The Effect of Spironolactone in Patients With Obesity at Risk for Heart Failure: Proteomic Insights from the HOMAGE Trial

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ABSTRACT

Background: Adipose tissue influences the expression and degradation of circulating biomarkers. We aimed to identify the biomarker profile and biological meaning of biomarkers associated with obesity to assess the effect of spironolactone on the circulating biomarkers and to explore whether obesity might modify the effect of spironolactone.

Methods and Results: Protein biomarkers ($n = 276$) from the Olink Proseek−Multiplex cardiovascular and inflammation panels were measured in plasma collected at baseline, 1 month and 9 months from the HOMAGE randomized controlled trial participants. Of the 510 participants, 299 had obesity defined as an increased waist circumference (≥102 cm in men and ≥88 cm in women). Biomarkers at baseline reflected adipogenesis, increased vascularization, decreased fibrinolysis, and glucose intolerance in patients with obesity at baseline. Treatment with spironolactone had only minor effects on this proteomic profile. Obesity modified the effect of spironolactone on systolic blood pressure ($P_{interaction} = 0.001$), showing a stronger decrease of blood pressure in obese patients (−14.8 mm Hg 95% confidence interval −18.45 to −11.12) compared with nonobese patients (−3.6 mm Hg 95% confidence interval −7.82 to 0.66).

Conclusions: Among patients at risk for heart failure, those with obesity have a characteristic proteomic profile reflecting adipogenesis and glucose intolerance. Spironolactone had only minor effects on this obesity-related proteomic profile, but obesity significantly modified the effect of spironolactone on systolic blood pressure. (*J Cardiac Fail 2022;28:778−786*)

Key Words: Obesity, biomarker, heart failure, spironolactone.

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Obesity is positively associated with incident heart failure (HF).[1] Body mass index (BMI) is the most commonly used measurement to define obesity, but it may not accurately reflect obesity because it does not take fat distribution into account. In this regard, waist circumference (WC) better reflects abdominal fat, which seems to be a stronger risk factor for the development of HF than BMI.[2] Obese patients may have higher aldosterone levels owing to hypersecretion of trophic factors from the visceral adipose tissue,[3] which may result in mineralocorticoid receptor overactivation. Obesity might therefore influence the effect of mineralocorticoid receptor antagonist (MRA) therapy. In the EMPHASIS-trial, eplerenone improved outcomes in patients with HF with and without obesity, although the benefit seemed to be more pronounced among those with abdominal obesity as defined by WC.[4]

Adipose tissue influences the expression and degradation of circulating biomarkers.[5] For example, obese patients have lower levels of circulating N-terminal pro brain natriuretic peptide (NT-proBNP). However, little is known regarding the obesity-related proteomic profile, especially in those at risk for HF.

The Heart OMics in AGEing (HOMAGE) trial showed that treatment with spironolactone (vs usual care) in patients at risk of developing HF led to a decrease in collagen synthesis markers, NT-proBNP, and blood pressure, and improved cardiac remodelling.[6] In this prespecified analysis of the HOMAGE trial, we aimed (1) to study the biomarker profile and biological meaning of biomarkers associated with obesity, as compared with patients without obesity; (2) to assess the effect of spironolactone on the circulating proteomic biomarkers in patients with obesity; and (3) to explore whether obesity might have modified the effect of spironolactone on the main outcomes assessed in the HOMAGE trial (level of collagen synthesis markers, NT-proBNP systolic and diastolic blood pressures, echocardiographic left atrial volume, and left ventricular mass).

