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A Randomized Study of the Beneficial Effects of Aldosterone Antagonism on LV Function, Structure, and Fibrosis Markers in Metabolic Syndrome

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OBJECTIVES The purpose of this study was to identify the effects of spironolactone on left ventricular (LV) structure and function, and serological fibrosis markers in patients with metabolic syndrome (MS) taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.

BACKGROUND Myocardial fibrosis may be an important contributor to myocardial impairment in MS, and aldosterone antagonism may reduce fibrosis.

METHODS Eighty patients (age 59 ± 11 years) with MS, already being treated with angiotensin II inhibition, were randomized to spironolactone 25 mg/day or placebo for 6 months. Each patient underwent baseline and follow-up conventional echocardiography and color tissue Doppler imaging. Raw data files were used to measure calibrated integrated backscatter and to calculate radial and longitudinal strain. Blood was obtained at baseline and follow-up to measure fibrosis markers (procollagen type III amino-terminal propeptide and procollagen type I carboxy-terminal propeptide [PICP]).

RESULTS The spironolactone group showed significant improvement of LV function, myocardial reflectivity, and LV hypertrophy, with a parallel decrease in levels of PICP and procollagen type III amino-terminal propeptide. No analogous changes were seen in the placebo group. Baseline strain ($\beta = 0.47$, $p < 0.0001$), spironolactone therapy ($\beta = -0.38$, $p < 0.0001$), and change in PICP level ($\beta = -0.19$, $p < 0.03$) were independently associated with LV systolic function improvement (increase in strain). Correlates of LV diastolic function improvement (increase in early diastolic mitral annular velocity) were baseline early diastolic mitral annular velocity ($\beta = 0.47$, $p < 0.0001$), spironolactone therapy ($\beta = -0.21$, $p < 0.03$), change in PICP level ($\beta = -0.23$, $p < 0.02$), and age ($\beta = 0.22$, $p < 0.04$). Favorable effects of spironolactone on cardiac function were not demonstrated in patients with less fibrosis (the lower baseline PICP tertile) or preserved function (the upper baseline strain tertile).

CONCLUSIONS Addition of spironolactone to standard angiotensin II inhibition improved myocardial abnormalities and decreased fibrotic markers in MS. The magnitude of benefit on cardiac performance is determined mainly by baseline LV dysfunction and collagen turnover as well its response to intervention. (J Am Coll Cardiol Img 2011;4:1239–49) © 2011 by the American College of Cardiology Foundation

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The constellation of cardiovascular risk factors represented by metabolic syndrome (MS) is associated with increased cardiovascular morbidity and mortality. Cardiac complications of MS include abnormal left ventricular (LV) structure and function, leading progressively to congestive heart failure (1–5). As the prevalence of this condition rises to nearly a quarter of the adult population, exceeding 40% in those older than 50 years (6), these cardiac ramifications are likely to become a major public health concern.

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ABBREVIATIONS AND ACRONYMS

A = peak late diastolic flow velocity

AA = aldosterone antagonist

ACEI = angiotensin-converting enzyme inhibitor

ARB = angiotensin receptor blocker

E = peak early diastolic flow velocity

e′ = peak early diastolic mitral annular velocity

Em = peak early diastolic myocardial velocity

IB = integrated backscatter

LV = left ventricular

MS = metabolic syndrome

PICP = procollagen type I carboxy-terminal propeptide

PIIINP = procollagen type III amino-terminal propeptide

RAA = renin-angiotensin-aldosterone

SR = peak systolic strain rate

TGF = transforming growth factor

The mechanisms behind cardiac derangements in MS are multifactorial, but one of the pivotal contributors is thought to be myocardial fibrosis (7). Accordingly, measures targeting this pathophysiology might improve outcomes by reducing the pathological substrate accounting for myocardial impairment. The renin-angiotensin-aldosterone (RAA) system has been incriminated in the process of cardiac fibroblast proliferation and collagen synthesis. However, although angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are widely used for the treatment of hypertension in MS, suboptimal aldosterone suppression due to "aldosterone escape" (8–10) may limit the potential suppression of myocardial fibrosis by these drugs.

Aldosterone antagonists (AAs) may improve LV systolic and diastolic function as well as reduce cardiac fibrosis in various heart diseases (11–17), although the combination of AAs and ACEIs/ARBs is less well explored (18,19). The effect on the extracellular matrix was a contributor to the improved outcomes in 2 large randomized trials that demonstrated that the addition of AAs to standard therapy (including ACEIs or ARBs) diminished the risk of mortality and morbidity in heart failure (20,21). Given the role of enhanced fibrosis in the pathophysiology of metabolic disorders, as well as growing evidence on the beneficial effect of AAs extending beyond patients with moderate to severe heart failure (17,22), we hypothesized that implementation of spironolactone in addition to drugs opposing angiotensin II would improve cardiac abnormalities in the population with MS regardless of the symptoms of heart decompensation. Circulating markers of collagen type I and type III turnover—procollagen type I carboxy-terminal propeptide (PICP) and procollagen type III amino-terminal propeptide (PIIINP)—may reflect the intensity of myocardial fibrosis and thus may provide insight into fibrous tissue accumulation in the heart muscle (23–25). In this randomized controlled trial, we sought to determine the effects of adding spironolactone to ACEIs or ARBs on LV structure and function and serological fibrosis markers in patients with MS.

