Plasma myo-inositol elevation in heart failure: clinical implications and prognostic significance. Results from the BElgian and CAnadian MEtabolomics in HFpEF (BECAME-HF) research project

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Summary

Background The metabolic environment plays a crucial role in the development of heart failure (HF). Our prior research demonstrated that *myo*-inositol, a metabolite transported by the sodium-*myo*-inositol co-transporter 1 (SMIT-1), can induce oxidative stress and may be detrimental to heart function. However, plasmatic *myo*-inositol concentration has not been comprehensively assessed in large cohorts of patients with heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF).

Methods Plasmatic *myo*-inositol levels were measured using mass spectrometry and correlated with clinical characteristics in no HF subjects and patients with HFrEF and HFpEF from Belgian (male, no HF, 53%; HFrEF, 84% and HFpEF, 40%) and Canadian cohorts (male, no HF, 51%; HFrEF, 92% and HFpEF, 62%).

Findings *Myo*-inositol levels were significantly elevated in patients with HF, with a more pronounced increase observed in the HFpEF population of both cohorts. After adjusting for age, sex, body mass index, hypertension, diabetes, and atrial fibrillation, we observed that both HFpEF status and impaired kidney function were associated with elevated plasma *myo*-inositol. Unlike HFrEF, abnormally high *myo*-inositol (≥69.8 μM) was linked to unfavourable clinical outcomes (hazard ratio, 1.62; 95% confidence interval, [1.05–2.5]) in patients with HFpEF. These elevated levels were correlated with NTproBNP, troponin, and cardiac fibrosis in this subset of patients.

Abbreviations: AUC, areas under the curve; CI, confidence interval; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; ePASP, estimated pulmonary artery systolic pressure; FGF-23, fibroblast growth factor-23; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; hsTnT, high sensitivity troponin T; LA, left atrial; LV, left ventricular; LVEF, left ventricular ejection fraction; LC-MS/MS, liquid chromatography–mass spectrometry; MHI, Montreal Heart Institute; MIOX, *myo*-inositol oxygenase; NYHA, New York Heart Association; NOX2, NADPH oxidase 2; NTproBNP, N-terminal pro-B type natriuretic peptide; OR, Odds ratio; ROC, Receiver operating characteristic; ROS, reactive oxygen species; SD, standard deviation; SGLT2i, sodium-glucose co-transporter-2 inhibitor; SMIT1, sodium-myo-inositol co-transporter 1; SMIT2, sodium-myo-inositol co-transporter 2; SNP, single nucleotide polymorphism

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Interpretation *Myo*-inositol is a metabolite elevated in patients with HF and strongly correlated to kidney failure. In patients with HFpEF, high *myo*-inositol levels predict poor clinical outcomes and are linked to markers of cardiac adverse remodelling. This suggests that *myo*-inositol and its transporter SMIT1 may have a role in the pathophysiology of HFpEF.

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Keywords: Heart failure; HFpEF; *Myo*-inositol; Prognosis; Metabolites; Kidney

Research in context

Evidence before this study

The availability of metabolites significantly impacts the pathophysiology of heart failure (HF). Despite its previous association with severe HF in a limited patient cohort and with cardiomyocyte death in isolated cells, *myo*-inositol is an infrequently detected plasma metabolite. To gain a comprehensive understanding of *myo*-inositol's role in HF, it was imperative to conduct large cohort studies involving patients with both HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF).

Added value of this study

This study presents compelling evidence that plasmatic *myo*-inositol is consistently elevated in all patients with HF, with even higher levels in HFpEF individuals. Notably, *myo*-inositol levels are closely linked to impaired renal function, which is the primary route for *myo*-inositol clearance, as well as the presence of HFpEF. Moreover, we demonstrate that *myo*-inositol can effectively predict adverse clinical outcomes in patients with HFpEF and is associated with adverse cardiac remodelling.