Methods

Trial Design and Population

The HOMAGE trial had a prospective, randomized, open-label, blinded end point, multicenter design. People at increased risk of developing HF were assigned randomly to receive either spironolactone or standard care (ClinicalTrials.gov Identifier: NCT02556450). The rationale, trial design, and main results have been published elsewhere.[6,7] The study was approved by all relevant ethics committees and regulatory bodies. All participants provided written informed consent prior to study-specific procedures. The main participating criteria included age 65 or older (amended to 60 years during the course of the trial), cardiovascular risk defined by either the presence of coronary artery disease or at least 2 of the following: diabetes, treated hypertension, microalbuminuria, or an abnormal electrocardiogram. Participants also had to have an NT-pro BNP between 125 and 1000 ng/L a or BNP between 35 and 280 ng/L. The main exclusion criteria were an estimated glomerular filtration rate of less than 30 mL/minute/1.73 m^2, serum potassium of more than 5.0 mmol/L, a left ventricular ejection fraction of less than 45%, a diagnosis of HF or treatment with loop diuretics, and atrial fibrillation or flutter. The primary and secondary outcomes measures in the HOMAGE trial were procollagen type I carboxy-terminal propeptide and procollagen III amino terminal propeptide (both of which are markers of collagen synthesis), NT-pro BNP, systolic and diastolic blood pressure, echocardiographic left atrial volume, and left ventricular mass.

For this analysis, patients were divided into 2 groups based on the presence of obesity as defined by a WC of 102 cm or greater for men and 88 cm or greater for women.[8] As an additional analysis, obesity was defined based on BMI and all patients were divided into 2 groups according to the World Health Organization BMI classification (BMI <30 and ≥ 30 kg/m^2).

Proteomic Biomarkers

The baseline, month 1, and month 9 (or last visit) plasma samples were analyzed for 276 protein biomarkers by the TATAA-biocenter using the Olink Proseek Multiplex cardiovascular II, cardiovascular III, and inflammation panels. The proteins were determined using high-throughput Olink Proseek Multiplex 96 $\times$ 96 kits, which measure 92 manually selected proteins simultaneously in 1 μL of plasma per kit. Each kit uses a proximity extension assay technology with dual-recognition DNA-coupled readout, where 92 oligonucleotide-labelled antibody probe pairs are allowed to bind to their respective targets in the sample. The platform provides Log2 normalized protein expression values with relative quantification. A detailed description of the Olink technology is depicted in the Supplemental Materials. The abbreviations, full names, and respective Olink multiplex panels of the studied proteins are described in the Supplemental Table 1. The assays were performed blinded to treatment allocation. The proteomic results were then merged into a database.

Statistical Analyses

We compared the characteristics of the patients with and without obesity using the appropriate tests for continuous and categorical variables. To assess whether the baseline biomarkers were expressed differently in patients with and without obesity, we performed logistic regression analyses with obesity as the outcome variable and adjusted for age, sex, coronary artery disease, hypertension, and estimated glomerular filtration rate. We corrected the findings for multiple testing using a false discovery rate of 5%. After selecting the proteins with differential expression by obesity, we tested whether spironolactone changed the levels of those proteins. For consistency with the primary report, we used analysis of covariance comparing the difference in any changes between the control and spironolactone groups in the regression model.[6]

Proteins that were differentially expressed between obese and nonobese patients were used to investigate further. A linear regression model was fitted, with the protein change (from baseline to last visit) as the outcome variable, a binary variable to indicate the treatment group (control/spironolactone), and the baseline normalized protein expression as covariates. The treatment effect was the coefficient that resulted from the comparison of spironolactone vs control in the regression model. A residual analysis was used to examine the fit of the model. No data transformation was required to meet the assumptions of linear regression. Similar analyses were performed for the protein change at 1 month. To study whether obesity influences the response to spironolactone on the main outcomes of the study, we performed an analysis of covariance with a treatment-by-obesity interaction term. Statistical analyses were performed using Stata (version 16, StataCorp LP, College Station, TX).

Bioinformatical and Network Analyses

Only those proteins that were discovered in both BMI and WC analyses were used for network analysis and to test for functional enrichment. The aims were to enhance consistency and limit the likelihood of

