.03 to 0.04)	0.871	−0.01 (0.3)	−0.01 (0.3)	0.01 (−0.04 to 0.06)	0.715
<Bas.Galectin-3	−0.02 (0.2)	−0.01 (0.2)	+0.01 (−0.04 to 0.06)	Interaction	0.00 (0.3)	+0.01 (0.2)	−0.00 (−0.05 to 0.05)	Interaction
>Bas.Galectin-3	+0.01 (0.2)	+0.01 (0.2)	0.00 (−0.05 to 0.05)	=0.849	−0.01 (0.2)	−0.03 (0.3)	−0.01 (−0.08 to 0.05)	=0.407

<Bas., less than median serum galectin-3 at baseline; >Bas., greater than median serum galectin-3 at baseline; CITP, collagen type-1 C-terminal telopeptide; MMP-I, matrix metalloproteinase-1; NT-proBNP, amino-terminal B-type natriuretic peptide; PICP, procollagen type-I C-terminal pro-peptide; PIIINP, procollagen type-III N-terminal pro-peptide; SD, standard deviation.
Values shown in bold are significant at a *P*-value <0.05.

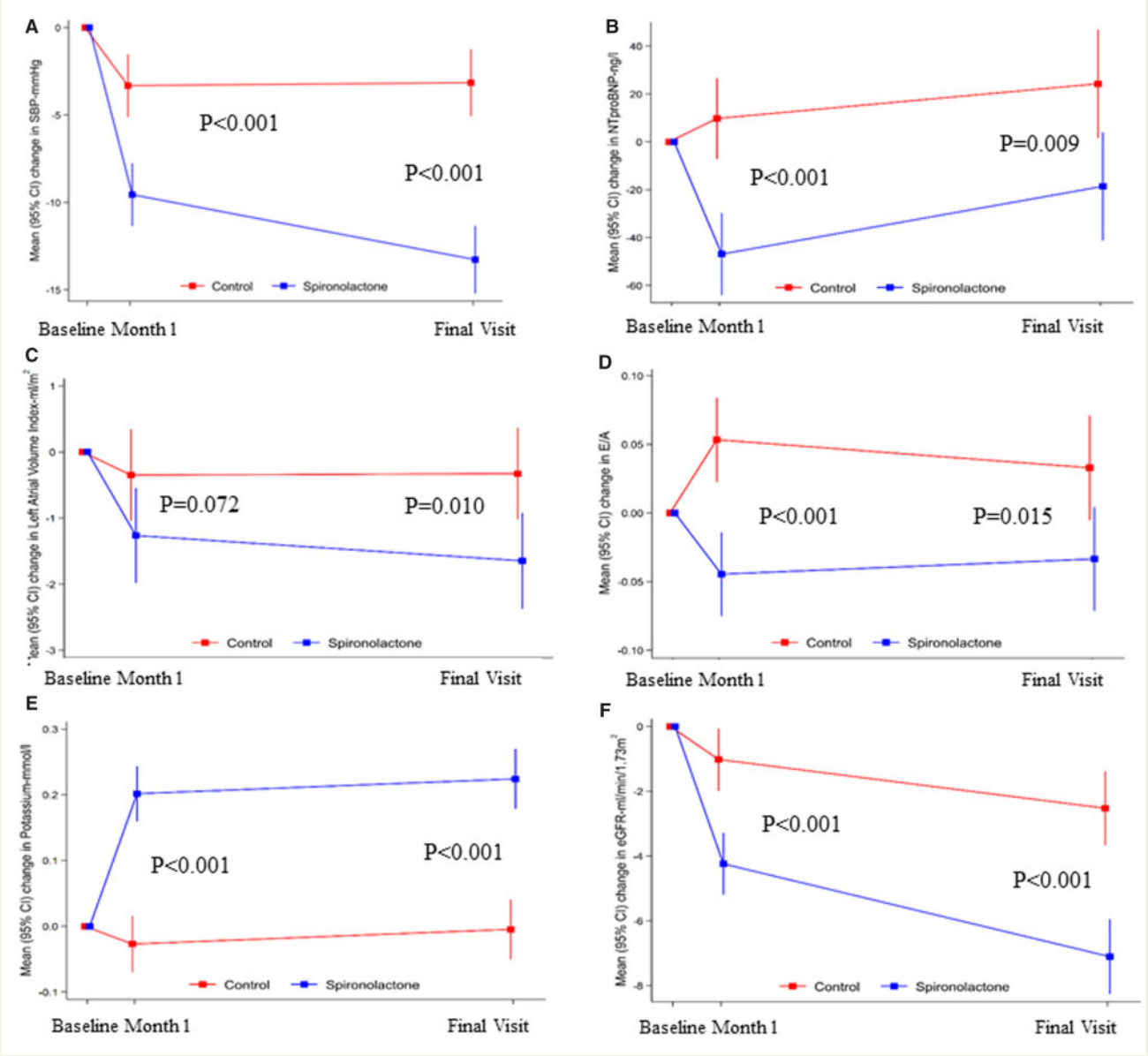


Figure 3 Changes from baseline to 1 month and to the final visit in (*A*) systolic blood pressure; (*B*) plasma concentrations of NT-proBNP; (*C*) left atrial volume index; (*D*) E/A ratio; (*E*) serum potassium; (*F*) estimated glomerular filtration rate. Data shown are mean change and standard deviation. *P*-values are for the comparison between intervention and control.

type-I compared with type-III collagen that, along with hypertrophy, contributes to a restrictive ventricular pathophysiology, leading to increases in diastolic ventricular pressures and atrial dilation that may culminate in HFpEF.[10,11,30,32] Our data suggest that MRA might reduce or reverse accumulation of type-I collagen but have little or no effect on type-III collagen, effects that might be considered advantageous for an intervention aimed at preventing or reversing pathological myocardial fibrosis. If changes in serum markers of type-1 collagen also indicate a favourable clinical response to spironolactone, this casts doubt on the utility of galectin-3 as a means of selecting patients for treatment with spironolactone, if future trials show that MRA can delay or prevent the onset of heart failure.

We are aware of only one study that obtained myocardial biopsies before and after administration of spironolactone in patients with heart failure,[33] where a serum PICP/CITP of >35, rare amongst participants in our trial, had more myocardial fibrosis and a greater abundance of both type-I and type-III collagen. Spironolactone reduced both PICP/CITP ratio and myocardial collagen fraction. Several other trials have investigated the effects of spironolactone on serum collagen markers in a broad range of cardiovascular diseases,[11] generally showing that serum PIIINP was not markedly different in people with and without cardiovascular disease but that administration of MRA to patients with severe HFrEF reduced serum PIIINP, which is why it was chosen as the primary efficacy marker for HOMAGE.[11]

Table 4 **Changes in clinical variables and laboratory tests at 1 month and final visit for participants randomized to spironolactone or control**

