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ORIGINAL RESEARCH ARTICLE

Elevated plasma sRAGE and IGFBP7 in heart failure decrease after heart transplantation in association with haemodynamics

Abdulla Ahmed[1,2]*, Salaheldin Ahmed[1,2], Mattias Arvidsson[1,2], Habib Bouzina[1,2], Jakob Lundgren[1,2] and Göran Rådegran[1,2]

[1]*Department of Clinical Sciences, Lund, The Section for Cardiology, Lund University, Lund, Sweden;* [2]*The Haemodynamic Lab, The Section for Heart Failure and Valvular Disease, VO Heart and Lung Medicine, Skåne University Hospital, Lund, Sweden*

Abstract

Aims Metabolic derangement is implicated in the pathophysiology of heart failure (HF) and pulmonary hypertension (PH). We aimed to identify the dynamics of metabolic plasma proteins linked to end-stage HF and associated PH in relation to haemodynamics, before and after heart transplantation (HT).

Methods and results Twenty-one metabolic plasma proteins were analysed with proximity extension assay in 20 controls and 26 patients before and 1 year after HT. Right heart catheterizations were performed in the HF patients pre-operatively and 1 year after HT. Plasma levels of soluble receptor for advanced glycation end products (sRAGE) and insulin-like growth factor-binding protein 7 (IGFBP7) were higher in HF patients compared with controls ($P < 0.0001$) and decreased after HT ($P < 0.0001$), matching controls' levels. The decrease in sRAGE after HT correlated with improved mean pulmonary arterial pressure ($r_s = 0.7$; $P < 0.0001$), pulmonary arterial wedge pressure ($r_s = 0.73$; $P < 0.0001$), pulmonary vascular resistance ($r_s = 0.65$; $P = 0.00062$), and pulmonary arterial compliance ($r_s = -0.52$; $P = 0.0074$). The change in plasma IGFBP7 after HT correlated with improved mean right atrial pressure ($r_s = 0.71$; $P = 0.00011$) and N-terminal pro-brain natriuretic peptide ($r_s = 0.71$; $P < 0.0001$).

Conclusions Our results indicate that plasma sRAGE may reflect passive pulmonary vascular congestion and the 'mechanical' state of the pulmonary vasculature in HF patients with or without related PH. Furthermore, sRAGE and IGFBP7 may provide additional insight into the pathophysiological mechanisms in HF and associated PH. Their potential clinical and therapeutic relevance in HF and associated PH need further investigation.

Keywords Biomarkers; Haemodynamics; Heart failure; Heart transplantation; Metabolism; Pulmonary hypertension

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**Correspondence to: Abdulla Ahmed, The Haemodynamic Lab, The Section for Heart Failure and Valvular Disease, VO Heart and Lung Medicine, Skåne University Hospital, Lund, Sweden. Fax: 0046-(0)46-307984. Email: abdulla.ahmed@med.lu.se; abdulla_896@hotmail.com*

Introduction

Altered energetics and metabolic inefficiency emerge in the failing heart in addition to a multitude of pathogenic mechanisms such as neurohormonal activation and matrix remodelling.[1–3] Metabolic derangement in heart failure (HF) may manifest as decreased oxidative metabolism and a shift to anaerobic glycolysis, leading to an energy deficit that maintain the progression in HF.[4,5] Furthermore, both cardiac and systemic insulin resistance may emerge, and lower insulin sensitivity correlates with increased morbidity and mortality.[2,6] Metabolic dysregulation has also been proposed to contribute to the development and progression of pulmonary hypertension (PH), a complicating condition present in up to 75% of patients with HF with reduced ejection fraction (HFrEF) and 83% of HF with preserved ejection fraction (HFpEF).[7] This dysregulation is associated with aberrant signalling in pulmonary vascular cells, from which endothelial dysfunction and vascular remodelling may arise.[8]

Biomarkers including proteomics may in this context aid in establishing diagnosis and prognosis and refine risk stratification in HF.[3,9,10] Given the complex interplay of different pathophysiological mechanisms in HF,[3] multimarker testing has been proposed as a future approach,[9,10] where each biomarker represents an important pathophysiological pathway.[9] In addition, as the heart is more energy consuming per gram tissue than any other organ,[1,11] adjuvant therapies aiming to attenuating the dysfunction of cardiac metabolism may be of great value.[4] In this regard, biomarkers that may reflect or provide information on the pathophysiology of HF[3] may potentially aid in the development of new treatment strategies in HF, urged for in current guidelines.[12]

Apart from metabolic derangement and haemodynamic overload, other functioning mechanisms in HF that influence the cardiac architecture include breakdown and synthesis of the extracellular matrix.[3,10] This may result in excessive fibrosis following myocardial infarction (MI) and/or ventricular remodelling in dilated cardiomyopathy, both of which affect haemodynamics adversely.[3] Accordingly, we recently mapped the dynamics of plasma extracellular matrix proteins in HF patients and found that MMP-2 and prolargin may reflect the aberrant extracellular matrix remodelling in HF and associated PH.[13] Given the complex and diverse pathophysiological mechanisms present in HF and PH,[3,8] we aimed in the present study to further investigate in end-stage HF patients the plasma levels of metabolic proteins in relation to haemodynamics, before and 1 year after heart transplantation (HT).

Methods

Study population

The study population consisted of 29 HF and heart transplant recipients in addition to 20 healthy controls ≥18 years, enrolled between October 2011 and February 2017 in the Lund Cardio Pulmonary Register (LCPR) cohort of Region Skåne biobank. Patients with missing haemodynamic values or exhibiting PH after HT were excluded (n = 3). Seventeen (65.4%) out of 26 patients had dilated cardiomyopathy, whereas 3 (11.5%) had ischaemic cardiomyopathy, hypertrophic cardiomyopathy, or other aetiology, respectively. Thirteen patients (50%) had atrial fibrillation, 3 (11.5%) diabetes mellitus, and 5 (19.2%) hypertension. Controls without a medical history of MI, HF, diabetes mellitus, or atrial fibrillation were enrolled in LCPR and included in the present study (*Table 1*).

All participants provided informed written consent, and the study was conducted in accordance with the Declaration of Helsinki and Istanbul and approved by the regional ethical board in Lund, Sweden (Dnr: 2010/114, 2010/442, 2011/368, 2011/777, 2014/92, and 2015/270).

Proteomic analysis

Venous plasma samples were collected from controls and in patients during right heart catheterization (RHC) prior to and at the 1 year follow-up after HT. The samples were stored at −80°C in LCPR. Twenty-one metabolic proteins and N-terminal pro-brain natriuretic peptide (NT-proBNP) were analysed with proximity extension assay (PEA) using Proseek Multiplex immunoassay reagent kits (Cardiovascular II, III and Oncology II panel, Olink Proteomics, Uppsala, Sweden). The selected panels included a variety of proteins that involved a broad range of mechanisms and pathways, including but not limited to extracellular matrix, metabolism, and inflammation. From the three panels, metabolic proteins were selected and defined as a protein category in the present study. PEA is a 96-plex immunoassay based on pairs of antibodies associated with unique oligonucleotides (probes). Upon target binding, the probes come into proximity and a DNA polymerase extends the two oligonucleotide pairs, creating a template for quantitative-PCR.[14] Adjustments for inter-plate variations and protein-level normalization were made with internal controls added to each sample as well as external controls added as separate samples. The panels are also validated for sensitivity, specificity, dynamic range, precision, and scalability. Protein levels are expressed in arbitrary units (AU) on a linear normalized protein expression scale (Olink Proteomics).