Table 1. Baseline Characteristics of the Patients According to WC

Characteristics	Normal Waist Circumference (n = 211)	High WC (n = 299)	P Value
Age (years)	73 (69-79)	73 (68−78)	.68
Male sex (%)	180 (85)	200 (67)	<**.001**
BMI (kg/m^2)	25.4 (23.7−27.2)	30.8 (28.1−34.2)	<**.001**
WC (cm)	95 (88−99)	109 (103−116)	–
Heart rate (bpm)	59 (54−65)	62 (56−69)	**.002**
Systolic blood pressure (mm Hg)	140 (125−153)	141 (130−157)	.05
Diastolic blood pressure (mm Hg)	78 (71−86)	78 (71−84)	.5
Medical history (%)			
Hypertension	148 (70)	253 (85)	<**.001**
Diabetes mellitus	55 (26)	153 (51)	<**.001**
Coronary artery disease	172 (82)	193 (65)	<**.001**
Stroke/TIA	7 (3)	20 (7)	.09
COPD	10 (5)	21 (7)	.29
Echocardiography			
LV ejection fraction (%)	62 (57−66)	64 (59−67)	.05
Indexed LV mass (g/m^2)	92 (80−106)	97 (82−114)	**.017**
LV hypertrophy	42 (21)	91 (35)	**.001**
Indexed LA volume (mL/m^2)	32 (27−38)	29 (24−35)	**.003**
E/e′	8.9 (7.3−10.8)	9.5 (7.8−11.8)	**.009**
Laboratory results			
Estimated glomerular filtration rate (mL/min/1.73 m^2)	73 (63−86)	72 (61−84)	.15
Urea (mmol/L)	8 (5.5−13.6)	9.3 (6−14.6)	.12
NT-proBNP (pg/mL)	279 (179−422)	219 (154−398)	**.03**
Hemoglobin (g/dL)	14.2 (13.3−14.9)	14 (13−14.8)	.09
Sodium (mmol/L)	140 (138−141)	139 (138−141)	.18
Potassium (mmol/L)	4.3 (4.1−4.5)	4.3 (4.1−4.6)	.59
Medication (%)			
Antiplatelet	173 (82)	228 (76)	.12
Beta-blocker	155 (74)	198 (66)	.08
ACE inhibitor or ARB	158 (75)	242 (81)	.1
Thiazides	23 (11)	64 (21)	**.002**
Lipid-lowering agent	176 (83)	243 (81)	.53

Values are median (interquartile range).

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor type II blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; LA, left atrium; LV, left ventricle; NT pro-BNP, N-terminal pro brain natriuretic peptide; PCI, percutaneous coronary intervention; TIA, transient ischemic attack; WC, waist circumference.

High WC (≥102 cm for men and ≥88 cm for women characterizing abdominal obesity); NWC, normal WC (<102 cm for men and <88 cm for women).

including incidental proteins based on the definition of obesity. The Search Tool for the Retrieval of Interacting Genes/Proteins database was used to analyze functional enrichment (GO biological processes and KEGG pathways) using proteins that were significantly increased or decreased in patients who were obese compared with nonobese at baseline.

Results

Clinical Characteristics of the Study Population

Among the 527 patients included in the HOMAGE trial, 510 were included in the WC analysis (17 patients had a missing WC value). In total, 299 patients (57%) had a high WC ($obese_{WC}$) (ie, WC $\geq$ 102 cm for men and $\geq$ 88 cm for women) (Table 1). Compared with patients with a normal WC, those with $obese_{WC}$ were more likely to be women and had more obesity-related comorbidities such as hypertension and diabetes ($P < .001$ for both). Regarding the BMI analysis, 525 were included (2 further patients had a missing value). Of these patients, 178 (34%) had a BMI of 30 kg/m^2 or greater ($obese_{bmi}$). Compared with patients with a BMI of less than 30 kg/m^2, those with $obese_{bmi}$ were younger; however, despite their younger age they had more obesity-related comorbidities (Supplemental Table 2). In contrast with $obese_{WC}$, patients with $obese_{bmi}$ did not differ from the nonobese regarding sex. There were no relevant significant differences in baseline characteristic between patients treated with spironolactone or standard care in either the $obese_{bmi}$ or $obese_{wc}$ subgroups (Supplemental Tables 3 and 4).