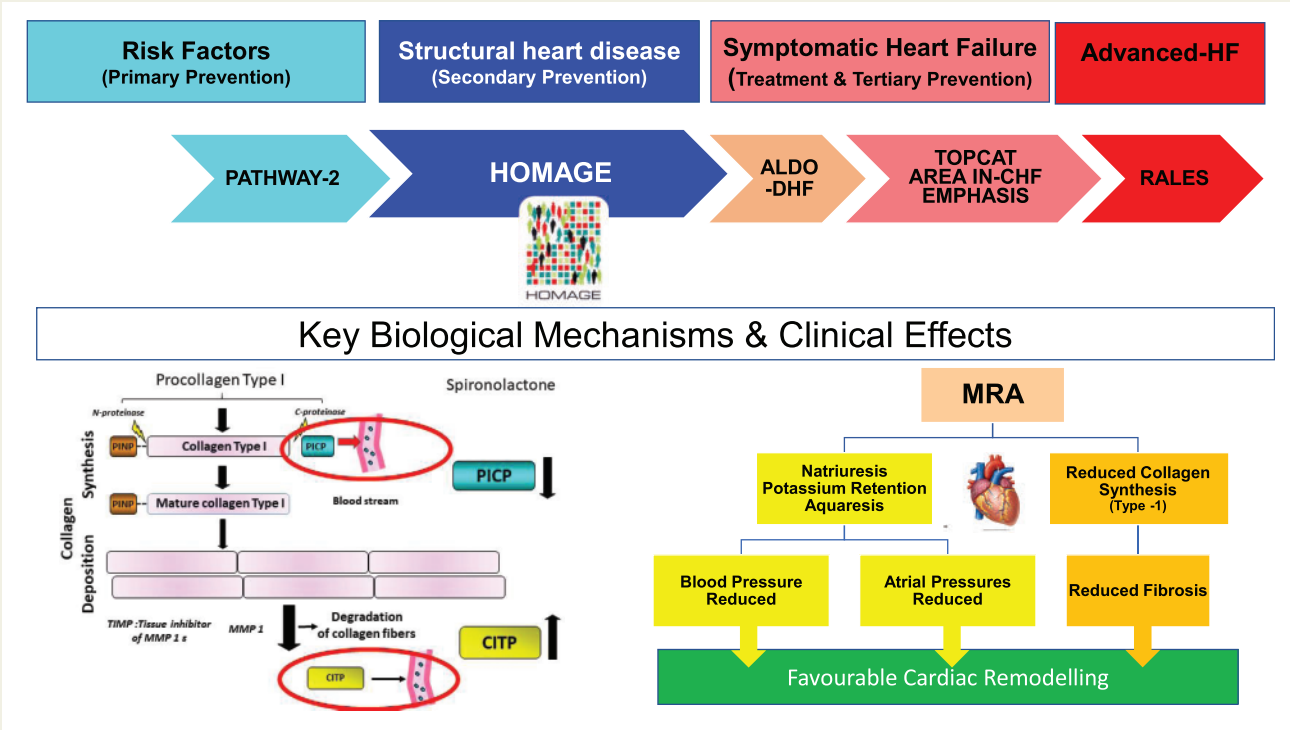
Clinical variables								
	One month				End of trial			
	Control	Spironolactone	Difference	*P*	Control	Spironolactone	Difference	*P*
N	257	259			255	251		
Days follow-up	31 (28 to 35)	32 (28 to 35)			267 (184 to 275)	266 (176 to 274)		
NYHA >1	*N* = 33	*N* = 40	+7	0.669	*N*=35	*N*=35	0	0.765
Loop diuretic	*N* = 2	*N* = 1	−1	0.558	*N*=7	*N*=2	−5	0.097
	Mean (SD) change		Mean difference (95% CI)		Mean (SD) change		Mean difference (95% CI)	
Breathlessness	−0.1 (2.1)	−0.1 (2.3)	+0.1 (−0.4 to +0.4)	0.884	0.0 (2.2)	+0.1 (2.4)	+0.1 (−0.3 to +0.4)	0.731
Ankle Swelling	−0.1 (1.6)	−0.1 (1.6)	+0.1 (−0.2 to +0.4)	0.547	−0.2 (1.8)	0.0 (2.0)	+0.3 (0.0 to +0.6)	0.061
EQ5D VAS	+2 (14)	+1 (15)	−2 (−4 to 0)	0.077	0 (15)	−1 (16)	−1 (−3 to +1)	0.380
Shuttles Completed	+0.5 (8.7)	+1.5 (9.5)	+1.0 (−0.9 to +2.8)	0.322	+1.6 (17.2)	+3.7 (20)	+1.6 (−1.7 to +5.0)	0.342
Physical signs								
Weight (kg)	**0.1 (1.5)**	**−0.5 (1.2)**	**−0.5 (−0.8 to −0.3)**	**<0.001**	−0.5 (2.8)	−0.9 (2.5)	−0.3 (−0.8 to 0.2)	0.178
Heart Rate (bpm)	0 (8)	0 (7)	0 (−1 to +2)	0.490	0 (8)	−1 (7)	−1 (−2 to +1)	0.329
Sys BP (mmHg)	**−3 (16)**	**−9 (16)**	**−6 (−9 to −3)**	**<0.001**	**−3 (19)**	**−13 (18)**	**−10 (−13 to −7)**	**<0.001**