METHODS

Study design. The present study was designed as a prospective, blinded, parallel-group, placebo-controlled trial evaluating the potential of 6 months of treatment with spironolactone 25 mg/day to improve cardiac function and morphology and reduce fibrosis intensity in patients with MS. The primary endpoints were alterations in LV function as assessed by systolic echocardiographic parameters (strain and peak systolic strain rate [SR]) and diastolic parameters (peak early diastolic velocity [Em] and the ratio of mitral inflow early diastolic velocity to peak early diastolic mitral annular velocity [E/e′]). Secondary endpoints included changes in collagen metabolism as reflected by serum levels of PICP and PIIINP, myocardial echodensity as estimated by integrated backscatter (IB), LV wall thicknesses, and LV mass. This report follows the recommendations of the 2010 Consolidated Standards of Reporting Trials Statement (26).

Sample size was calculated on the basis of previous data from patients with MS (1). Assuming a significant difference in peak strain of 10% between spironolactone and placebo and applying the variance seen in our patients, the predicted sample size was 37 per group at 95% power and 2-sided alpha level of 0.05. To allow for possible dropouts, we increased the number of patients in each group to 40.

Randomization to spironolactone or matching placebo was done in blocks of 10 with an allocation ratio of 1:1 using sequentially numbered, opaque, sealed envelopes. The randomization list and the study drugs were prepared by the assigned person coordinating the study, who was not involved in the procedures. Patients' selection and randomization were carried out by the designated investigators. The study participants and the investigators assessing the outcomes were blinded to group assignment.

Study medication was withheld in the presence of significant hyperkalemia (>5.5 mmol/l), renal impair-

ment (serum creatinine >310 μmol/l [3.5 mg/dl]), or serious side effects.

Patient selection. We screened 132 patients with cardiovascular risk factors (hypertension, diabetes mellitus, and obesity) who were referred to specialist clinics for management of MS and recruited 80 consecutive patients satisfying the selection criteria (Fig. 1). The diagnosis of MS was established as recommended by the amended National Cholesterol Education Program's Adult Treatment Panel III guidelines in patients meeting ≥3 of the following criteria: 1) increased waist circumference (≥102 cm in men or ≥88 cm in women); 2) increased fasting triglycerides (≥1.7 mmol/l) or drug treatment for elevated triglycerides; 3) high blood pressure (≥130/≥85 mm Hg) or antihypertensive therapy; 4) decreased high-density lipoprotein cholesterol (<1.03 mmol/l in men or <1.3 mmol/l in women) or drug treatment for reduced high-density lipoprotein cholesterol; 5) impaired fasting glucose (≥5.6 mmol/l) or drug treatment for elevated glucose (27). To be eligible, patients had to remain on stable pharmacotherapy including ACEIs or ARBs for ≥6 months before entering the study.

Coronary artery disease was excluded on the basis of a negative history and normal stress echocardiogram. Other exclusion criteria included moderate and severe valvular heart disease; absence of stable sinus rhythm; adrenocortical disorders; pulmonary, hepatic, rheumatic, neoplastic, skeletal, thyroid, and renal diseases (including renal insufficiency with serum creatinine >220 μmol/l [2.5 mg/dl]), hyperkalemia >5.0 mmol/l; and treatment with AAs.

All participants were informed of the purpose of the study and provided written informed consent. Investigations were in accordance with the Declaration of Helsinki and were approved by the institutional ethics committee.

Study protocol. At baseline, enrolled patients underwent physical examination (with blood pressure measurement), blood specimen collection, and echocardiography. Thereafter, patients were randomized to receive either 25 mg of spironolactone or placebo once daily. Previous pharmacotherapy was maintained unchanged. Patients' status, compliance with the treatment, and serum electrolyte levels were monitored at visits every 2 weeks during the first 2 months and then monthly. The baseline investigations were repeated after 6 months.

Blood assays. Peripheral venous blood was obtained between 8:00 AM and 9:00 AM after 30 min of rest. Serum PICP level was measured with an enzyme-linked immunoadsorbent assay (Takara Bio, Shiga, Japan); intra-assay and interassay coefficients of variation (CVs) were 4.9% and 5.5%, respectively. Serum PIIINP level was quantified by radioimmunoassay (Orion Diagnostica, Espoo, Finland); intra-assay and interassay CVs were 3.9% and 5.3%, respectively. Serum level of transforming growth factor (TGF)-β_1 was estimated using an enzyme-linked immunoadsorbent assay (R&D Systems, Minneapolis, Minnesota); intra-assay and interassay CVs were 2.7% and 7.0%, respectively. Serum aldosterone level was assessed by radioimmunoassay (Immunotech SAS, Marseille, France); intra-assay and interassay CVs were 7.6% and 8.8%, respectively. Plasma angiotensin II levels were quantified