Table 2. Proteins Associated With a High WC in the Blood Sample at Baseline (Before Spironolactone Treatment)

Protein		WC
Proteins associated with high WC and high BMI*		
Adrenomedullin	adm	9.03 (6.35 to 11.70)
Thrombospondin-2	thbs2	8.68 (4.61 to 12.74)
Retinoic acid receptor responder protein-2	rarres2	8.61 (5.51 to 11.71)
Fatty-acid binding protein-4	fabp4	7.52 (6.24 to 8.81)
Perlecan	plc	7.51 (4.09 to 10.92)
Leptin	lep	7.24 (6.43 to 8.05)
IL receptor 1 receptor antagonist	il1ra	6.94 (5.35 to 8.53)
Procollagen III amino terminal propeptide	p3np	3.05 (1.31 to 4.79)
Plasminogen activator inhibitor-1	pai	2.71 (1.73 to 3.7)
Insulin-like growth factor-binding protein-1	igfbp1	−3.65 (−4.63 to −2.67)
Insulin-like growth factor-binding protein-2	igfbp2	−4.22 (−5.91 to −2.52)
Paraoxonase-3	pon3	−4.35 (−6.14 to −2.55)
Proteins associated with high WC but not with a high BMI		
Hepatocyte growth factor	hgf	4.44 (2.09 to 6.78)
Cathepsin D	ctsd	4.21 (1.95 to 6.46)
IL-18 receptor-1	Il18r1	4.3 (2 to 6.61)
Cathepsin B	ctsb	3.69 (1.66 to 5.72)
IL-6	Il6	1.99 (0.88 to 3.11)
Tumor necrosis factor receptor superfamily member-11a	tnfrsf11a	3.99 (1.74 to 6.25)
c-c motif chemokine ligand-16	ccl16	2.74 (1.17 to 4.31)
Cluster of differentiation 8a	cd8a	3.12 (1.39 to 4.85)
Highly sensitive troponin T	hstnt	3.21 (1.41 to 5.00)
Tumor necrosis factor receptor superfamily member-6	fas	4.99 (2.19 to 7.80)

Values are the β-coefficient (95% confidence interval). All reported values were significant using a false discovery rate (FDR) of 5%. The β-coefficients represent the changes in Log2 normalized protein **expression** values, as per Olink standard reporting of protein concentrations. Abbreviations as in Table 1.

*See supplemental Table 5 for the proteins associated with a high BMI ($\geq$30 kg/m^2). The 12 proteins that were associated with both measures for obesity were selected for biological pathway analysis.High WC ($\geq$102 cm for men and $\geq$88 cm for women characterizing abdominal obesity).

Proteomic Profile at Baseline

At baseline, patients with $obese_{WC}$ had greater expression of 19 proteins and lower expression of 3 proteins compared with $nonobese_{WC}$ patients (Table 2). Twelve of these proteins (9 increased, 3 decreased) were also found in $obese_{bmi}$ patients, suggesting that of those proteins tested, these 12 are the most robustly associated with obesity irrespective of definition (Supplemental Table 5).

The 12 proteins strongly clustered in biological processes related to (1) lipid metabolism, (2) angiogenesis, and (3) insulin signaling (Fig. 1). Adrenomedullin (ADM) showed the strongest positive correlation with obesity (β-coefficient 9.03 [6.35−11.7], Table 2) and is involved in all processes, as is also the case for leptin. The proteomic profile of the obese patient reflects increased adipogenesis, increased vascularization, decreased fibrinolysis, and greater glucose intolerance (Fig. 2).

Proteomic Changes With Spironolactone in Obese Patients

Among the differentially expressed proteins in obese patients (Table 2), spironolactone induced a mild but significant increase in leptin and fatty acid-binding protein 4 (FABP4) at 1 month, a mild increase in FABP4, chemerin (RARRES2), perlecan, and ADM at 9 months, and a mild decrease of thrombospondin-2 (THBS2) at 9 months (Supplemental Table 6).

Interaction of Obesity With the Main Trial Outcomes

The modifying effect of obesity on the impact of spironolactone for each of the main HOMAGE trial