Dia BP (mmHg)	**0 (11)**	**−3 (8)**	**−2 (−4 to +1)**	**0.003**	**−1 (10)**	**−4 (9)**	**−3 (−5 to −2)**	**<0.001**
Post-exercise	**−3 (25)**	**−11 (26)**	**−8 (−14 to −3)**	**0.001**	**−5 (27)**	**−16 (27)**	**−11 (−16 to −7)**	**<0.001**
Sys. BP (mmHg)								
Laboratory tests								
Haemoglobin (g/dL)	−0.2 (0.6)	−0.1 (0.6)	+0.1 (0 to +0.2)	0.110	−0.3 (0.8)	−0.4 (0.9)	−0.1 (−0.3 to 0.0)	0.089
Sodium (mmol/L)	**+0.2 (2.1)**	**−1.0 (2.4)**	**−1.2 (−1.5 to −0.8)**	**<0.001**	**0.0 (2.4)**	**−1.1 (2.5)**	**−1.2 (−1.6 to −0.8)**	**<0.001**
Potassium (mmol/L)	**0.0 (0.3)**	**+0.2 (0.4)**	**+0.2 (+0.2 to +0.3)**	**<0.001**	**0.0 (0.4)**	**+0.2 (0.4)**	**+0.2 (+0.2. +0.3)**	**<0.001**
>5.5 mmol/L	*N* = 1	*N* = 7		0.110	*N*= 1	*N* = 4		0.079
Urea (mmol/L)	**+0.4 (2.8)**	**+1.4 (3.2)**	**+1.0 (+0.5 to +1.6)**	**<0.001**	**+0.5 (3.4)**	**+1.8 (4.1)**	**+1.3 (+0.7 to +2.0)**	**<0.001**
Creatinine (μmol/L)	**+1 (9)**	**+5 (10)**	**+4 (+3 to +6)**	**<0.001**	**+4 (17)**	**+9 (13)**	**+5 (+2 to +8)**	**<0.001**
eGFR mL/min/1.73m^2	**−1 (8)**	**−4 (8)**	**−3 (−5 to −2)**	**<0.001**	**−2 (9)**	**−7 (10)**	**−5 (−6 to −3)**	**<0.001**
Number <45 mL/min/1.73m^2	*N* = 13	*N* = 25		0.143	***N* = 20**	***N* =32**		**0.032**