The metabolic plasma proteins included 2,4-dienoyl-CoA reductase (DECR-1), fatty acid binding protein 2 (FABP2), 4 (FABP4), fibroblast growth factor-binding protein 21 (FGF-21), folate receptor gamma (FR-gamma), insulin-like growth factor 1 receptor (IGF1R), insulin-like growth factor-binding protein 1 (IGFBP1), 2 (IGFBP2), 7 (IGFBP7), low-density lipoprotein receptor (LDLR), leptin, oxidized low-density lipoprotein receptor 1 (LOX-1), lipoprotein lipase (LPL), proprotein convertase subtilisin/kexin type 9 (PCSK9), serum paraoxonase 3 (PON3), retinoic acid receptor responder protein 2 (RARRES2), resistin, serpin A12, soluble receptor for advanced glycation end products (sRAGE; the total pool of all soluble forms of RAGE), pappalysin-1, and transferrin receptor 1 (TR-1).

Right heart catheterization and population characteristics

RHC was performed in the patients in supine position prior to and at the follow-up 1 year after HT, using a Swan–Ganz catheter (Baxter Healthcare Corp., Santa Ana, CA, USA) inserted

Table 1 Characteristics of study population

	Controls (n = 20)		Pre-HT (n = 26)		Post-HT (n = 26)	
Variable	n (%)	Median (IQR)	n (%)	Median (IQR)	n (%)	Median (IQR)
Female, n (%)	10 (50)		5 (19.2)			
Age (years)	20 (100)	41 (27–51)	26 (100)	50 (45–61)*	26 (100)	52 (47–63)
BSA (m^2)	19 (95)	1.9 (1.8–2.0)	25 (96.2)	2 (1.8–2.1)	26 (100)	2 (1.8–2.1)
Creatinine (μmol/L)			25 (96.2)	108 (90–123)	26 (100)	114 (97–142)
eGFR (mL/min/1.73 m^2)			25 (96.2)	63 (55–71)	26 (100)	53 (43–72)
NT-proBNP (AU)	20 (100)	1.1 (1.1–1.2)	26 (100)	24 (11–40)*	26 (100)	2 (1.4–5.8)*,**
HFrEF (EF < 50%)			24 (92.3)			
HFpEF (EF ≥ 50%)			2 (7.7)			
PH-LHD			19 (73.1)			
Ipc-PH			10 (52.6)[a]			
Cpc-PH			9 (47.4)			
Atrial fibrillation			13 (50)		—	
Diabetes mellitus			3 (11.5)		9 (34.6)	
Hypertension			5 (19.2)		3 (11.5)	
Medications			n (%)		n (%)	
Beta-blockers			25 (96.2)		9 (34.6)	
Angiotensin-converting enzyme inhibitor			11 (42.5)		—	
Angiotensin II receptor blocker			11 (42.5)		10 (38.5)	
Mineralocorticoid receptor antagonist			22 (84.6)		3 (11.5)	
Furosemide			24 (92.3)		12 (46.2)	
Cordarone			4 (15.4)		—	
Prednisolone			1 (3.8)		25 (96.2)	
Cyclosporine			—		3 (11.5)	
Tacrolimus			—		23 (88.5)	
Mycophenolate mofetil			—		21 (80.8)	
Azathioprine			—		5 (19.2)	
Sildenafil			—		1 (3.8)	
Levosimendan			—		—	
Diabetic patients' characteristics			Non-diabetic (n = 23)	Diabetic (n = 3)	Non-diabetic (n = 17)	Diabetic (n = 9)
Fasting glucose (mmol/L)			—	6.5 (6.2–6.7)[b]	—	6.7 (5.8–7.6)[c]
HbA1C (mmol/mol)			—	47 (44–49)[b]	—	37 (36–42)
NT-proBNP (AU)			21 (11–37)	63 (13–85)	1.9 (1.5–4.8)	2.8 (1.2–6.9)

AU, arbitrary units; BSA, body surface area; Cpc-PH, combined post-capillary and pre-capillary pulmonary hypertension; eGFR, estimated glomerular filtration rate; HbA1C, glycated haemoglobin; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HT, heart transplantation; Ipc-PH, isolated post-capillary pulmonary hypertension; IQR, inter-quartile range; NT-proBNP, N-terminal pro-brain natriuretic peptide; PH-LHD, pulmonary hypertension associated with left heart disease.
$P < 0.0003$ was considered statistically significant; false discovery rate <0.01.
[a]One patient exhibited severe orthopnoea during evaluation with right heart catheterization, and pulmonary arterial wedge pressure could not be measured. The patient underwent a second right heart catheterization as a part of the pre-HT evaluation, which revealed isolated post-capillary pulmonary hypertension.
[b]Indicates $n - 1$.
[c]Indicates $n - 2$.
*$P < 0.0003$; false discovery rate <0.01, vs. controls.
**$P < 0.0003$; false discovery rate <0.01, vs. pre-HT.

predominantly via the right internal jugular vein. PH due to left heart disease was diagnosed by experienced cardiologists and defined as a resting mean pulmonary arterial pressure (mPAP) ≥25 mmHg with a pulmonary arterial wedge pressure (PAWP) >15 mmHg.[15] Further classification of isolated post-capillary PH vs. combined post-capillary and pre-capillary PH was defined as a diastolic pulmonary pressure gradient (DPG) <7 mmHg and/or pulmonary vascular resistance (PVR) ≤3 WU vs. DPG ≥ 7 mmHg and/or PVR > 3 WU, respectively.[15] The haemodynamic data closest to HT or prior to left ventricular (LV) assist device implantation were used if more than one RHC was performed.

During RHC, the mean arterial pressure (MAP), mPAP, mean right atrial pressure (MRAP), PAWP, systolic pulmonary arterial pressure (SPAP), diastolic pulmonary arterial pressure (DPAP), arterial oxygen saturation (SaO_2), and mixed venous oxygen blood saturation (SvO_2) were measured. Calculated haemodynamic parameters and the corresponding formulae include cardiac index (CI) = cardiac output (CO)/body surface area (BSA); stroke volume (SV) = CO/HR; stroke volume index (SVI) = CI/HR; DPG = DPAP − PAWP; transpulmonary pressure gradient (TPG) = mPAP − PAWP; pulmonary arterial compliance (PAC) = SV/(SPAP − DPAP); PVR = TPG/CO, LV stroke work index (LVSWI) = (MAP − PAWP) × SVI, and right

ventricular stroke work index (RVSWI) = (mPAP − MRAP) × SVI (*Table 2*).