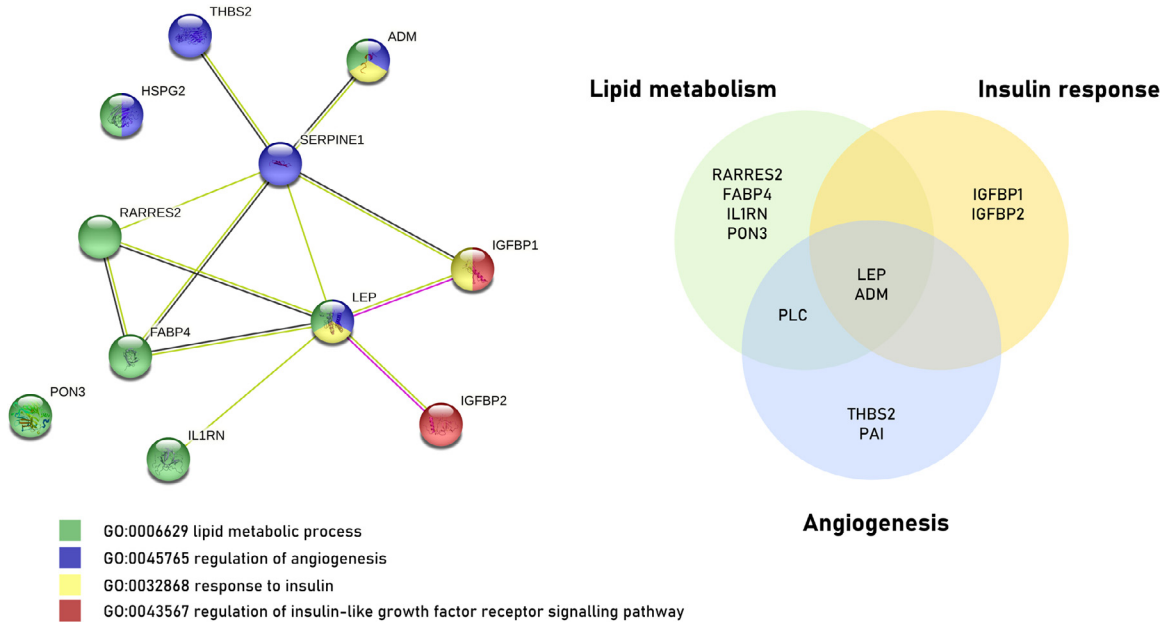

Fig. 1. Network of protein biomarkers which were significantly differentially detected in patients with obesity compared with nonobese patients (false discovery rate q value < 0.05). The full names of the proteins are depicted in Supplemental Table 1. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to analyze functional enrichment (GO biological processes and KEGG pathways) using these proteins. Four major pathways could be retrieved from this analysis as reflected by corresponding colors (left). Metaclustering revealed involvement of proteins in single, or in multiple biological processes (right). P3NP was not depicted in the figure as it is a product of collagen production which could not be analyzed for protein-protein interactions.