Dia BP, diastolic blood pressure; eGFR, estimated glomerular filtration rate by the four-variable modified diet in renal disease (MDRD) equation; NYHA, New York Heart Association Class; Sys BP, systolic blood pressure; VAS, visual analogue score.
Values shown in bold are significant at a *P*-value <0.05

Table 5 Changes in cardiac biomarkers and echocardiography at 1 month and final visit for participants randomised to spironolactone or control

Biomarkers of cardiac function and echocardiographic variables								
	One month				End of trial			
	Control	Spironolactone		*P*	Control	Spironolactone		*P*
N	257	259			255	251		
	Mean change (SD)		Mean difference (95% CI)		Mean (SD) change		Mean difference (95% CI)	
NT-proBNP ng/L	**+9**	**−47 (143)**	**−57 (−81; −33)**	**<0.001**	**+26 (212)**	**−20 (167)**	**−43 (−75 to −11)**	**0.009**
hsTnT ng/L	0.0	0.0	0.0	0.809	0.0	0.1	0.0 (0.0 to +0.1	0.113
GDF-15 ng/L	0.0	0.9	0.0	0.939	0.1	0.1	0.0 (0.0 to +0.1)	0.095
	Mean (SD) change				Mean (SD) change			
QRS (ms)	0 (10)	0 (14)	0 (−2 to +2)	0.345	**+1 (10)**	**−2 (10)**	**−3 (−5 to −1)**	**0.003**
LVEDVi (mL/m^2)	−2 (5)	−1 (5)	0 (−1 to +2)	0.551	−1 (4)	−1 (4)	0 (−1 to +1)	0.984
LVEF (%)	+0.7 (4.8)	+0.3 (5.3)	−0.5 (−1.7 to +0.7)	0.404	**−0.4 (4.4)**	**+0.8 (4.2)**	**+1.2 (+0.2 to +2.2)**	**0.022**
LVMi (g/m^2)	0 (12)	−1 (14)	−1 (−4 to +1)	0.296	−1 (12)	−3 (13)	−2 (−4 to 0)	0.079
LAVI (mL/m^2)	0 (5)	−1 (4)	−1 (−2 to 0)	0.072	**0 (5)**	**−2 (5)**	**−1 (−2 to 0)**	**0.010**
E m/s	**+0.02 (0.13)**	**−0.04 (0.14)**	**−0.06 (−0.08 to −0.03)**	**<0.001**	**+0.01 (0.14)**	**−0.04 (0.14)**	**−0.05 (−0.07 to −0.02)**	**<0.001**
A m/s	−0.01 (0.12)	−0.02 (0.12)	0.00 (−0.03 to 0.02)	0.855	0.00 (0.013)	−0.02 (0.14)	−0.01 (−0.04 to +0.01)	0.268
E/A	**+0.05 (0.22)**	**−0.04 (0.23)**	**−0.10 (−0.14 to −0.05)**	**<0.001**	**+0.03 (0.30)**	**−0.04 (0.28)**	**−0.07 (−0.12 to −0.01)**	**0.015**
E/e'	+0.05 (2.43)	−0.22 (2.3)	−0.22 (−0.68 to +0.25)	0.363	−0.02 (2.66)	−0.34 (2.30)	−0.38 (−0.83 to 0.08)	0.103
TAPSE	−0.6 (5.2)	−0.3 (5.2)	−0.1 (−1.0 to +0.9)	0.887	0.0 (5.3)	−0.3 (5.3)	−0.5 (−1.4 to +0.5)	0.305