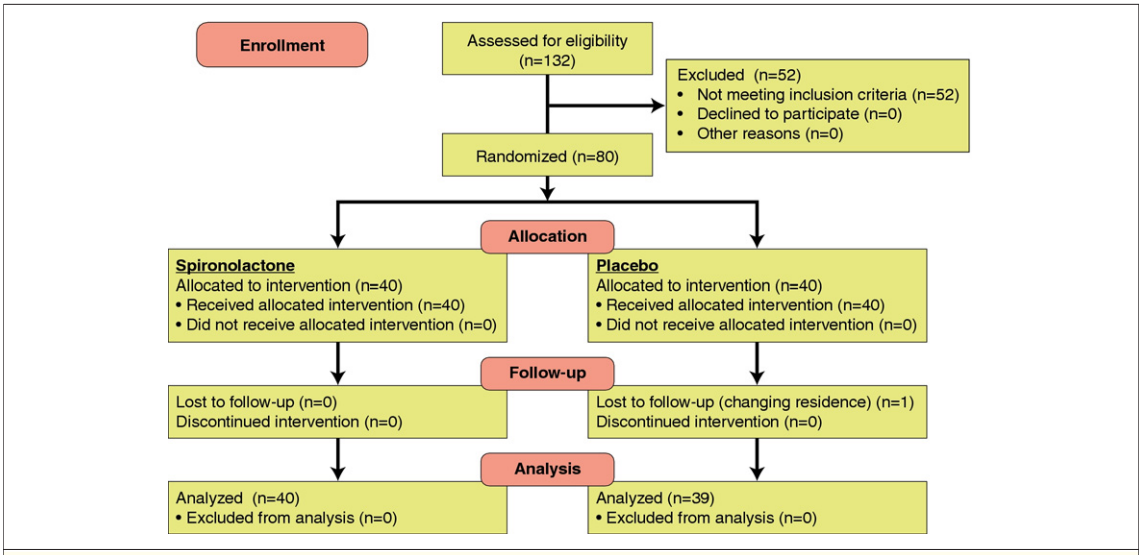


Figure 1. Study Flow Diagram

by radioimmunoassay (DIAsource ImmunoAssays, Nivelles, Belgium); intra-assay and interassay CVs were 3.1% and 4.3%, respectively.

Echocardiography. Imaging was performed using standard equipment (Vivid 7 and System 5, GE Ultrasound, Horten, Norway) with phased-array 2.5-MHz multifrequency transducers.

CONVENTIONAL ECHOCARDIOGRAPHY. Cardiac dimensions and wall thickness were measured according to standard recommendations (28). LV mass was derived from the modified American Society of Echocardiography cube formula and indexed for height to the power of 2.7 (29). LV ejection fraction was assessed by a modified Simpson biplane method.

Pulsed-wave Doppler recordings of the LV inflow were obtained from the apical 4-chamber view with the sample volume placed between the tips of the mitral leaflets. E and late diastolic flow velocity (A) (E/A) and deceleration time of early diastolic flow wave were estimated.

CALIBRATED IB. IB is a marker of myocardial reflectivity that has previously been correlated with histological evidence of fibrosis (30). IB curves were obtained from a 9 × 9-pixel sample volume in end-diastole in the basal anteroseptum, posterior wall, and pericardium in the parasternal long-axis view. Calibrated IB was calculated by subtracting average pericardial IB from average myocardial IB intensity in 3 consecutive cardiac cycles.

PULSED-WAVE TISSUE DOPPLER. Recordings of the septal and lateral portion of annulus were used for the assessment of e′. E/e′ obtained from the septal and lateral sides of the mitral annulus was calculated to approximate LV filling pressure.

MYOCARDIAL DEFORMATION. Color tissue Doppler myocardial imaging data were acquired in the 3 apical views to evaluate LV longitudinal function and in the parasternal short-axis view to quantify LV radial performance. The narrowest possible image sector and the optimal depth of imaging were applied to achieve maximal frame rate. Pulse repetition frequency was set at the lowest value without aliasing. The ultrasound beam was kept as parallel as possible to the myocardial segment of interest to ensure an insonation angle $<20^{\circ}$. The sampling window was placed in the central part of each segment and maintained at the same position by manually tracking the wall motion during the cardiac cycle.

Digital data were analyzed offline (Echopac, GE Medical). Regional myocardial velocity curves were extracted from color Doppler data obtained in the basal segment of the interventricular septum to estimate peak systolic velocity and Em. LV longitudinal myocardial deformation was assessed in the apical, mid, and basal segments of each wall, whereas LV radial myocardial deformation was assessed in the basal posterior segment. SR was evaluated from the spatial velocity gradient over a 12-mm computation length in the longitudinal direction and 1 mm less than the end-diastolic wall thickness in the radial direction. SR profiles were then integrated over time to obtain strain curves triggered to the R-peak of the electrocardiogram. Parameters estimated from myocardial deformation curves included peak strain, defined as the greatest negative value on the strain curve, and SR. All strain, SR, and myocardial velocity profiles were averaged from 3 consecutive cardiac cycles. The results for strain and SR are presented as the average values from all segments subjected to analysis.

REPRODUCIBILITY. The reproducibility of myocardial measurements was calculated using values averaged from all of the segments assessed in 15 randomly selected examinations. The intraobserver and interobserver variability was 0.7 ± 0.4% and 0.8 ± 0.5% for strain, 0.05 ± 0.02 s^{-1} and 0.07 ± 0.04 s^{-1} for strain rate, 0.2 ± 0.3 cm/s and 0.3 ± 0.3 cm/s for myocardial velocities, and 1.2 ± 1.3 dB and 1.4 ± 1.4 dB for calibrated IB, respectively.

The evaluation of baseline collagen metabolism in patients with MS was based on a comparison with serum levels of PICP and PIIINP obtained from 34 healthy volunteers of comparable age (57.1 ± 8.5 years) with normal cardiac function and morphology.

Statistical analysis. Data are presented as mean ± SD; skewed variables (C-reactive protein, serum aldosterone) were log-transformed. Between-group comparisons were performed with an unpaired 2-sided Student *t* test or, when more than 2 groups were included, by 1-way analysis of variance with the Scheffe post hoc test for continuous variables and chi-square test for categorical variables. Homogeneity of variances was evaluated by the Levene test. Longitudinal analyses were carried out using a repeated-measures 2-way analysis of variance with planned comparisons by the linear contrasts method. Associations between variables were expressed with Pearson or Spearman correlation coefficients and stepwise multiple regression analysis. Effect size was evaluated using the Cohen d method. Changes in particular parameters with intervention were calculated by subtracting the baseline value from the follow-up value. All analy-

ses were performed with standard statistical software (Statistica for Windows 8, StatSoft Inc., Tulsa, Oklahoma). The level of statistical significance was set at $p < 0.05$.

