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
Introduction

Many people with cardiovascular disease will develop heart failure, leading to substantial disability, demands on health-services and mortality. Early identification of cardiac dysfunction and therapeutic targeting of specific pathways of disease progression, such as myocardial and vascular fibrosis, 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). MRA also reduce serum markers of collagen synthesis in patients with a range of cardiovascular diseases, including HFrEF and HfPEF, which might reflect favourable effects on fibrosis. Galectin-3 is a proposed marker of fibrotic activity and, in experimental models, a mediator of aldosterone-induced fibrosis. In the general population, higher plasma concentrations of galectin-3 predict the development of heart failure and, subsequently, a worse outcome.

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 (HOMEAGE) 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. The HOMEAGE clinical trial was a prospective, randomized, open-label, blinded-endpoint (PROBE) multi-centre trial, investigating the 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. 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 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/m²; P = 0.10), 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

Trials 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 125–1000ng/L or BNP 35–280ng/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 m², 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. Serum galectin-3 was chosen as a marker of fibrotic activity. 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 pro-peptide; 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. 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 Diagnostica3), PICP by enzyme immunoassay (METRA; Quidel Corporation) and matrix metalloproteinase-1 (MMP-1) by an amplified luminescent proximity homogenous assay (ALPHA-LISA) (PerkinElmer6). 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 (ELECSYS6; 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 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.25 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 m2. 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/m2], 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 increased19 (Table 2). Serum concentrations of markers of collagen metabolism were not greater than published normal values but this could reflect differences amongst assays11,28 (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).
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...
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

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### Table 1  Patient characteristics at baseline

| Demographics and lifestyle | Control \( N = 262 \) | Quartiles 1 & 3 | Spironolactone \( N = 265 \) | Quartiles 1 & 3 |
|---------------------------|---------------------|----------------|-----------------------------|----------------|
| Age (years)               | 73 (68 to 78)       | N =           | 73 (69 to 79)               | N =           |
| Women                     | 75 (29)             | 60 (23)       | 24 (9)                      |
| Current smoker            | 20 (8)              | 24 (9)        |
| Prior medical history, N (%) | N =               | N =           |
| Hypertension              | 199 (76.0)          | 214 (80.8)    | 110 (41.5)                  |
| Diabetes mellitus         | 109 (41.6)          | 110 (41.5)    | 103 (39.3)                  |
| Coronary artery disease   | 190 (72.5)          | 190 (71.7)    | 138 (52.7)                  |
| Myocardial infarction     | 103 (39.3)          | 113 (42.6)    | 129 (48.7)                  |
| Percutaneous coronary intervention | 138 (52.7)          | 129 (48.7)    | 69 (26.3)                   |
| Coronary artery bypass graft | 69 (26.3)          | 67 (25.3)     | 13 (5.0)                    |
| Stroke/transient ischaemic attack | 13 (5.0)          | 15 (5.7)      |
| Medications               | N =                 | N =           |
| Thiazide diuretics        | 41 (15.6)           | 46 (17.4)     | 140 (53.4)                  |
| ACE inhibitors            | 140 (53.4)          | 135 (50.9)    | 71 (27.1)                   |
| Angiotensin receptor blockers | 71 (27.1)        | 74 (27.9)     | 180 (68.7)                  |
| Beta-blockers             | 180 (68.7)          | 186 (70.2)    | 51 (19.5)                   |
| Calcium channel blockers  | 51 (19.5)           | 59 (23.3)     | 217 (82.8)                  |
| Lipid-lowering therapy    | 217 (82.8)          | 218 (82.3)    | 181 (69.1)                  |
| Aspirin                   | 181 (69.1)          | 195 (73.6)    | 205 (78.2)                  |
| New York Heart Association Class | N =                | N =           |
| I                         | 219 (83.6)          | 223 (84.2)    | 35 (13.4)                   |
| II                        | 35 (13.4)           | 33 (12.5)     | 5 (1.9)                     |
| III                       | 5 (1.9)             | 5 (1.9)       |
| Patients self-rated symptoms | Median            | Median        |
| Breathless on moderate exertion | 5 (2 to 6)       | 5 (2 to 6)    | 0 (0 to 2)                  |
| Ankle swelling            | 0 (0 to 2)          | 0 (0 to 2)    | 80 (70 to 90)               |
| EQSD VAS                  | 80 (70 to 90)       | 76 (66 to 85) | 205 (78.2) |
| Physical examination      | Median              | Median        |
| Weight, kg                | 81.7 (71.0 to 91.0) | 82.0 (73.0 to 92.3) |
| BMI, kg/m²                | 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) | 140 (128 to 155)            |
| Systolic blood pressure, mmHg | 140 (128 to 155) | 140 (126 to 155) | 77 (71 to 84) |
| 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)     | 45 (17.2)                   |
| >160 mmHg                 | 45 (17.2)           | 45 (17.0)     |