Implications of all the available evidence

Myo-inositol emerges as a diagnostic and prognostic tool for HFpEF. Its exclusive transport by SMIT1 within the heart renders the *myo*-inositol/SMIT1 axis an intriguing subject for future research and a potential therapeutic target in the treatment of HF.

Introduction

Heart failure (HF) is a prevalent and complex clinical syndrome characterised by significant morbidity and mortality. Patients with HF are classified into two major groups based on their left ventricular ejection fraction (LVEF): heart failure with reduced ejection fraction (HFrEF) (LVEF < 40%) and heart failure with preserved ejection fraction (HFpEF) (LVEF ≥ 50%). These groups exhibit distinct clinical and biological characteristics, and their underlying pathophysiology have been thoroughly investigated. However, HFpEF, which represents more than 50% of HF cases, remains a poorly understood disease with limited therapeutic options, except for sodium-glucose co-transporter-2 inhibitors (SGLT2i), being the only drugs with a proven clinical benefit.[1,2] Several proposed mechanisms contribute to the development of HFpEF, including systemic inflammation, microvascular dysfunction, cardiometabolic abnormalities, and interstitial fibrosis.[3] The strongest supportive evidence accumulated to date indicates that co-morbidities are key elements of HFpEF pathophysiology, favouring systemic inflammation and coronary microvascular endothelial dysfunction, driving to LV remodelling, interstitial fibrosis, and diastolic dysfunction.[4] The "extramyocardial" origin of HFpEF differs from the "intramyocardial" origin of HFrEF, which mainly results from cardiomyocyte damages because of ischaemia-reperfusion injury, infection, or toxicity. One could hypothesise that circulating metabolic factors, influenced by co-morbidities, may promote HFpEF by acting on various cardiac cell types, such as cardiomyocytes, endothelial cells, and fibroblasts. The identification of plasma metabolites associated with HFpEF could improve the understanding of its pathophysiology, provide a better definition of clinical phenotypes and their prognosis, and possibly help in designing targeted therapies for patient subgroups. Several studies have been conducted to compare HFrEF and HFpEF using plasmas metabolomics predominantly using a targeted approach focusing on organic and amino acids and acylcarnitines, in search of such biomarker. Although they revealed noteworthy differences between these two conditions, such in levels of long-chain acylcarnitines being higher in HFrEF *vs.* HFpEF, other metabolites potentially relevant to HF do not emerge prominently in the few untargeted studies conducted to date.[5–8]

Among them, *myo*-inositol is a metabolite that needs specifically designed mass spectrometry analysis to be detected and quantified in plasma or urine, as it is often contaminated by co-elution of numerous isomers as well as abundant glucose causing ion suppression. It is

the most abundant stereoisomer of the cyclic polyols and is the precursor of phosphoinositides, which regulates various cellular functions such as cell growth and proliferation.[9] *Myo*-inositol is also controlling intracellular osmolarity, mainly in the proximal tubule of kidneys, where it is catabolized into D-glucuronic acid by the *myo*-inositol oxygenase (MIOX).[10] Its membrane transport is regulated by a subgroup of the SGLT's family, including sodium-*myo*-inositol co-transporter 1, and 2 (SMIT1, and SMIT2), which are both characterised by a low affinity for glucose (>50 and 30 mM respectively) and a high affinity for *myo*-inositol (17 and 120 μM).[11–13] We recently demonstrated that SMIT1 was the only functional SGLT isoform fully expressed in human, murine and rodent hearts.[14,15] Interestingly, SMIT1 confers cardiomyocytes the ability to detect hyperglycaemia or an increase in *myo*-inositol levels, leading to NADPH oxidase 2 (NOX2) activation, reactive oxygen species (ROS) production and eventually cell death.[14] Hence, elevated plasma *myo*-inositol levels may be harmful for the heart. Although previous studies have associated plasma *myo*-inositol levels with HF severity in small patient cohorts,[16] comprehensive investigations in large populations of patients with HF are lacking. The role of *myo*-inositol and its transporter, SMIT1, in cardiovascular disease remains largely unexplored.