RESULTS

Patient characteristics. The baseline characteristics of the spironolactone and placebo groups (demographics and clinical and laboratory parameters) are summarized in Tables 1 and 2. Three components of MS were present in 38% of the treatment group and in 36% of the placebo group, with 4 components in 33% and 38% and 5 components in 30% and 26%, respectively. All of the randomized patients were asymptomatic or had minor limitation of physical activity (New York Heart Association functional class I to II). Comparisons with healthy controls revealed increased levels of the fibrosis markers in patients with MS—PICP (117.9 ± 41.2 μg/l in the referent group, $p < 0.01$ vs. spironolactone and $p < 0.002$ vs. placebo group) and PIIINP (4.39 ± 0.96 μg/l in controls, $p < 0.002$ vs. spironolactone and $p < 0.03$ vs. placebo group).

Adherence and drug side effects. One patient in the placebo group did not complete the study protocol because of changing residence. Mild gynecomastia developed in 2 male patients in the spironolactone group but did not lead to drug discontinuation. No other adverse effects or complications occurred.

Baseline echocardiography. No differences between the treatment and placebo groups in all of the baseline echocardiographic indexes of cardiac structure and function were demonstrated (Table 3). Patients with MS presented LV structural and functional abnormalities as compared with healthy referents: increased LV end-diastolic dimension, LV mass index, left atrial dimension, E/e′ and IB in the basal anteroseptum, and decreased Em, longitudinal strain, and longitudinal SR (the values in the control group were 46.7 ± 3.0 mm for LV end-diastolic dimension, 41.0 ± 5.8 g/m$^{2.7}$ for LV mass index, 36.0 ± 2.7 mm for left atrial dimension, 8.9 ± 1.5 for E/e′, −20.6 ± 5.3 dB for the basal septal IB, 8.3 ± 2.6 cm/s for Em, 20.4 ± 2.3% for longitudinal strain, and 1.61 ± 0.18 s^{-1} for SR; all $p < 0.001$ vs. the MS groups).

Clinical and laboratory parameters. No significant changes were noted from baseline to 6 months in either group in parameters related to adipose tissue content, blood pressure, plasma lipid level, C-reactive protein level, renal function and metabolic control

Table 1. Demographic Data, Baseline and Follow-Up Values, and Change During Follow-Up of Clinical Characteristics

	Spironolactone (n = 40)			Placebo (n = 39)			p Value
	Baseline	Follow-Up	p Value	Baseline	Follow-Up	p Value	ΔSpironolactone vs. ΔPlacebo
Clinical features							
Age, yrs	58.4 ± 11.5	—	—	60.6 ± 11.4	—	—	
Male	19 (48)			17 (44)			
Diabetes mellitus	23 (58)			21 (54)			
BMI, kg/m^2	32.8 ± 4.8	32.4 ± 4.7	0.37	31.6 ± 4.0	31.3 ± 4.1	0.28	0.69
Waist circumference, cm	101 ± 11	100 ± 11	0.26	99 ± 10	99 ± 10	0.39	0.24
Waist/hip ratio	0.97 ± 0.14	0.96 ± 0.13	0.30	0.96 ± 0.10	0.96 ± 0.10	0.50	0.28
Systolic blood pressure, mm Hg	143 ± 16	140 ± 14	0.21	139 ± 12	137 ± 11	0.32	0.67
Diastolic blood pressure, mm Hg	82 ± 9	80 ± 6	0.23	82 ± 6	80 ± 6	0.22	0.82
Medications							
ACEI	35 (88)			32 (82)			
ARB	5 (13)			7 (18)			
Beta-adrenolitics	24 (60)			20 (51)			
Calcium antagonist	13 (33)			10 (26)			
Diuretic (hydrochlorothiazide or indapamide)	19 (48)			21 (54)			
Statin	27 (68)			22 (56)			
Fibrate	3 (8)			3 (8)			
Sulfonylurea	11 (28)			9 (23)			
Biguanide	13 (33)			10 (26)			

Values are mean ± SD or n (%).
ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; BMI = body mass index; ΔSpironolactone = change in the spironolactone group during follow-up; ΔPlacebo = change in the placebo group during follow-up.

Table 2. Baseline, Follow-Up Values, and Change During Follow-Up of Laboratory Characteristics

	Spironolactone (n = 40)			Placebo (n = 39)			p Value
	Baseline	Follow-Up	p Value	Baseline	Follow-Up	p Value	ΔSpironolactone vs. ΔPlacebo
Fasting insulin, mIU/l	13.3 ± 9.5	13.6 ± 9.7	0.88	12.8 ± 9.8	11.1 ± 8.7	0.35	0.45
Fasting glucose, mg/dl	123 ± 33	119 ± 31	0.36	118 ± 31	113 ± 19	0.23	0.81
HOMA-IR	4.1 ± 3.0	4.1 ± 3.7	0.87	4.1 ± 4.1	3.1 ± 2.7	0.29	0.29
Creatinine, mg/dl	0.93 ± 0.21	0.90 ± 0.23	0.27	0.93 ± 0.21	0.93 ± 0.25	0.82	0.41
Uric acid, mg/dl	6.7 ± 1.7	6.8 ± 1.5	0.82	6.3 ± 1.3	6.3 ± 1.0	0.86	0.97
Potassium, mmol/l	4.2 ± 0.3	4.5 ± 0.3	0.001	4.1 ± 0.3	4.2 ± 0.3	0.56	0.01
Total cholesterol, mg/dl	200 ± 43	194 ± 36	0.38	198 ± 41	194 ± 32	0.41	0.68
Low-density lipoprotein, mg/dl	121 ± 39	113 ± 40	0.36	116 ± 32	115 ± 32	0.91	0.36
High-density lipoprotein, mg/dl	49 ± 13	49 ± 13	0.64	52 ± 15	50 ± 13	0.36	0.19
Triglycerides, mg/dl	167 ± 78	157 ± 76	0.28	158 ± 57	151 ± 46	0.24	0.75
C-reactive protein, mg/l	3.5 ± 2.7	4.3 ± 4.3	0.30	3.1 ± 2.9	3.6 ± 2.6	0.26	0.59
PIIINP, μg/l	5.76 ± 1.99	4.91 ± 1.73	0.003	5.32 ± 1.92	5.20 ± 1.96	0.50	0.02
PICP, μg/l	142.1 ± 33.2	122.1 ± 23.1	0.001	150.1 ± 43.6	145.9 ± 47.4	0.38	0.02
TGF-β_1, μg/ml	33.8 ± 7.1	30.7 ± 6.0	0.01	31.9 ± 7.0	31.6 ± 6.4	0.57	0.02
Aldosterone, pg/ml	80.5 ± 64.4	92.6 ± 77.1	0.22	68.2 ± 54.6	63.2 ± 45.2	0.77	0.09
Angiotensin II, pmol/l	11.7 ± 4.7	12.7 ± 6.6	0.40	13.9 ± 7.4	11.8 ± 6.4	0.64	0.13