BMI, body mass index; VAS, visual analogue score.
Table 2  Investigations at baseline

| Baseline investigations | Control  | Spironolactone |
|-------------------------|----------|----------------|
|                         | N = 262  | N = 265        |
| Shuttle walk-test       | Median   | Quartiles 1 & 3| Median   | Quartiles 1 & 3|
| Number of shuttles      | 50       | (32 to 70)     | 48       | (33 to 65)     |
| completed               |          |                |          |                |
| Breathlessness score     | 5        | (3 to 7)       | 5        | (3 to 7)       |
| after exercise           |          |                |          |                |
| Post-exercise heart rate | 76       | (64 to 93)     | 76       | (64 to 90)     |
| b.p.m.                  |          |                |          |                |
| Post-exercise, systolic  | 165      | (144 to 187)   | 165      | (144 to 188)   |
| BP, mmHg                |          |                |          |                |
| Electrocardiogram and   |          |                |          |                |
| echocardiography         |          |                |          |                |
| QRS duration (ms)        | 92       | (84 to 102)    | 92       | (84 to 108)    |
| LV end-diastolic volume  | 42       | (36 to 49)     | 41       | (35 to 48)     |
| index (mL/m²)            |          |                |          |                |
| LV ejection fraction (%) | 63       | (57 to 66)     | 63       | (59 to 67)     |
| LV left ventricular mass | 95       | (81 to 113)    | 94       | (81 to 111)    |
| index (g/m²)             |          |                |          |                |
| Men                      | 97       | (79 to 103)    | 94       | (82 to 111)    |
| Left atrial volume       | 88       | (79 to 103)    | 96       | (81 to 109)    |
| index (mL/m²)            | 31       | (26 to 37)     | 30       | (26 to 35)     |
| Early mitral flow        | 0.7      | (0.6 to 0.8)   | 0.7      | (0.6 to 0.8)   |
| velocity (E) m/s         |          |                |          |                |
| Late (atral) mitral      | 0.8      | (0.7 to 0.9)   | 0.8      | (0.7 to 0.9)   |
| flow velocity (A) m/s    |          |                |          |                |
| E/A ratio                | 0.8      | (0.7 to 1.0)   | 0.8      | (0.7 to 1.0)   |
| E/ early diastolic        | 9.4      | (7.5 to 11.8)  | 9.2      | (7.6 to 11.3)  |
| tissue velocity (e') ratio|        |                |          |                |
| Tricuspid annular plane  | 22.4     | (16.7 to 26.6) | 21.7     | (17.5 to 26.2) |
| systolic excursion       |          |                |          |                |
| 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         | 12.9     | (9.2 to 18.5)  | 12.7     | (8.4 to 17.6)  |
| troponin-T, ng/L         |          |                |          |                |
| Growth differentiation,  | 1,421    | (1,011 to 1,954)| 1,467   | (1,076 to 2,207)|
| factor, 15 ng/L          |          |                |          |                |
| 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 25.3) | 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.
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. 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. 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. 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 | Control | Spironolactone |
|                                     | n=257     | n=259         | n=255   | n=251          |
| n                                   | Mean (SD) | Mean difference (95% CI) | P       | Mean (SD) | Mean difference (95% CI) | P       |
| 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) | =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-I C-terminal telopeptide; MMP-1, 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.
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 HfPEx.\textsuperscript{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,\textsuperscript{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,\textsuperscript{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.\textsuperscript{11}