Here, we quantified plasma levels of *myo*-inositol in patients with HFpEF and HFrEF, as well as in individuals without HF, across a discovery and confirmation cohort. We investigated its association with comorbidities and clinical prognosis in patients with HF, expecting *myo*-inositol to be elevated in patients with HF, and possibly harmful in its pathophysiology.

Methods

This investigation was conducted in agreement with the principles outlined in the Declaration of Helsinki. Local ethics committee approved the study (Comité d'éthique Hospitalo-Facultaire, Cliniques universitaires Saint Luc and UCLouvain, Brussels, 2012/23AVR/199 and 2018/19MAR/118), and all patients provided written informed consent before study enrolment (Clinical trial NCT03197350, MHI protocol #2017-2316).

Belgian discovery cohort

Between December 2015 and June 2017, consecutive patients from the Cliniques universitaires Saint Luc (Brussels, Belgium) with HFpEF and HFrEF were prospectively considered for inclusion into the study. The inclusion criteria were typical symptoms and signs of HF, New York Heart Association (NYHA) functional class II or higher, N-terminal pro-B type natriuretic peptide (NT-proBNP) >350 pg/mL, or hospitalization for HF within the previous 12 months. Left ventricular ejection fraction (LVEF) was required to be lower than 40% in patients with HFrEF and 50% or higher in HFpEF. For patients with HFpEF, at least 2 of the following echocardiographic parameters of diastolic dysfunction were required prior to inclusion: LA >34 mL/m^2; E/e' ratio >14; triscupid regurgitation >2.8 ms, septal e' velocity <7 cm/s or lateral e' velocity <10 cm/s.[17] The exclusion criteria for all patients with HF were severe valvular disease, infiltrative or hypertrophic cardiomyopathy, acute coronary syndrome in the previous 30 days, chronic obstructive pulmonary disease GOLD 3 or 4, congenital heart disease, pericardial disease, terminal renal failure (eGFR < 15 mL/min/1.73 m^2) or subjects requiring dialysis, atrial fibrillation with a ventricular response >140 bpm, severe anaemia (haemoglobin <8 g/dL), cirrhosis, and active cancer. A total of 221 patients with HFrEF and 136 with HFpEF satisfied the inclusion criteria.

Patients were compared with 94 individuals without HF (no HF group), prospectively recruited. These subjects had no history of cardiovascular events, including HF, and underwent a full clinical exam, electrocardiogram, echocardiography, and exercise stress test, which all had to be normal prior to inclusion. Nevertheless, these no HF subjects may exhibit a range of cardiovascular risk factors frequently encountered in an elderly population, such as hypertension, diabetes, and hypercholesterolemia.

Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) score was calculated for each patient with HFpEF and HFrEF to assess interaction between comorbidities, HF status and *myo*-inositol. MAGGIC score is a predictive risk validation tool for mortality in HF that integrates multiple demographic, clinical and laboratory variables (age, sex, diabetes, chronic obstructive pulmonary disease (COPD), HF diagnostic within the last 18 months, current smoker, NYHA class, the use of beta blocker, angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, body mass index, systolic blood pressure, creatinine, and LVEF).[18,19]

Echocardiography

All 451 participants of the Belgian cohort underwent transthoracic echocardiography (iE33 system Philips Healthcare, Best, The Netherlands) to assess ventricle structure, systolic and diastolic function, and measurements of left and right atrial volumes, in addition to a valvular evaluation. Pulmonary pressures were estimated using tricuspid regurgitation velocity. All echocardiographic measurements were averaged over three beats in the presence of atrial fibrillation.