Values are mean ± SD, unless otherwise indicated.
HOMA-IR = homeostasis model assessment of insulin resistance; PICP = procollagen type I carboxy-terminal propeptide; PIIINP = procollagen type III amino-terminal propeptide; TGF = transforming growth factor; other abbreviations as in Table 1.

parameters, blood aldosterone level, and angiotensin II level. There was a mild increase in serum potassium in the spironolactone group but no significant change with placebo (Tables 1 and 2). Circulating levels of the fibrotic markers PICP, PIIINP, and TGF-β_1 significantly decreased in the treatment group. No analogous changes were found with placebo (Table 2).

Table 3. Baseline, Follow-Up Values, and Change During Follow-Up of Echocardiographic Characteristics

	Spironolactone (n = 40)			Placebo (n = 39)			p Value
	Baseline	Follow-Up	p Value	Baseline	Follow-Up	p Value	ΔSpironolactone vs. ΔPlacebo
LV end-diastolic dimension, mm	51.8 ± 4.2	52.0 ± 4.1	0.41	51.1 ± 4.2	50.7 ± 4.0	0.30	0.14
IVS, mm	13.4 ± 1.9	12.8 ± 1.7	0.001	13.2 ± 1.7	13.1 ± 1.7	0.49	0.04
PW, mm	10.5 ± 1.4	10.1 ± 1.5	0.02	10.1 ± 1.3	10.0 ± 1.3	0.38	0.30
LV mass index, g/m$^{2.7}$	62.3 ± 10.1	59.6 ± 10.9	0.003	60.4 ± 11.5	59.0 ± 12.2	0.35	0.47
Left atrial dimension, mm	44.0 ± 3.2	42.9 ± 3.3	0.001	42.8 ± 4.2	42.7 ± 4.7	0.77	0.01
LV ejection fraction, %	67.6 ± 3.9	67.9 ± 4.1	0.52	67.1 ± 4.6	67.3 ± 4.3	0.66	0.64
E/e′	12.4 ± 2.9	11.6 ± 3.2	0.01	11.4 ± 2.7	11.5 ± 2.4	0.52	0.01
E/A	0.95 ± 0.19	0.95 ± 0.21	0.93	0.93 ± 0.42	0.92 ± 0.31	0.67	0.77
DT, ms	239 ± 43	238 ± 44	0.91	238 ± 43	230 ± 37	0.21	0.30
Sm, cm/s	5.6 ± 1.4	5.8 ± 1.2	0.08	5.8 ± 1.3	5.8 ± 1.3	0.95	0.13
Em, cm/s	5.7 ± 1.5	6.3 ± 1.4	0.03	5.9 ± 1.6	5.8 ± 1.7	0.47	0.005
Longitudinal strain, %	17.5 ± 2.7	19.2 ± 2.3	0.001	17.9 ± 2.6	17.8 ± 2.6	0.52	0.001
Longitudinal SR, s^{-1}	1.41 ± 0.25	1.55 ± 0.20	0.001	1.43 ± 0.21	1.43 ± 0.22	0.55	0.001
Radial strain, %	41.4 ± 14.0	39.6 ± 10.4	0.58	39.6 ± 13.1	40.3 ± 13.6	0.37	0.28
Radial SR, s^{-1}	2.87 ± 0.92	2.77 ± 0.70	0.55	2.84 ± 0.71	2.87 ± 0.72	0.33	0.36
IB AS, dB	−13.6 ± 5.0	−14.4 ± 4.2	0.37	−15.1 ± 5.3	−15.0 ± 4.4	0.80	0.45
IB post, dB	−25.3 ± 5.0	−27.6 ± 5.2	0.04	−23.1 ± 5.7	−24.1 ± 4.4	0.25	0.30

Values are mean ± SD.
DT = deceleration time of early diastolic flow wave; E/A = ratio of peak early and late diastolic flow velocities; E/e′ = ratio of peak early mitral inflow velocity and peak early diastolic mitral annular velocity; Em = peak early diastolic velocity; IB AS = calibrated integrated backscatter in the basal anteroseptum; IB post = calibrated integrated backscatter in the basal posterior wall; IVS = interventricular septal thickness; LV = left ventricular; PW = LV posterior wall thickness; Sm = peak systolic velocity; SR = peak systolic strain rate; other abbreviations as in Figure 1.