A, late (atrial) mitral flow velocity; E, early mitral flow velocity; e', early diastolic tissue velocity; GDF-15, growth differentiation factor-15; hsTnT, high-sensitivity troponin-T; LAVI, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; SD, standard deviation; TAPSE, tricuspid annular plane systolic excursion.
Values shown in bold are significant at a *P*-value <0.05



Take home figure: Diagram showing the evolution from risk factors, through structural heart disease to heart failure and the randomized controlled trials of mineralo-corticoid receptor antagonists (MRA) that have addressed each stage. The PATHWAY-2 trial[39] demonstrated the effects of spironolactone on blood pressure, a key risk factor for heart failure. HOMAGE is the only trial, to date, that has focused on patients with structural heart disease with few or no symptoms of heart failure. The ALDO-DHF trial[14] showed favourable effects on ventricular filling in patients with a preserved left ventricular ejection fraction (LVEF) and heart failure (HFpEF). Many patients in ALDO-DHF had less severe cardiac dysfunction than in HOMAGE; there is substantial overlap in the patient characteristics of these two trials. TOPCAT[15] investigated the effects of spironolactone in HFpEF with equivocal results. EMPHASIS,[13] AREA IN-CHF,[34] and RALES[9] investigated the effects of mineralo-corticoid receptor antagonists in HFrEF (heart failure with a reduced left ventricular ejection fraction). In HOMAGE, spironolactone caused an early reduction in weight, blood pressure, and natriuretic peptides, suggesting a natriuretic and diuretic effect. Changes in serum markers of type-1, although not type-III, collagen metabolism were also observed within 1 month. This combination of effects was followed by favourable cardiac remodelling. Type-1 collagen is the more important contributor to myocardial stiffness. The magnitude of changes in collagen metabolites observed suggests a systemic effect of spironolactone rather than only on the myocardium.

However, in one substantial trial of patients with less severe HFrEF, canrenone, the active metabolite of spironolactone, did not reduce serum PIIINP, despite reducing left ventricular mass, left atrial diameter, and B-type natriuretic peptide and increasing LVEF.[34] Differences between assays or disease-state may account for these apparent inconsistencies.

Serum PICP is raised in people with cardiovascular disease and also declines with administration of an MRA.[11,35] Fewer trials have investigated the effects of MRA on serum CITP and found no consistent effect.[11] Duration of treatment may be important; we observed clearer reductions in CITP with spironolactone at 1 month compared with the final visit, which might reflect an early increase in the rate of collagen turnover before it subsides to a new steady-state. We observed a rise in galectin-3 with the administration of spironolactone as have others.[36] This might reflect increased galectin-3 production due to potassium-mediated increases in aldosterone subsequent to administration of an MRA,[37] or reduced galectin-3 clearance due to the decline in eGFR.