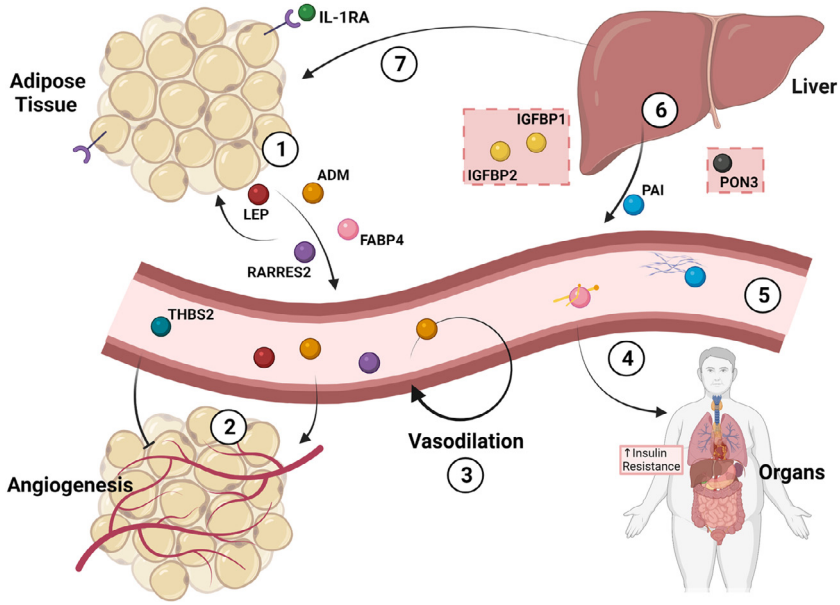

Fig. 2. Summary of the biological processes of the biomarkers which are differentially expressed in obese patient compared with nonobese patients. (1) The adipokines leptin (LEP), adrenomedullin (ADM), fatty acid-binding protein 4 (FABP4), and chemerin (RARRES2) are secreted from the adipocytes into the circulation. Leptin and chemerin work in an autocrine feedback loop to stimulate oxidative substrate metabolism. (2) Leptin, adrenomedullin, and chemerin all stimulate angiogenesis, thrombospondin-2 (THBS2) is a well-known modulator of extracellular matrix remodeling during angiogenesis. (3) Adrenomedullin causes dilation of the blood vessels by exerting its effect on the vascular smooth muscle cells. (4) Fatty acid-binding protein 4 acts as a chaperone for long-chain fatty acids (FA) in the circulation, and transports these to the organs. (5) Plasminogen activator (PAI)-1 blocks the activation of fibrinolysis, thereby limiting the dissolution of the fibrin clot. (6) Paraoxonase-3 (PON3) is anchored to high-density lipoprotein (HDL) particles in the circulation, and prevent low-density lipoprotein (LPL) particles from oxidation. (7) Insulin-like growth factor-binding protein-1 and 2 (IGFBP-1 and IGFBP-2) are strongly associated with insulin sensitivity and prevent adipocyte differentiation and adipogenesis. The proteins in the red squares (IGFBP-1, IGFBP-2, and PON3) are decreased in obese patients. P3NP was not depicted in the figure as it is a product of collagen production which could not be analyzed for protein−protein interactions. (Figure created with BioRender.com.)

Table 3. Association Between Spironolactone and Primary Outcomes From the HOMAGE Trial Depending on WC

Characteristics	β-Coefficient (95% CI)	*P* value
Procollagen type I carboxy-terminal propeptide (μg/L)		
NWC	−4.61 (−10.44 to 1.21)	.12
HWC	−9.91 (−15.05 to −4.76)	**.0002**
Interaction spironolactone*WC	.26	
Procollagen III amino terminal propeptide (μg/L)		
NWC	0.15 (−0.23 to 0.52)	.44
HWC	−0.09 (−0.54 to 0.36)	.69
Interaction spironolactone*WC	.55	
Carboxy-terminal telopeptide of collagen type I (μg/L)		
NWC	0.22 (−0.53 to 0.97)	.56
HWC	0.13 (−0.51 to 0.78)	.69
Interaction spironolactone*WC	.82	
N-terminal pro brain natriuretic peptide (ng/L)		
NWC	−0.03 (−0.23 to 0.17)	.78
HWC	−0.25 (−0.46 to −0.04)	**.022**
Interaction spironolactone*WC	.14	
Systolic blood pressure (mm Hg)		
NWC	−3.58 (−7.82 to 0.66)	.1
HWC	−14.79 (−18.45 to −11.12)	**.001**
Interaction spironolactone*WC	**.001**	
Diastolic blood pressure (mm Hg)		
NWC	−1.5 (−3.77 to 0.78)	.2
HWC	−4.53 (−6.66 to −2.4)	**.001**
Interaction spironolactone*WC	.06	
Indexed left atrial volume (mL/m^2)		
NWC	−2.03 (−3.86 to −0.20)	**.03**
HWC	−2.83 (−4.59 to −1.08)	**.002**
Interaction spironolactone*WC	.54	
Indexed left ventricular mass (g/m^2)		
NWC	−0.3 (−3.51 to 2.92)	.86
HWC	−3.49 (−6.81 to −0.16)	**.04**
Interaction spironolactone*WC	.19	

HWC, high WC; NWC, normal WC. Other abbreviations as in Table 1.
High WC (≥102 cm for men and ≥88 cm for women characterizing abdominal obesity).

outcomes[6] is shown in Table 3. The interaction between spironolactone and obese$_{WC}$ reached statistical significance only for systolic blood pressure (P_{int} = .001), meaning that obese$_{WC}$ patients had a significant greater decrease of systolic blood pressure (−14.8 mm Hg) in response to spironolactone compared with nonobese$_{WC}$ patients (−3.6 mm Hg). There was also a trend indicating a greater decrease in diastolic blood pressure in obese$_{WC}$ patients (P_{int} = .06).