The creatinine-based estimation of glomerular filtration rate of the patients during RHC was calculated using the revised Lund–Malmö formula.[16] HF and immunosuppression therapies were in accordance with the guidelines of European Society Cardiology and International Society for Heart and Lung Transplantation[12,17] (*Table 1*).

Statistical analysis

Non-parametric data were presented in median (inter-quartile range) unless otherwise stated. Wilcoxon signed-rank test and Mann–Whitney *U* test were used as appropriate. Outliers were defined by Tukey's fence. Correlations were expressed by Spearman's rank coefficient (r_s). To accommodate for mass significance, the two-stage step-up method of Benjamini, Krieger, and Yekutieli was used to calculate the false discovery rate (FDR) with (Q = 0.01) for comparisons of plasma proteins, haemodynamic parameters, and demographic characteristics and (Q = 0.1) for correlations. *P* values less than the FDR thresholds were considered statistically significant. Statistical analyses were performed using GraphPad Prism Version 8.00 (GraphPad Software, La Jolla, CA, USA, www.graphpad.com).

Study set-up

Two criteria were applied to determine the relative importance of the metabolic plasma proteins: (i) a significant change in plasma levels after vs. before HT, as well as in before HT vs. the controls, and (ii) post-HT protein levels develop towards the controls' plasma levels, displaying a pattern of normalization or matching controls' levels (*Figure 1A*). These criteria excluded proteins whose development did not correspond to NT-proBNP levels and haemodynamic improvement. The primary aim of the control group was to assess the general normal ranges of the plasma proteins and to limit the number of statistical tests performed. Next, correlations of changes between metabolic plasma protein levels and improvement in haemodynamics were analysed (*Figure 1B*). Subsequently, plasma proteins were ranked based on the strength of r_s and the number of correlations present (*Figure 1C* and *1D*).

Results

Study population

The characteristics of the controls and heart transplant recipients have previously been described (*Table 1*).[13,18]

Table 2 Haemodynamic characteristics of patients

Haemodynamic parameter	Pre-HT (n = 26)		Post-HT (n = 26)		Δ (post-HT − pre-HT)		*P* value
	n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	Post-HT vs. Pre-HT
MAP (mmHg)	25	82 (77–93)	26	102 (91–108)	25	15 (9–27)	<0.0001*
mPAP (mmHg)	25	29 (24–38)	26	14 (12–17)	25	−15 (−26 to 7.5)	<0.0001*
PAWP (mmHg)	24	20 (18–25)	26	7 (4–9.3)	24	−17 (−21 to 6.5)	<0.0001*
MRAP (mmHg)	25	14 (7.5–18)	25	3 (1–4)	24	−12 (−15 to 3.3)	<0.0001*
HR (b.p.m.)	25	73 (69–82)	26	82 (73–89)	25	7 (−4 to 15)	0.063
CO (L/min)	25	3.3 (2.6–4.1)	26	5.5 (5–6.5)	25	2.2 (1.2–2.9)	<0.0001*
CI (L/min/m^2)	25	1.8 (1.4–2.2)	26	2.8 (2.6–3.2)	25	1.1 (0.65–1.6)	<0.0001*
SV (mL/beat)	25	48 (35–58)	26	72 (66–78)	25	23 (14–34)	<0.0001*
SVI (mL/beat/m^2)	25	25 (18–29)	26	36 (33–40)	25	12 (6.5–18)	<0.0001*
DPG (mmHg)	24	1 (0–3.8)	26	2 (−0.25 to 4)	24	0 (−2 to 3.5)	0.8
TPG (mmHg)	24	8.5 (6–12)	26	8 (5–10)	24	−1.5 (−6 to 2)	0.17
PAC (mL/mmHg)	25	2.2 (1.8–3.1)	26	5.4 (4.1–6.6)	25	3.2 (1.3–4)	0.00029*
PVR (WU)	24	2.4 (1.4–3.5)	26	1.4 (0.89–1.9)	24	−1.3 (−1.9 to 0.036)	<0.0001*
PVRI (WU/m^2)	24	5.1 (2.9–6.9)	26	2.8 (1.7–3.7)	24	−2.4 (−4 to 0.42)	<0.0001*
LVSWI (mmHg × mL/m^2)	24	1541 (1052–2007)	26	3344 (3167–3810)	24	1675 (1224–2532)	<0.0001*
RVSWI (mmHg × mL/m^2)	25	362 (294–615)	25	429 (317–516)	24	62 (−119 to 245)	0.64
a-vO_2diff (mL O_2/L)	25	74 (63–81)	23	42 (40–51)	22	−32 (−40 to 19)	<0.0001*
SaO_2 (%)	25	96 (94–97)	23	97 (96–98)	22	1.7 (−0.2 to 2.8)	0.046
SvO_2 (%)	25	52 (47–60)	26	69 (66–72)	25	17 (11–24)	<0.0001*

a-vO_2 diff, arteriovenous oxygen difference; CI, cardiac index; CO, cardiac output; DPG, diastolic pulmonary pressure gradient; HR, heart rate; LVSWI, left ventricular stroke work index; MAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; MRAP, mean right atrial pressure; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; PVRI, pulmonary vascular resistance index; RVSWI, right ventricular stroke work index; SaO_2, arterial oxygen saturation; SV, stroke volume; SVI, stroke volume index; SvO_2, mixed venous oxygen saturation; TPG, transpulmonary pressure gradient; WU, Wood units. Other abbreviations as in *Table 1*.

One CO value was calculated with indirect Fick before HT.

*Indicates statistically significant values ($P < 0.0003$; false discovery rate <0.01).