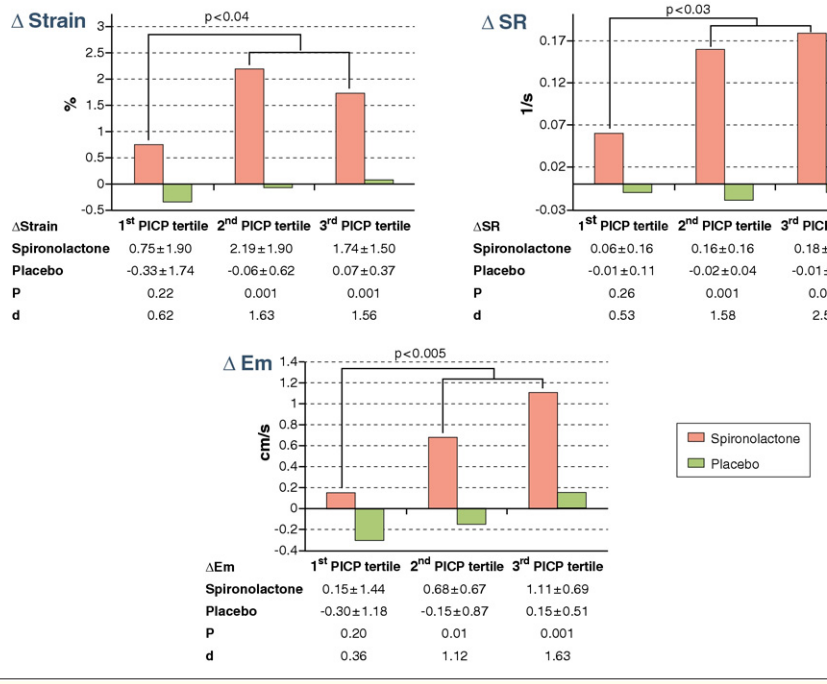
Echocardiographic parameters of cardiac function and morphology. The spironolactone group demonstrated a significant decrease in LV mass index, interventricular septal thickness, LV posterior wall thickness, and left atrial diameter at follow-up (Table 3). The improvement in LV systolic function in the spironolactone group was evidenced by increase in longitudinal strain and SR, whereas that in LV diastolic function was characterized by increase in Em and decrease in E/e′ (Table 3). The improvement in myocardial tissue density with spironolactone therapy was reflected by a decrease in calibrated IB in the basal posterior wall (Table 3).

All echocardiographic parameters of cardiac function and morphology remained unchanged over the intervention period in the placebo group.

Response to spironolactone therapy according to baseline PICP tertiles. Significant differences in changes in strain, SR, and Em between the spironolactone and placebo groups, reflecting LV function improvement with intervention, were seen in the middle and upper but not in the lower baseline PICP tertile. The effect size was medium in the first tertile and large in the second and third tertiles for all 3 variables. Changes in strain, SR, and Em in the treatment group were lower in the first PICP tertile than in the second and third PICP tertiles. No differences in this regard were shown in the placebo group (Fig. 2).

Response to spironolactone therapy according to baseline longitudinal strain tertiles. Significant differences in changes in PICP and PIIINP levels between the spironolactone and placebo groups were revealed in the first baseline longitudinal strain tertile, whereas those of LV function parameters (i.e., longitudinal strain, SR, and Em) were seen in the first and second tertiles. The effect was found to be large in the first and second tertiles and small in the third tertile for cardiac functional indexes and large in the first tertile and small in the second and third tertiles for the procollagen peptides. Changes in LV longitudinal deformation parameters in the spironolactone group were higher in the lower and middle than in the upper strain tertile. The placebo group demonstrated no differences in this respect (Fig. 3).

Relation of changes in cardiac and fibrosis markers. A series of models was developed to identify the independent associations of LV systolic and diastolic function improvement (as indicated by increase in strain, SR, and Em and decrease in E/e′). The components of these models, selected on the basis of anticipated association included age, sex, blood pressure, baseline body mass index, homeostasis model assessment of insulin resis-



ΔStrain	1st PICP tertile	2nd PICP tertile	3rd PICP tertile
Spironolactone	0.75±1.90	2.19±1.90	1.74±1.50
Placebo	-0.33±1.74	-0.06±0.62	0.07±0.37
P	0.22	0.001	0.001
d	0.62	1.63	1.56

ΔSR	1st PICP tertile	2nd PICP tertile	3rd PICP tertile
Spironolactone	0.06±0.16	0.16±0.16	0.18±0.10
Placebo	-0.01±0.11	-0.02±0.04	-0.01±0.04
P	0.26	0.001	0.001
d	0.53	1.58	2.56

ΔEm	1st PICP tertile	2nd PICP tertile	3rd PICP tertile
Spironolactone	0.15±1.44	0.68±0.67	1.11±0.69
Placebo	-0.30±1.18	-0.15±0.87	0.15±0.51
P	0.20	0.01	0.001
d	0.36	1.12	1.63

Figure 2. Left Ventricular Function Response to Spironolactone Therapy Across Baseline PICP Tertiles

The range of procollagen type I carboxy-terminal propeptide (PICP) was 58.5 to 129.5 μg/l in the first tertile, 133.1 to 169.3 μg/l in the second tertile, and 170.0 to 215.7 μg/l in the third tertile. ΔEm = change in peak early diastolic velocity with intervention; ΔSR = change in longitudinal peak systolic strain rate with intervention; ΔStrain = change in longitudinal strain with intervention.