Discussion

Investigating the clinical and proteomic response to spironolactone in patients with obesity showed that (i) obese individuals have a plasma proteomic profile reflecting adipogenesis, increased vascularization, decreased fibrinolysis, and glucose intolerance and (ii) spironolactone has only a minor influence on this proteomic profile, but (iii) obesity modifies the antihypertensive effect of spironolactone. Obesity might, therefore, modify the effects of spironolactone in patients with an elevated cardiovascular risk.

Spironolactone Treatment in Patients With Obesity

We observed a significant interaction between obesity and a decrease in systolic blood pressure; patients with obesity had a stronger antihypertensive response to spironolactone compared with nonobese patients. Obesity is characterized by high circulating levels of aldosterone, which can be secreted directly by adipocytes or may be released from the adrenal gland in response to leptin.[9] The increased levels of leptin in obese patients may be responsible for the excessive mineralocorticoid receptor signaling that is the hallmark of obesity-

related HF.[4] Rat studies showed that interference with the mineralocorticoid receptor signaling by using antagonists (eg, MRAs) delayed HF onset in both lean and obese rats, but the obese rats showed an additional improvement regarding to cardiac fibrosis, obesity, and dyslipidaemia.[10] Also in mice, MRA treatment attenuated the obesity-related insulin resistance through a decrease in inflammation and oxidative stress.[11] In the EMPHASIS-HF trial, eplerenone improved outcome in patients with HF with reduced ejection fraction, with the most benefit in those with abdominal obesity.[4] The same observation was made in the TOPCAT trial, which investigated spironolactone treatment in patients with HF with preserved ejection fraction; the favorable effect of MRA was most apparent in obese patients who had elevated levels of aldosterone.[12] Treatment with a MRA can thus be considered as a first-line agent for hypertension in obese patients at risk for HF.

In our study, we analyzed the influence of spironolactone on the proteomic profile of obese patients at risk for HF. Spironolactone had only a minor influence on the proteomic profile of obese patients, only leptin, FABP4, RARRES2, THBS2, ADM, and perlecan were minimally altered after 1 and 9 months of treatment. The increase in leptin could be a positive feedback loop as a result of interfering in the leptin−aldosterone−neprilysin axis.[9] The other proteomic markers in the circulation are mostly secreted by the adipose tissue or as a response to increased fatty acids in the circulation. Although spironolactone has pleiotropic effects, it does not influence the substrate metabolism, which partly explains the minor influence of spironolactone on the proteomic profile.[13]

Obesity did modify the antihypertensive effect of spironolactone, showing that obese patients at risk for HF derive greater benefit from spironolactone compared with those who are not overweight. Although aldosterone was not measured in the HOMAGE trial, a relative hyperaldosteronism in obese patients may explain the beneficial effect of spironolactone on blood pressure.[14] However, even if the effects of spironolactone on blood pressure are greater in those with obesity, spironolactone still improved cardiac remodeling in those without obesity, suggesting additional beneficial mechanisms in people at risk beyond blood pressure reduction.[13]

Biological Proteome Profile of Patients With Obesity

The most commonly used measurement to define obesity is BMI, although this metric does not take fat distribution into account. Abdominal fat is a potential risk factor in the onset of HF and is better reflected by measuring the WC.[2] We used both BMI and WC to find the most consequent proteins associated with obesity to minimize incidental findings. Twelve biomarkers were strongly associated with obesity, of which 9 circulating proteins were elevated and 3 were decreased in obese people. Collectively, these biomarkers reflect a metabolic profile of insulin resistance, (adipose) angiogenesis, expansion of adipocytes, thrombosis, and a dysregulated substrate metabolism, which is summarized step by step in Fig. 2.