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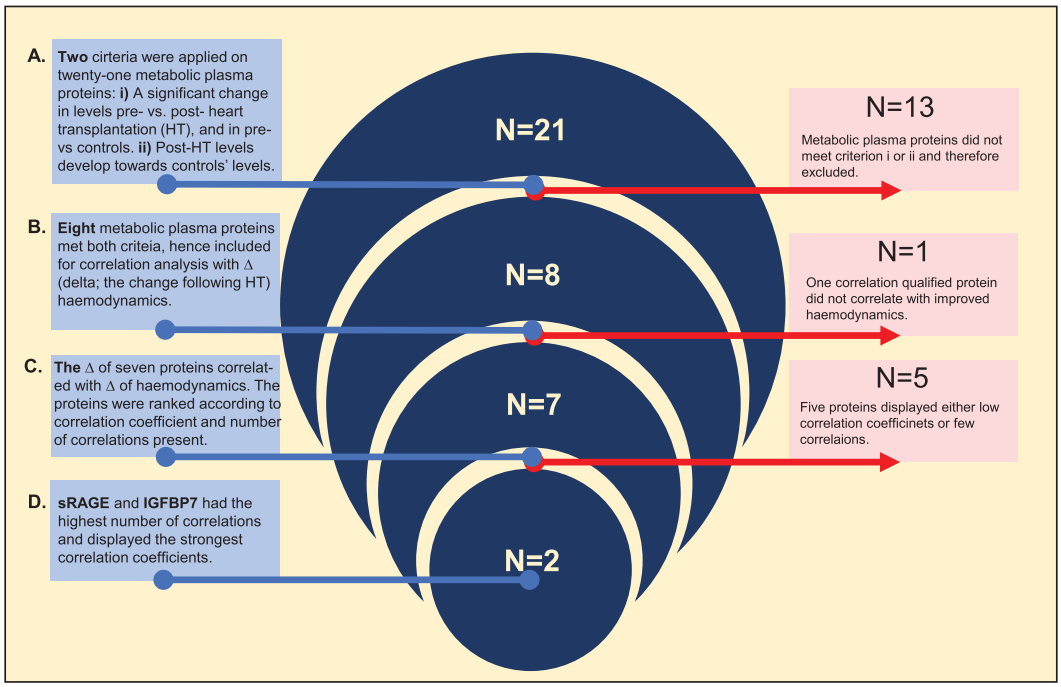
Figure 1 Overview of the study set-up. Statistical significance in (A) was $P < 0.008$; false discovery rate <0.01. Statistical significance for correlations of change in (B)–(D) was $P < 0.021$; false discovery rate <0.1. IGFBP7, insulin-like growth factor-binding protein 7; sRAGE, soluble receptor for advanced glycation end products.



Plasma NT-proBNP levels were higher in the patients prior to HT compared with controls and decreased after HT, towards controls' levels ($P < 0.001$; FDR < 0.01). Plasma creatinine ($P = 0.49$) and the creatinine-based estimation of glomerular filtration rate (eGFR) ($P = 0.15$) remained unchanged following HT (FDR < 0.01) (*Table 1*).

High plasma sRAGE and IGFBP7 in end-stage HF decreased after HT towards controls' levels and correlated with improved haemodynamics

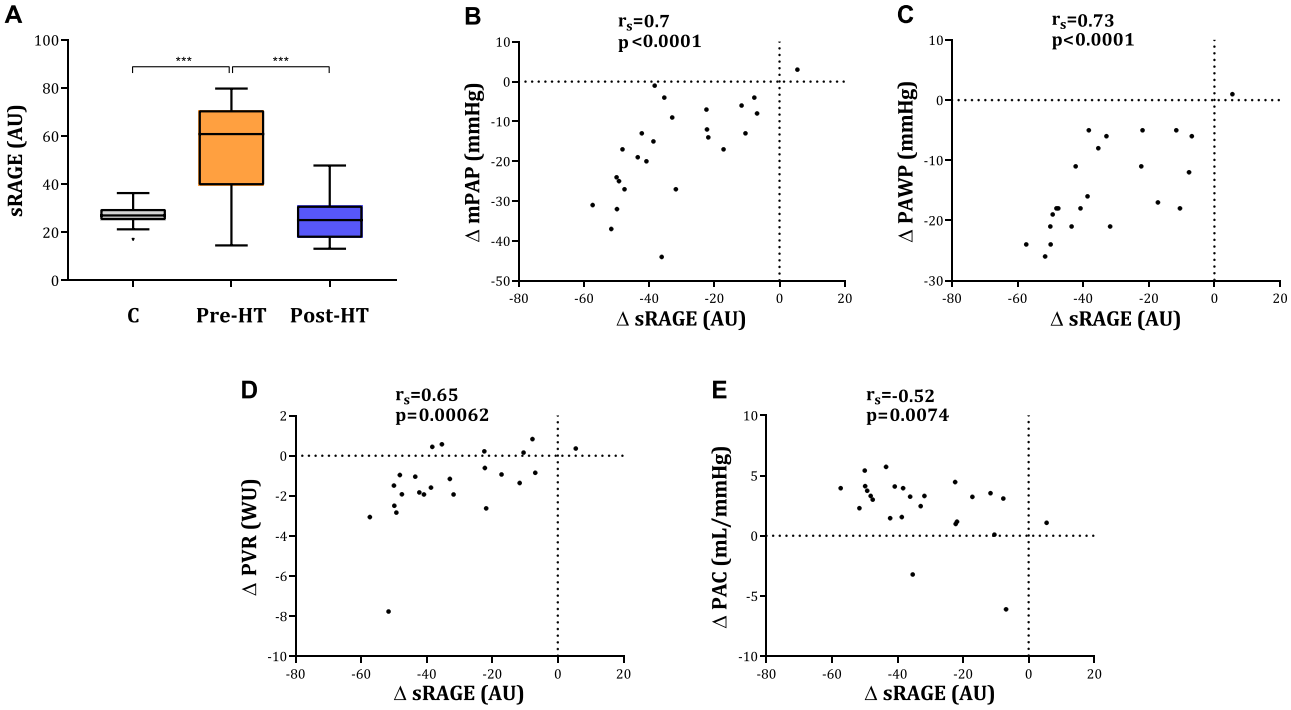
Plasma sRAGE and IGFBP7 were higher in end-stage HF patients prior to HT vs. controls and decreased after HT, matching controls' levels (*Figures 2A* and *3A*), meeting the inclusion criteria for correlations (*Figure 1A* and *1B* and *Table 3*) ($P < 0.009$; FDR < 0.01). Haemodynamic improvement after HT was described previously in detail and characterized by a decrease after HT in mPAP, PAWP, MRAP, and PVR, as well as an increase in PAC, CI, SVI, and LVSWI (*Table 2*),[13] ($P < 0.0003$; FDR < 0.01). The Δ (post-HT − pre-HT values) of sRAGE correlated with improvement in the highest number of haemodynamic parameters including ΔmPAP ($r_s = 0.7$; $P < 0.0001$), ΔPAWP ($r_s = 0.73$; $P < 0.0001$), ΔPVR ($r_s = 0.65$; $P = 0.00062$), and ΔPAC ($r_s = -0.52$; $P = 0.0074$) (FDR < 0.1) (*Figure 2B–2E*). IGFBP7 displayed the highest correlation coefficients for ΔMRAP ($r_s = 0.71$; $P = 0.00011$) and ΔNT-proBNP ($r_s = 0.71$; $P < 0.0001$) (FDR < 0.1) among all qualified metabolic plasma proteins (*Figure 3B* and *3C*).

Next, to address the potential role of post-operative medications including the immunosuppressive agents on protein levels, correlations between pre-operative levels of sRAGE, IGFBP7, and haemodynamics were made. Pre-HT plasma sRAGE correlated with mPAP ($r_s = 0.69$; $P = 0.00014$), PAWP ($r_s = 0.62$; $P = 0.0012$), PVR ($r_s = 0.61$; $P = 0.0017$), and PAC ($r_s = -0.65$; $P = 0.00045$). Pre-HT plasma IGFBP7 correlated with MRAP ($r_s = 0.75$; $P < 0.0001$) and NT-proBNP ($r_s = 0.70$; $P < 0.0001$).