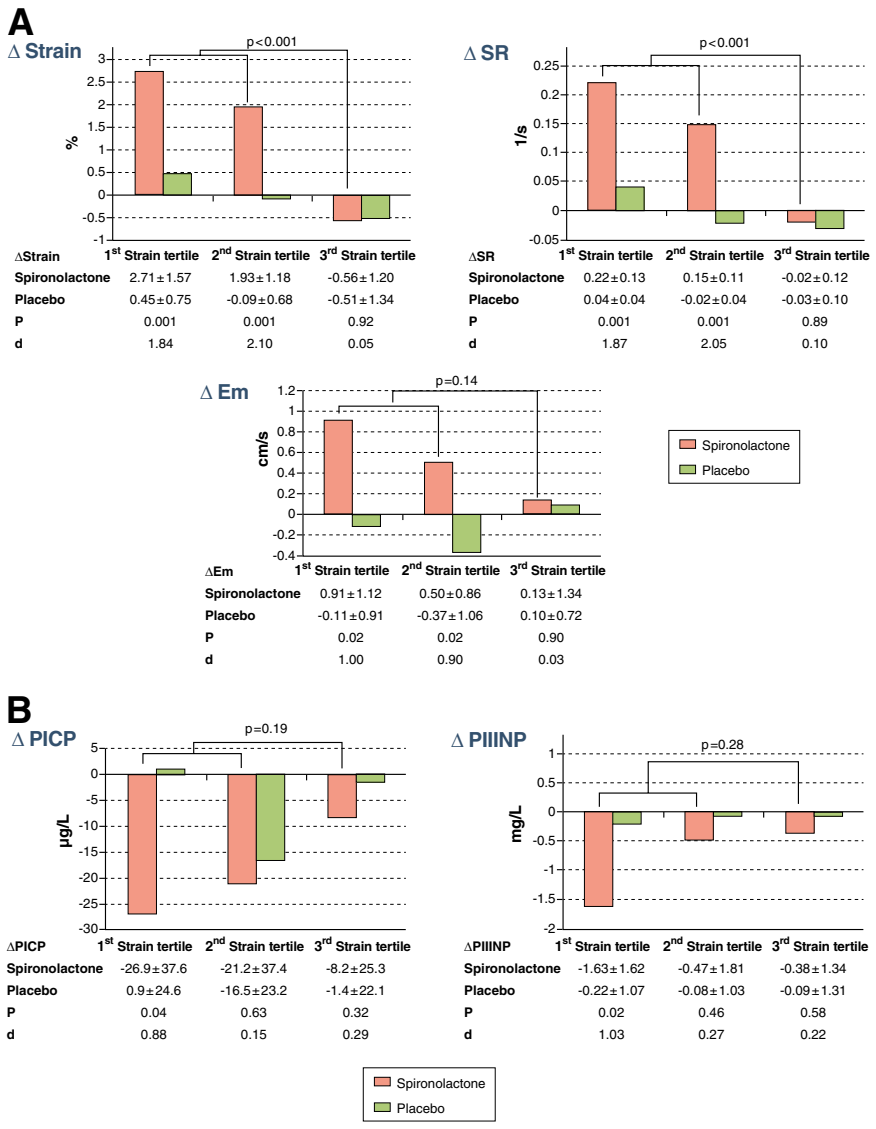


Figure 3. Left Ventricular Function (A) and Propeptide (B) Response to Spironolactone Therapy Across Baseline Longitudinal Strain Tertiles

The range of strain was 13.5% to 16.7% in the first tertile, 16.8% to 18.8% in the second tertile, and 18.9% to 21.7% in the third tertile. The greatest change of systolic and diastolic function were in the segments with worst baseline function. ΔPICP = change in procollagen type I carboxy-terminal propeptide level with intervention; ΔPIIIINP = change in procollagen type III amino-terminal propeptide level with intervention; other abbreviations as in Figure 2.

tance, markers of fibrosis (baseline values and follow-up changes of PICP and PIIINP levels), myocardial echodensity (baseline values and follow-up changes of IB in the basal posterior wall), baseline LV performance, and treatment with spironolactone. The independent determinants (Table 4) were baseline LV performance, baseline PICP level and its change at follow-up, treatment with spironolactone, change in calibrated IB in the basal posterior wall at follow-up, and patient age.

A model was also developed to identify the independent associations of myocardial density, a marker of fibrosis (as reflected by decrease in calibrated IB in the basal posterior wall). After adjustment for potential associations (including age, sex, treatment group, homeostasis model assessment of insulin resistance, blood pressure, and baseline and follow-up alterations in PICP level, PIIINP level, and LV mass index), the only independent correlate was a change in PICP ($\beta = 0.25$, $p < 0.02$).

Table 4. Multivariable Predictors of LV Function Improvement

	β	p Value
ΔStrain (R^2 = 0.52)		
Baseline strain	−0.47	0.0001
Spironolactone treatment	0.38	0.0001
ΔPICP	−0.19	0.03
ΔSR (R^2 = 0.56)		
Baseline SR	−0.52	0.0001
Spironolactone treatment	0.52	0.0001
Baseline PICP	0.16	0.04
BMI >30 kg/m^2	0.12	0.11
ΔEm (R^2 = 0.32)		
Baseline Em	−0.47	0.0001
ΔPICP	−0.23	0.02
Spironolactone treatment	0.21	0.03
Age	−0.22	0.04
ΔE/e′ (R^2 = 0.22)		
Baseline E/e′	−0.30	0.006
Spironolactone treatment	−0.22	0.04
ΔIB post	0.21	0.04
Baseline PICP	−0.19	0.07

Δ indicates change with intervention.
Abbreviations as in Tables 1 and 3.

Moreover, significant univariate correlations between spironolactone therapy and changes in the levels of the procollagen peptide—PICP and PIIINP were demonstrated (both $r = -0.22$, $p < 0.05$).

DISCUSSION

This randomized placebo-controlled study demonstrated that the addition of spironolactone to standard treatment including ACEIs or ARBs for 6 months improved myocardial abnormalities, with a concurrent decrease in fibrotic markers in patients with MS. The magnitude of benefit on cardiac performance was determined mainly by the degree of baseline LV dysfunction and the extent of collagen turnover at baseline as well as its response to intervention.