The biological function of these 12 proteins can be associated directly with obesity, and most of the described proteins can be linked directly or indirectly to HF or cardiac disease. The increase in adipose tissue is associated with an elevated serum concentration of the adipokines *ADM, leptin, FABP4*, and *chemerin* (*RARRES2*).[15−18] By autocrine signaling, leptin can influence the metabolism of adipocytes directly (via the leptin receptors on adipocytes) and indirectly, as it modifies the insulin sensitivity of adipocytes to stimulate oxidative substrate metabolism and to inhibit lipid accumulation.[18] In addition, increased leptin signaling can promote cardiac inflammation, microcirculatory abnormalities, and cardiac fibrosis.[9] Chemerin also works in an autocrine loop to induce differentiation and expansion of preadipocytes towards adipocytes.[17] Leptin, ADM, and chemerin all stimulate angiogenesis, thereby ensuring sufficient blood supply for the adipocyte expansion.[19−21] *THBS2* is a well-known modulator of extracellular matrix remodeling during angiogenesis.[22] ADM improves the integrity of the blood vessels, and thereby decrease vascular permeability. In addition, it causes dilation of the blood vessels by exerting its effect on the vascular smooth muscle cells.[23,24] FABP4 acts as a chaperone for long-chain fatty acids in the circulation, and transports these to the organs.[15,25] The oxidative stress associated with increasing visceral fat leads to a conformational change of FABP4, which prevents the protein to bind fatty acids effectively. This process eventually leads to a dysregulation of communication between energy storage systems and organs, disrupting glucose homeostasis and mediating insulin resistance. *Plasminogen activator* (*PAI*)*-1*, expressed by the *SERPINE1* gene, is mainly secreted from the liver and the adipose tissue and blocks the activation of fibrinolysis, thereby limiting the dissolution of the fibrin clot.[26,27] Obesity is associated with a marked increase of plasma PAI-1 levels, mainly because PAI-1 is implicated in adipose tissue development and insulin signaling in adipocytes. The impaired fibrinolysis, together with endothelial dysfunction, hyperreactivity of platelets and hypercoagulability contribute to a prothrombotic state in patients with obesity.[26]

The *paraoxonase-3* concentration is decreased in patients with obesity. This protein is secreted by the

liver and is anchored to high-density lipoprotein particles in the circulation, and prevent low-density lipoprotein particles from oxidation.[28] Low levels of circulating PON3 are a risk factor for atherosclerosis and obesity owing to disturbed lipid metabolism.[29] Levels of *insulin-like growth factor-binding protein 1 and 2* (*IGFBP1 and IGFBP2)* are strongly associated with insulin sensitivity; thus, the low levels of IGFBP-1 and -2 reflect insulin resistance.[30] The IGFBPs prevent adipocyte differentiation and adipogenesis. Levels of *IL-1 receptor antagonist (IL1RN)* are associated with measures of obesity and insulin resistance.[31] The increased levels of inflammatory cytokines compete with insulin for binding of the insulin receptor. As a consequence, the substrate metabolism is disturbed, leading to decreased fat to carbohydrate oxidation rate.[32]

Limitations

We tested the effect of spironolactone on multiple proteins applying a correction for test multiplicity to limit the occurrence of false positive findings; however, because HOMAGE was a randomized controlled trial, other proteins, whose levels were also significantly changed with spironolactone, might also be implicated in relevant pathways and biological processes and might be worth exploring in further studies. Additionally, many of the highlighted mechanisms should be replicated and confirmed at the cellular level. The effect of spironolactone (vs standard of care) on the circulating proteins associated with obesity was tested as an exploratory analysis and not corrected for multiplicity of tests. Such results require further replication and should be interpreted with caution. About 68% of the included subjects in the HOMAGE trial had more than 250 days of follow-up, meaning that a minority of patients did not have 9 months of follow-up. However, in the HOMAGE clinical trial, we observed that most of the effects of spironolactone on biomarkers occurred after 1 month of treatment.[6]

Conclusions

Among patients at risk for HF, those with obesity have a characteristic proteomic profile reflecting adipogenesis and glucose intolerance. Spironolactone had only a minor effect on this obesity-related proteomic profile, but obesity modified the effect of spironolactone on systolic blood pressure.

LAY SUMMARY

Obesity is a well-known risk factor for developing heart failure. Biomarkers measured in the blood show increased adipogenesis and glucose intolerance in patients with obesity. Treatment with spironolactone does not change this biomarker profile. However, patients with obesity have a greater decrease in their systolic blood pressure when treated with spironolactone compared with nonobese patients.

Declaration of Competing Interest

The authors have no relevant conflicts of interest to disclose with regards to the content of this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cardfail.2021.12.005.

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