Plasma sRAGE, IGFBP7, and haemodynamics in the diabetic population

Descriptive statistics on patients exhibiting diabetes ($n = 3$ pre-HT and $n = 9$ post-HT) displayed a tendency towards higher plasma sRAGE levels both before HT and after HT, whereas IGFBP7 levels were higher in diabetic patients before HT, and no difference was observed after HT. In pre-HT and post-HT, diabetic patients displayed lower LVSWI and higher levels of mPAP, CI, SVI, and NT-proBNP compared with non-diabetic patients (Supporting Information, *Table S1* and *Table 1*). The low number of patients in the diabetic subgroups did not allow for statistical testing.

Figure 2 (A) Plasma levels of soluble receptor for advanced glycation end products (sRAGE) in controls, pre-heart transplantation (HT), and post-HT. (B–E) Correlations between changes in plasma sRAGE and changes in haemodynamics. In (A), statistical significance was $P < 0.008$; false discovery rate <0.01. ***$P < 0.0001$. Statistical significance for correlations of change was considered $P < 0.021$; false discovery rate <0.1. mPAP, mean pulmonary arterial pressure; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; WU, Wood units.



Other metabolic plasma proteins

In addition to sRAGE and IGFBP7, six metabolic plasma proteins (FABP4, IGF1R, IGFBP2, LOX-1, pappalysin-1, and PON3) met both inclusion criteria for correlations (*Table 3*). All but LOX-1 correlated with changes in at least one haemodynamic parameter and/or ΔNT-proBNP (*Table 4*) ($P < 0.021$; FDR < 0.1). The metabolic plasma proteins that failed to meet either of the criteria were disqualified for correlation analysis (*Figure 1* and *Table 3*).

Discussion

Heart failure, characterized by impaired haemodynamics, associated with deranged metabolic milieu, and aggravated by

Figure 3 (A) Plasma levels of insulin-like growth factor-binding protein 7 (IGFBP7) in controls, pre-heart transplantation (HT), and post-HT. (B) Correlations between changes in plasma IGFBP7 and changes in mean right atrial pressure (MRAP), as well as (C) N-terminal pro-brain natriuretic peptide (NT-proBNP). In (A), statistical significance was $P < 0.008$; false discovery rate <0.01. ***$P < 0.0001$. Statistical significance for correlations of change was considered $P < 0.021$; false discovery rate <0.1.

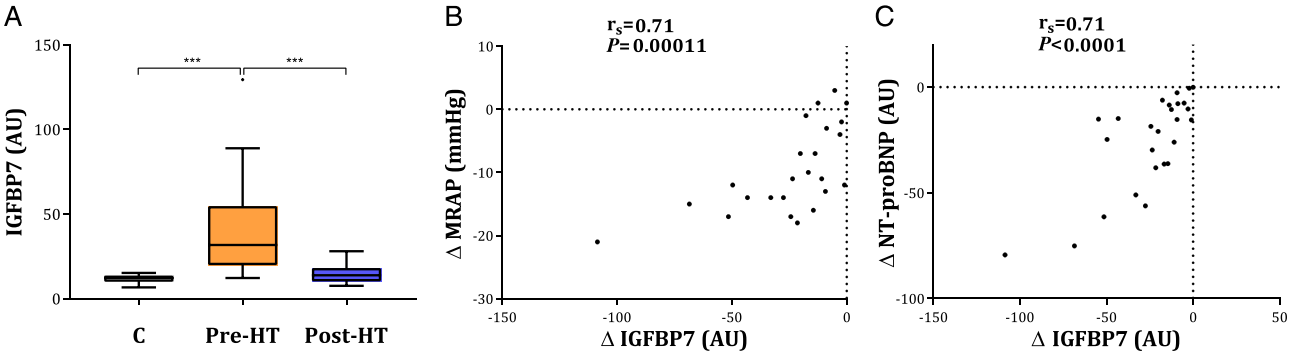


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Table 3 Characteristics of metabolic plasma proteins and NT-proBNP in study population

Plasma protein (AU)	Controls (n = 20)	Pre-HT (n = 26)	Post-HT (n = 26)	Δ (post-HT − pre-HT)	*P* values		
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Pre-HT vs. Post-HT	C vs. Pre-HT	C vs. Post-HT
DECR-1	95 (40–200)	38 (29–69)	37 (23–81)	−2.2 (−27 to 45)	0.90	0.010	0.0095
FABP2	252 (149–359)	375 (238–586)	565 (390–776)	211 (−46 to 400)	0.0051	0.021	<0.0001*
FABP4	9.8 (7.9–20)	42 (29–85)	28 (21–41)	−15 (−48 to 1.9)	0.00028*	<0.0001*	<0.0001*
FGF-21	34 (12–66)	230 (133–425)	130 (68–437)	−95 (−218 to 258)	0.41	<0.0001*	<0.0001*
FR-gamma	79 (73–89)[a]	100 (81–2972)[a]	83 (71–2331)	−9.4 (−22 to 5.4)[a]	0.16	0.043	0.64
IGF1R	8.6 (7.9–9.8)[a]	14 (11–18)[a]	11 (8.9–13)	−3.7 (−5.7 to 2.4)[a]	<0.0001*	<0.0001*	0.024
IGFBP1	8.2 (4.2–24)	41 (20–68)	27 (14–44)	−13 (−43 to 2.3)	0.016	0.00018*	0.0027*
IGFBP2	131 (92–184)	250 (161–394)	217 (110–323)	−44 (−160 to 0.15)	0.0047*	<0.0001*	0.025
IGFBP7	12 (10–14)	32 (20–55)	14 (10–18)	−17 (−36 to 9.1)	<0.0001*	<0.0001*	0.124
LDL-R	12 (8.1–15)	9.8 (6.5–14)	14 (11–17)	3.5 (−0.39 to 5.8)	0.0047*	0.23	0.36
LEPTIN	31 (15–84)	69 (22–109)	63 (35–113)	7.6 (−8.9 to 24)	0.17	0.37	0.10
LOX-1	63 (52–80)	117 (89–141)	68 (52–88)	−54 (−84 to 16)	<0.0001*	<0.0001*	0.55
LPL	987 (925–1143)	926 (844–1278)	1155 (909–1436)	121 (15–319)	0.0079*	0.48	0.14
Pappalysin-1	9.3 (7.8–11)	15 (11–20)	12 (11–15)	−2.6 (−8.3 to 0.57)	0.0022*	<0.0001*	0.00024*
PCSK9	3.1 (2.9–3.7)	3.6 (3.1–4.1)	3.9 (3.5–4.4)	0.43 (0.0091–0.86)	0.0022*	0.027	<0.0001*
PON3	57 (43–104)	26 (19–33)	38 (31–50)	14 (3.2–20)	<0.0001*	<0.0001*	0.0037*
RARRES2	2164 (1901–2241)	2492 (2215–2795)	2374 (1962–2611)	−115 (−487 to 114)	0.056	0.0062*	0.21
Resistin	53 (43–66)	61 (51–89)	72 (54–98)	1.3 (−11 to 19)	0.71	0.042	0.010
SERPIN-A12	6.9 (3.7–12)	18 (11–36)	20 (10–37)	0.83 (−8.5 to 9.8)	0.76	0.00024*	0.0017*
sRAGE	27 (25–30)	61 (40–71)	25 (18–31)	−36 (−48 to 16)	<0.0001*	<0.0001*	0.14
TR-1	15 (13–17)	20 (15–38)	22 (13–42)	2.6 (−9.2 to 15)	0.55	0.0050*	0.012
NT-proBNP (AU)	1.1 (1.1–1.2)	24 (11–40)	2 (1.4–5.8)	−17 (−37 to 8.4)	<0.0001*	<0.0001*	<0.0001*