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.
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 | Spironolactone | Difference | Control | Spironolactone | Difference | P | End of trial | Spironolactone | Difference | P |
|--------------------|-----------|---------------|------------|---------|---------------|------------|----|--------------|---------------|------------|----|
| N                  | 257       | 259           |            |         |               |            |    |              | 255           | 251        |    |
| Days follow-up     | 31 (28 to 35) | 32 (28 to 35) | +1         | 0.669   | 267 (184 to 275) | +6 (176 to 274) | 0.765 |
| NYHA >1            | N = 33    | N = 40        | -7         | 0.558   | N=35          | N=35       | 0.097 |
| Loop diuretic      | N = 2     | N = 1         | -1         |          | N=7           | N=2        | -5 |
| 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) | 0.31 |
| 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) | 0.061 |
| EQSD VAS           | +2 (14)   | +1 (15)       | -2 (-4 to 0) | 0.077 | 0 (15) | -1 (16) | 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) | 0.342 |
| Physical signs     | Weight (kg) | 0.1 (1.5) | -0.5 (-0.8 to -0.3) | <0.001 | -0.5 (2.8) | -0.9 (2.5) | 0.178 |
|                    | Heart Rate (bpm) | 0 (8) | 0 (-1 to +2) | 0.490 | 0 (8) | -1 (7) | 0.329 |
|                    | Sys BP (mmHg) | -3 (16) | -9 (16) | -6 (-9 to -3) | <0.001 | -3 (19) | -13 (18) | <0.001 |
|                    | Dia BP (mmHg) | 0 (11) | -3 (8) | -2 (-4 to +1) | 0.003 | -1 (10) | -4 (9) | <0.001 |
|                    | Post-exercise | -3 (25) | -11 (26) | -8 (-14 to -3) | 0.001 | -5 (27) | -16 (27) | <0.001 |
| 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) | 0.089 |
|                    | Sodium (mmol/L) | +0.2 (2.1) | -1.0 (2.4) | -1.2 (-1.5 to -0.8) | <0.001 | 0.0 (2.4) | -1.2 (2.5) | <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) | <0.001 |
|                    | >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) | <0.001 |
|                    | Creatinine (μmol/L) | +1 (9) | +5 (10) | +4 (+3 to +6) | <0.001 | +4 (17) | +9 (13) | <0.001 |
|                    | eGFR mL/min/1.73m² | -1 (8) | -4 (8) | -3 (-5 to -2) | <0.001 | -2 (9) | -7 (10) | <0.001 |
| Number             | 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)                                              |           |               |    |           |                |    |
| 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 | 0.0       | 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            | 0.0 (0.0 to +0.1) | 0.095 |
| Mean change (SD)                                             |           |               |    |           |                |    |
| QRS (ms)                                                     | 0 (10)    | 0 (14)        | 0 (−2 to +2) | 0.345 | +1 (10)       | −2 (10)        | −3 (−5 to −1) | 0.003 |
| LVESD (mL/m²)                                                | −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/m²²)                                                 | 0 (12)    | −1 (14)       | −1 (−4 to +1) | 0.296 | −1 (12) | −3 (13) | −2 (−4 to 0) | 0.079 |
| LAVI (mL/m²²)                                                | 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 (3.3) | −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; LVESD, 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.
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. 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. Fewer trials have investigated the effects of MRA on serum CITP and found no consistent effect. 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. This might reflect increased galectin-3 production due to potassium-mediated increases in aldosterone subsequent to administration of an MRA, 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 after-load or a direct effect of spironolactone on collagen production or processing. Many of our participants had inadequately controlled blood pressure, which fell substantially in those assigned to spironolactone, as observed in trials of resistant hypertension, 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.\textsuperscript{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,\textsuperscript{10} potassium must be transferred to the intra-cellular space and, therefore, assuming no change in intra-cellular potassium concentration, intra-cellular water must increase.\textsuperscript{45} 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 HFpEF; symptoms, reduced exercise capacity were observed. 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 of 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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