Potential mechanisms. Cardiac morphofunctional derangements in MS are due to a complex interplay of the multiple components of this disease condition. The mechanisms responsible for cardiac pathologies in MS include increased myocardial fibrosis, inflammation, oxidative stress, hyperinsulinemia and insulin resistance (causing abnormal myocardial substrate use), intracellular lipid accumulation and lipotoxicity (causing cardiomyocyte apoptosis), neurohormonal activation (especially the RAA and sympathetic systems), endothelial dysfunction, disturbances of adipokine release and signaling, and altered ventricular load (2,4,5,7,31). Of these, RAA system upregulation is an important part of the pathophysiological process that promotes free radical formation and cell proliferation and exerts proinflammatory and profibrotic effects (32). Both angiotensin II and aldosterone participate in extracellular matrix remodeling by enhancing collagen synthesis: angiotensin II via TGF-β_1 and aldosterone stimulation and aldosterone directly and through TGF-β_1 (33,34). These observations provide the rationale for aldosterone antagonism to reduce myocardial fibrosis.

The present study supports the favorable cardiac effect of aldosterone antagonism in MS by revealing an incremental benefit to angiotensin II inhibition. The 6-month follow-up duration in the current study was established in accordance with the fibrillar collagen half-life to be long enough to allow for the elimination of a considerable amount of collagen from the myocardium and hence the identification of the effects of treatment. The amelioration of LV function and structure abnormalities was paralleled by a decrease in the levels of serological markers of fibrosis (PICP and PIIINP) and the fibrotic mediator TGF-β_1. The independent association of PICP with significant LV systolic and diastolic function improvement supports the concept that inhibition of myocardial fibrosis with intervention might have contributed to functional recovery. Likewise, independent associations between the favorable changes in PICP level and an index of ultrasonic tissue characterization directly related to the morphometrically assessed myocardial collagen volume fraction (calibrated IB) as well as between the latter and diastolic improvement (E/e′) are consistent with the antifibrotic properties of spironolactone. The observation that the beneficial effects of aldosterone antagonism were more pronounced in patients with greater initial intensity of collagen anabolism is in line with previous findings in other disease populations (17).

Collagen markers. Our investigations suggest that PICP is superior to PIIINP in predicting changes of LV function and echodensity. This is not an unexpected finding because collagen type I represents more than 80% of total cardiac collagen and is the major component of myocardial extracellular matrix (35). The moderate improvement of LV function with spironolactone in this study was less than that reported previously in a hypertensive cohort (15), which might be attributable to a lesser intensity of fibrosis in this setting or the widespread use of ACEIs/ARBs limiting the baseline extent of fibrosis. The observed decrease in TGF-β_1 level associated with spironolactone antagonism, concordant with experimental evidence (11,12), was not independently associated with the demonstrated improvement in LV abnormalities.

Study limitations. In this study, echocardiographic markers of cardiac function were used as physiological endpoints. The association of these endpoints with clinical manifestations of heart failure and events has been shown in a number of previous investigations. Whether use of AAs in MS over a longer time span could improve outcome is a crucial question that needs to be verified in subsequent studies.

The characterization of fibrosis remains challenging. The imaging tests for this process are imperfect—we used tissue Doppler–based strain in this and our previous work on obesity because the signal/noise ratio of Doppler is less susceptible to imaging problems posed by obesity than are speckle-tracking–based approaches; however, the direction sensitivity of Doppler is an acknowledged limitation. Calibrated IB, although previously documented to correlate with myocardial collagen content, requires further validation to be considered as a reliable index of cardiac fibrosis. Similarly, biochemical tests may not be cardiac specific and are less markers of collagen synthesis than collagen turnover. This is less the case for circulating PICP, with a cardiac origin that has been shown in humans and which is completely cleaved during the conversion of procollagen type I into collagen type I. It is an important consideration for PIIINP, the cardiac origin of which remains to be proven, and PIIINP is not completely cleaved during the conversion of procollagen type III into collagen type III. Consequently, PIIINP remains in the collagen fibers and may also be released during fiber degradation.

Our results suggest a blood pressure–independent effect of spironolactone, supported by the absence of changes in blood pressure with intervention as well as the lack of contribution from blood pressure to multivariable models of improved cardiac function. However, these observations are based on clinic blood pressure measurements. Ambulatory blood pressure monitoring would offer a more comprehensive method of studying this association.

The present investigations did not shed light on the mechanisms underlying the regression of LV hypertrophy with spironolactone therapy, showing no associations between the reduction in LV mass and wall thicknesses with intervention and the evaluated laboratory and clinical factors. Considering the importance of the problem, the influence of aldosterone antagonism on cardiac morphologic abnormalities merits further exploration.

CONCLUSIONS

The addition of an AA to an ACEI or ARB improves cardiac functional and structural disturbances in patients with MS. The mechanisms contributing to this salutary effect appear consistent with a limitation of excessive collagen synthesis. In patients complying with prespecified selection criteria, our investigations confirmed previous observations that the dose of spironolactone 25 mg/day in conjunction with drugs antagonizing the action of angiotensin II was safe and well tolerated. Post hoc analyses based on tertiles of PICP and longitudinal strain suggest that the favorable effects of spironolactone may not be evident in patients with MS with the lowest fibrosis intensity as well as in those with less profound LV function abnormalities. Conversely, larger benefits gained from spironolactone therapy were associated with more severe baseline LV dysfunction and myocardial fibrosis (evidenced by PICP) and younger patient age (for diastolic impairment). These observations may guide the selection of patients with MS who would most benefit from treatment, but this issue needs to be addressed in separate, dedicated studies.

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