DECR-1, 2,4-dienoyl-CoA reductase; FABP2, fatty acid binding protein 2; FABP4, fatty acid binding protein 4; FGF-21, fibroblast growth factor-binding protein 21; FR-gamma, folate receptor gamma; HT, heart transplantation; IGF1R, insulin-like growth factor 1 receptor, IGFBP1, insulin-like growth factor-binding protein 1; IGFBP2, insulin-like growth factor-binding protein 2; IGFBP7, insulin-like growth factor-binding protein 7; IQR, inter-quartile range; LDL-R, low-density lipoprotein receptor; LOX-1, oxidized low-density lipoprotein receptor 1; LPL, lipoprotein lipase; NT-proBNP, N-terminal pro-brain natriuretic peptide; PCSK9, proprotein convertase subtilisin/kexin type 9; PON3, serum paraoxonase 3; RARRES2, retinoic acid receptor responder protein 2; sRAGE, soluble receptor for advanced glycation end products; TR-1, transferrin receptor 1.

[a]Indicates $n - 1$.

*Indicates statistically significant values ($P < 0.008$; false discovery rate <0.01).

Table 4 Correlations of changes (Δ) between metabolic plasma proteins and changes in haemodynamics as well as NT-proBNP

	Pulmonary parameters						Cardiac parameters											
	mPAP (mmHg)		PVR (WU)		PAC (mL/mmHg)		MRAP (mmHg)		NT-proBNP (AU)		PAWP (mmHg)		CI (L/min/m^2)		SVI (mL/beat/m^2)		LVSWI (mmHg × mL/m^2)	
Variable (Δ)	*n*	r_s (*P* value)	*n*	r_s (*P* value)	*n*	r_s (*P* value)	*n*	r_s (*P* value)	*n*	r_s (*P* value)	*n*	r_s (*P* value)	*n*	r_s (*P* value)	*n*	r_s (*P* value)	*n*	r_s (*P* value)
FABP4 (AU)	25	0.26 (0.21)	24	0.3 (0.16)	25	0.34 (0.092)	24	0.58 (0.0031)*	26	0.66 (0.00024)*	24	0.13 (0.55)	25	−0.027 (0.9)	25	−0.16 (0.46)	24	−0.27 (0.2)
IGF1R (AU)	24	0.16 (0.47)	23	0.14 (0.52)	24	0.27 (0.21)	23	0.49 (0.018)*	25	0.58 (0.0023)*	23	0.057 (0.8)	24	−0.098 (0.65)	24	−0.077 (0.72)	23	−0.04 (0.86)
IGFBP2 (AU)	25	0.16 (0.44)	24	−0.05 (0.82)	25	−0.019 (0.93)	24	0.47 (0.02)*	26	0.43 (0.027)	24	0.34 (0.11)	25	0.087 (0.68)	25	0.00077 (0.99)	24	−0.26 (0.23)
IGFBP7 (AU)	25	0.041 (0.85)	24	0.14 (0.52)	25	0.27 (0.19)	24	0.71 (0.00011)*	26	0.71 (<0.0001)*	24	0.12 (0.57)	25	−0.19 (0.37)	25	−0.15 (0.47)	24	−0.33 (0.12)
LOX-1 (AU)	25	0.33 (0.11)	24	0.24 (0.27)	25	−0.24 (0.25)	24	0.22 (0.29)	26	0.24 (0.24)	24	0.4 (0.051)	25	0.015 (0.95)	25	−0.081 (0.7)	24	−0.2 (0.35)
Pappalysin-1 (AU)	25	0.47 (0.018)*	24	0.4 (0.053)	25	0.032 (0.88)	24	0.37 (0.075)	26	0.35 (0.084)	24	0.35 (0.09)	25	−0.092 (0.66)	25	−0.16 (0.46)	24	−0.19 (0.36)
PON3 (AU)	25	−0.11 (0.61)	24	−0.053 (0.81)	25	−0.045 (0.83)	24	−0.53 (0.0072)*	26	−0.56 (0.0028)*	24	−0.031 (0.89)	25	0.29 (0.16)	25	0.41 (0.043)	24	0.3 (0.16)
sRAGE (AU)	25	0.7 (<0.0001)*	24	0.65 (0.00062)*	25	−0.52 (0.0074)*	24	0.18 (0.4)	26	0.3 (0.14)	24	0.73 (<0.0001)*	25	−0.078 (0.71)	25	−0.16 (0.46)	24	−0.22 (0.31)

r_s indicates Spearman's rank correlation coefficient. Other abbreviations as in *Table 2* and *3*.
*Indicates statistically significant correlations ($P < 0.021$; false discovery rate <0.1).

PH, may be resolved by HT.[2,15,19] Accordingly, the present study identified metabolic plasma proteins related to haemodynamic improvement in severe HF patients following HT. We specifically found that sRAGE and IGFBP7 correlated with most improved haemodynamic parameters and expressed the strongest correlations. Therefore, sRAGE and IGFBP7 may collectively be future novel biomarkers in HF and related passive pulmonary congestion in addition to their potential pathophysiological importance, which may be a means to guide future therapies.

RAGE is a multiligand receptor belonging to the immunoglobulin superfamily for cell surface molecules, expressed by a variety of cell types including cardiomyocytes, smooth muscle cells, and endothelial cells.[20,21] It interacts with advanced glycation end products (AGEs), formed by a non-enzymatic reaction between proteins and carbohydrate residues, as well as other ligands.[22,23] RAGE can be expressed as a soluble form, soluble RAGE or sRAGE, acting as a decoy receptor reflecting the extent of RAGE activation.[24] AGE-mediated activation of RAGE may contribute to the development and progression of HF as well as vascular dysfunction.[23] Key factors contributing to increased expression of AGEs include the degree of hyperglycaemia and oxidative stress,[25] both of which have been shown to be associated with HF.[10,26] In a mouse model, overexpression of RAGE reduced intracellular calcium concentration in cardiomyocytes, and RAGE activation delayed calcium reuptake, potentially reducing myocardial contractility.[27] In addition, AGE–RAGE interaction induced fibrosis via up-regulation of tumour growth factor-β[28,29] and connective tissue growth factor.[30] Correspondingly, inhibition of RAGE in pressure-overloaded mice attenuated cardiac remodelling including fibrosis and hypertrophy.[31]