Spironolactone reduced collagen marker within a few weeks, suggesting that they reflect changes in the rate of turnover rather than the mass of collagen. However, a favourable effect on turnover should eventually reduce fibrosis. Blood concentrations of biomarkers reflect the equilibrium between production and disposal. Plasma concentrations of biomarkers cleared by the kidney should rise because eGFR declines with the introduction of MRA. This suggests that the decline in PICP is due to reduced production, which could reflect favourable effects of MRA on ventricular pre- and afterload or a direct effect of spironolactone on collagen production or processing.[29,38] Many of our participants had inadequately controlled blood pressure, which fell substantially in those assigned to spironolactone, as observed in trials of resistant hypertension,[39] and was accompanied by a decline in NT-proBNP and, in the longer term, echocardiographic evidence of cardiac remodelling. However, the heart contains only a small proportion of the body's collagen. If spironolactone has a specific effect only on cardiovascular collagen, then the effect would have to be very large in order to change serum

marker concentrations. It is more likely that changes in serum PICP indicate an effect on fibroblasts in many organs.[10]

Assuming an extra-cellular fluid volume of 15 L, a rise in serum potassium concentration of just 0.2 mmol/L, as we observed, would require retention of only 3–4 mmol of potassium. If MRA cause retention of much larger amounts, as is likely,[40] potassium must be transferred to the intra-cellular space and, therefore, assuming no change in intra-cellular potassium concentration, intra-cellular water must increase.[41] Accordingly, changes in weight may underestimate the effect of spironolactone on extra-cellular water volume. NT-proBNP and left atrial volume both declined on spironolactone, implying a reduction in cardiac filling pressures that might be due to a subtle reduction in plasma volume, although haemoglobin did not change, or a reduction in afterload or improved myocardial function. Serum sodium concentration fell, implying even greater loss of extra-cellular sodium than of water.

Our population had normal left ventricular end-diastolic volumes and LVEF but left atrial volume and left ventricular mass were increased. We excluded patients with a diagnosis of heart failure or atrial fibrillation and those taking loop diuretics but, with the exception of *E*/*e*′, our patients had more severe cardiac dysfunction than many of those enrolled in trials of HFpEF.[11] Many of our patients also reported breathlessness on moderate exertion and had reduced exercise capacity and therefore fulfilled all of the diagnostic criteria for HFpEF; symptoms, reduced exercise capacity, cardiac dysfunction and raised NT-proBNP. Spironolactone reduced left atrial volume and NT-proBNP, suggesting favourable effects on cardiac structure and function that could delay or prevent the onset of clinically overt heart failure, although our trial was neither large nor long enough to demonstrate such an effect and no differences in symptoms or exercise capacity were observed.

The HOMAGE trial did not enrol the pre-specified number of participants or follow all those included for the intended duration. However, even if we had conducted the trial as originally planned a different result is unlikely. Interpretation of secondary endpoints in a clinical trial with a neutral primary endpoint should be considered hypothesis-generating rather than definitive. Also, we did not make adjustments for multiple hypothesis testing. Many patients had poorly controlled hypertension and, as discussed above, reduction in blood pressure could have made an important contribution to our results. We cannot exclude the possibility that some of our patients had transthyretin or other types of cardiac amyloidosis. Our trial was a mechanistic proof-of-concept trial and should be interpreted in the context of information from other trials.

In conclusion, spironolactone did not reduce serum concentrations PIIINP and serum galectin-3, measured at baseline, did not identify a subgroup with a greater response. The effect of spironolactone on PICP/CITP ratio appears early and is sustained, suggesting that the effects of MRA on type-I collagen metabolism may be underappreciated. Future research on the effects of MRA and other interventions on collagen metabolism in cardiac disease should consider measuring PICP/CITP as evidence of biological activity. The effects of spironolactone on type-I collagen turnover, blood pressure and cardiac function in people with a cardiovascular profile consistent with clinically occult HFpEF, indicate the need for clinical trials to determine whether timely intervention with an MRA can delay or prevent progression to clinically overt heart failure.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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