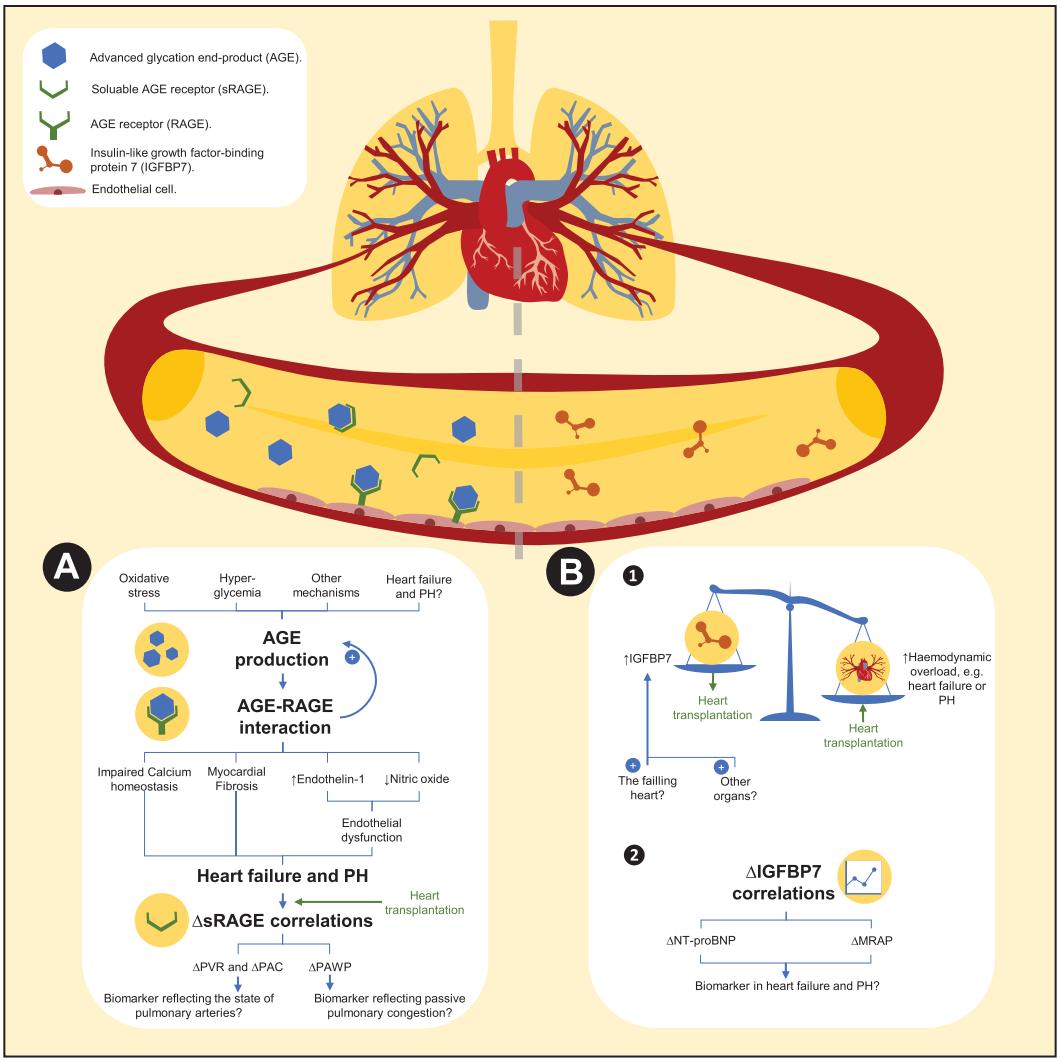
Chronic HF patients' functional capacity is closely associated to vascular compliance and endothelial dysfunction.[23,32] Endothelial dysfunction is an important predictor of major clinical events in HF including mortality,[33,34] and preservation of endothelial functionality is associated with improved LV function[35] and outcome.[33] Intriguingly, endothelial dysfunction is a main hallmark defined in the pathophysiology of HF and PH. Depending on the diagnostic method used, PH is present in up to 75% of patients with HFrEF and 83% of HFpEF.[7] Endothelial dysfunction is characterized by up-regulated endothelin-1 expression and reduced nitric oxide availability, two principal mechanisms in PH[8,15] that are implicated in the AGE–RAGE axis. For example, RAGE activation reduces the nitric oxide availability in human coronary artery endothelial cells,[36] and up-regulates endothelin-1 expression in human endothelial cells via nuclear factor-κB.[37]

Sodium–glucose cotransporter 2 inhibitors (SGLT2i) have been proposed to influence myocardial metabolism, fibrosis, and vascular function. A recent randomized placebo-controlled trial on patients with HFrEF using SGLT2i showed a decrease in the risk of HF aggravation and death from cardiovascular events, irrespective of diabetes.[38] The fact that HF patients without diabetes mellitus improved with SGLT2i treatment underlines the central role of disturbed energetics in HF.[1] Another study found elevated levels of sRAGE in diabetic rats following treatment with SGLT2i compared with untreated ditto.[39] In the present study, sRAGE levels were greatly elevated in HF, irrespective of diabetes (*Table 3* and Supporting Information, *Table S1*). However, we found lower levels of sRAGE 1 year after HT in comparison with pre-HT levels, a pattern that followed the controls' levels. Theoretically, the inconsistency of sRAGE levels after SGTL2i treatment and HT may be ascribed to potential effects of SGLT2i, including up-regulation of sRAGE levels, down-regulation of AGE levels, or both. Nevertheless, the common pathways, affected by SGTL2i treatment and involved in the AGE–RAGE interaction, warrant for further investigation as interfering with the AGE–RAGE axis may be a future alternative to explore in HF and PH studies.

We furthermore found higher levels of sRAGE in severe HF patients before HT, which may indicate increased activation by RAGE ligands, particularly by AGEs, as AGEs up-regulate the expression of RAGE.[40] Furthermore, the sRAGE levels decreased 1 year after HT, matching controls' levels. This decrease in sRAGE correlated with improvement in haemodynamic parameters reflecting passive pulmonary congestion and PH, namely, PAWP and mPAP, but correlated also with PVR and PAC. Changes in PAC and PVR, representing the mechanical components of the vasculature, are a consequence of the structural changes induced by PH and HF. Decreased compliance (i.e. stiffening) and increased resistance (i.e. narrowing) of the vasculature are characteristics of vascular remodelling.[41] Accordingly, it was recently shown that RAGE activation is critical in vascular remodelling induced by extracellular matrix accumulation in pulmonary arterial hypertension.[22] Interestingly, vascular remodelling also emerges in PH due to left-sided HF irrespective of ejection fraction.[42] Thus, plasma sRAGE may be a potential biomarker for passive pulmonary congestion in HF, as well as reflecting the mechanical state of the pulmonary arteries (i.e. PAC and PVR). Furthermore, we hypothesize that RAGE may have a role in endothelial dysfunction and vascular remodelling in PH due to left heart disease (*Figure 4A*).

IGFBP7, angiomodulin, or prostacyclin-stimulating factor is a secreted glycoprotein of the IGFBP superfamily, which binds and regulates the activity of insulin, insulin-like growth factor (IGF)-1 and IGF-2.[43–46] Additionally, IGFBP7 can bind IGF1R, inhibiting further downstream activation by IGF-1 and IGF-2.[47] IGFBP7 is associated with insulin resistance, cardiac hypertrophy,[48] vascular homeostasis,[49] and possibly fibrosis though interaction with tumour growth factor-β.[50] IGFBP7 has been found to be elevated patients with HFpEF and HFrEF,[9] congruent with our results. We, however, provide additional evidence on the dynamics of IGFBP7 in relation to HT, as the elevated levels of plasma IGFBP7 in severe HF decreased to match healthy controls' levels after HT.

Figure 4 (A) Different mechanisms may stimulate the production of AGEs. AGE binds to its receptor RAGE, whereof diverse pathways involved in heart failure and pulmonary hypertension (PH) pathophysiology and progression are activated. Heart failure patients with or without PH were heart transplanted, and correlations of the improved haemodynamic profile along with the change (Δ) in sRAGE were present. sRAGE may be a biomarker reflecting the mechanical state of pulmonary vasculature as well as pulmonary passive congestion, typically found in PH secondary to heart failure. (B1) Elevated IGFBP7 was present during haemodynamic overload. This imbalance was restored by heart transplantation (green). The source of plasma IGFBP7 may be the failing heart or other organs. (B2) ΔIGFBP7 correlated with improved haemodynamics, suggesting it to be a biomarker in heart failure and PH. MRAP, mean right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance.



Furthermore, we show that this decrease in plasma IGFBP7 after resolving of HF and PH correlated with improved MRAP and decreased NT-proBNP. Our results demonstrate a link between dynamics in IGFBP7, MRAP, and NT-proBNP, where the latter is prognostic of short-term and long-term outcome in HF patients.[9] Interestingly, IGFBP7 levels appeared to be independently predictive of mortality in HF patients,[50,51] indicating that this potential biomarker may provide additional insight into new pathophysiological mechanisms, possibly related to aberrant energetics, in HF and potentially secondary PH. Although urinary IGFBP7 has been associated with acute kidney injury, the cellular source of this glycoprotein remains unknown.[52] Given that we found no difference in the patients' eGFR before vs. after HT (P = 0.15) and that high IGFBP7 levels decreased following HT, the failing heart may provide clues to its expression. However, whether the failing heart is the main source of IGFBP7 or if IGFBP7 expression is ascribed to different tissues depending on the underlying pathophysiological condition need further investigation (*Figure 4B*).

In diabetic patients, a previous study found higher levels of sRAGE,[53] congruent with our findings (Supporting Information, *Table S1*). As for IGFBP7, higher serum levels in diabetic patients are associated with insulin resistance.[54] Another study found no difference in serum levels of IGFBP7 between type 2 diabetic and non-diabetic patients.[55] Accordingly, although we found a tendency of higher plasma IGFBP7 in the diabetic patients before HT, no difference was observed after HT between diabetic and non-diabetic patients. Descriptive statistics revealed that diabetic patients tended to have higher CI, SVI, and NT-proBNP but lower LVSWI before HT and after HT (Supporting Information, *Table S1* and *Table 1*). Notably, statistical testing was not performed in the diabetic subpopulation due to the low number of patients. Therefore, these findings remain inconclusive.

The other metabolic plasma proteins that did not display strong correlations with improved haemodynamics and/or NT-proBNP, including FABP4, IGF1R, IGFBP2, LOX-1, pappalysin-1, and PON3, may nonetheless be linked to the severity, progression, or distinct pathophysiological processes in HF and/or PH, as their levels developed towards the levels of healthy controls in response to HT.

Although we consider the invasive assessment of haemodynamics and the use of PEA as major strengths in our study, our work has limitations. The present study was small and single centred, and the controls were somewhat younger than the patients. However, the primary aim of the control group was to assess the general normal range of the proteins, and their data served to refine the study set-up by excluding proteins whose development was not associated with improved haemodynamics. In addition, tests for correlation of sRAGE and IGFBP7 levels were performed in relation to age in each group (controls, pre-HT, and post-HT), and the pattern of the correlations was compared between the groups for consistency. No consistent pattern due to age was found. Moreover, even though we found no difference in patients' eGFR and plasma creatinine in response to HT, it is, however, possible that medical therapies and patients' characteristics may influence our findings. Despite pre-operative correlations between sRAGE, IGFBP7, and haemodynamics, the precise effect of medications including immunosuppressive agents has not been investigated. Although a previous study found no difference in sRAGE levels among patients with or without prednisolone treatment,[56] it is established that prednisolone may induce diabetes mellitus, which in turn is associated with higher sRAGE levels.[53,57] There is a tendency towards lower levels of plasma sRAGE and treatment with mycophenolate mofetil, whereas treatment with azathioprine is associated with higher sRAGE levels.[58] There is a paucity of studies investigating the role of immunosuppressive treatment on the plasma levels of IGFBP7. Another limitiation is that the present study included patients with or without PH in a single cohort. However, most of the patients without PH either had a PAWP $>$ 15 mmHg or were in the borderline zone with PAWP values between 12 and 15 mmHg.

Notably, the present study does not address causality and is hypothesis generating. Hence, future studies are needed to provide evidence into the proteins' origin of expression as well as their potential pathophysiological mechanisms in HF and PH. Despite these limitations, we provide novel insights into the dynamics of metabolic plasma proteins including sRAGE and IGFBP7 in relation to HT and associated haemodynamics.

Conclusion

In conclusion, the present study provides evidence on the dynamics of metabolic plasma proteins in relation to HT and associated haemodynamics. In specific, plasma sRAGE and IGFBP7 were elevated in severe HF patients and decreased to match controls' levels after HT. The decrease in sRAGE correlated with mPAP, PAWP, PAC, and PVR, indicating that sRAGE may be a potential biomarker reflecting passive pulmonary congestion and the mechanical state of the pulmonary vasculature in HF patients. Although more future studies are needed to draw final conclusions, we suggest that sRAGE may be involved in endothelial dysfunction and vascular remodelling in HF and associated PH. Furthermore, the dynamics in IGFBP7 correlated with improved MRAP and NT-proBNP. Thus, IGFBP7 may provide further information on HF as this protein may provide additional insight into new pathophysiological mechanisms in HF and potentially associated PH, which may guide future therapies.

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Conflict of interest

A.A. and S.A. report no conflicts of interest. M.A. reports an unrestricted research grant from the Swedish Society of Pulmonary Hypertension. H.B. reports an unrestricted research

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Characteristics of patients with or without diabetes before and 1-year after heart transplantation.

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