Follow-up

All Belgian patients were prospectively followed-up during ambulatory visits and/or phone calls at 6-month intervals for at least 5 years. Clinical and vital status was obtained with the patients, their relatives, or treating physician. The primary endpoint was a

composite of death from any cause or hospitalization for HF, whichever occurred first. Hospitalization for HF was defined as treatment in the emergency room or admission to a hospital, with a diagnosis of decompensated HF requiring an intravenous diuretic.

Biomarker analysis

NTproBNP, high-sensitive troponin T (hsTnT), and fibroblast growth factor-23 (FGF-23) levels were measured in blood samples from Belgian patients. Briefly, after centrifugation at 3500 rpm for 10 min, serum aliquots were stored at –80 °C until automated electrochemiluminescence immunoassay on the Cobas® 8000 platform (NTproBNP and hsTnT), or immunoassay (intact FGF-23).

Cardiac magnetic resonance

Cardiac magnetic resonance (cMR) was performed using a 3-T system (Ingenia, Philips Medical Systems, Best, The Netherlands) in all no HF and HFpEF individuals who agreed and had no contraindication, and/or contingent upon MRI accessibility (79 no HF and 112 patients with HFpEF from the Belgian cohort), as described previously.[20] Briefly, pre- and post-contrast modified look-locker inversion recovery images were processed using the open-source software MRmap v1.4 under Interactive Data Language software®. Pre- and post-myocardial T1 times were measured in the anterior, anterolateral, inferolateral, inferior, inferoseptal, and anteroseptal myocardial regions. We calculated the average T1 time of the different regions of interest. Extracellular volume (ECV), a parameter reflecting myocardial interstitial fibrosis, was then computed according to the formula[21]

Confirmation cohort from Canada

The confirmation cohort included participants from the Montreal Heart Institute (MHI) Biobank (Protocol #2017-2316). Among the 17,000 patients from the MHI biobank, 987 suffered from HF. Based on the inclusion and exclusion criteria from a separate study of biomarkers in HF (see Supplemental material and Supplementary Fig. S1), the study population consisted of 77 patients with HFpEF, 103 patients with HFrEF and 105 age- and sex-matched no HF participants (without history of HF). *Myo*-inositol measurements were not available in three patients with HFpEF, four patients with HFrEF and two no HF subjects.

Plasma *myo*-inositol measurements

Blood samples at inclusion were obtained using venepuncture. Aliquots of serum and plasma were stored at –80 °C. *Myo*-inositol extraction was performed using 50 µL of plasma, which was mixed with 500 µL of a solution containing 85% acetonitrile and the internal standard (*myo*-inositol d-6; CDN Isotopes, Pointe-Claire, Canada) at 15 µM, passed through a protein precipitation plate (Phenomenex). 1 µL aliquots of the flow through were injected into the liquid chromatography–mass spectrometry (LC-MS/MS) instrument (Agilent Technologies, Santa Clara, USA). Calibration standards were obtained by spiking *myo*-inositol in a commercial plasma, at concentrations ranging from 0 to 200 µM. Endogenous *myo*-inositol concentrations, calculated by standard additions, were used to generate actual standard concentrations (20.4–220.4 µM). Chromatographic separations were carried out on an Xbridge amide analytical column maintained at 40 °C. The eluents consisted of 0.1% ammonium hydroxide in water (eluent A) and 0.1% ammonium hydroxide in acetonitrile (eluent B). The following gradient elution was applied at a flow rate of 500 µL/min: 90% B from 0 to 0.5 min; 90 to 75% B from 0.5 to 7 min; 75 to 50% B from 7 to 7.5 min and held constant until 8.5 min. Eluent B was increased to 90% from 8.5 to 9 min, then kept constant until 16 min to allow for column equilibration. Data acquisition was performed by multiple reaction monitoring using the following transitions: 179 > 87 for quantitation, 179 > 161 for confirmation for *myo*-inositol, 185 > 89 as quantifier, and 185 > 167 as qualifier for *myo*-inositol d-6. LC-MS measurements of *myo*-inositol were highly reproducible, as demonstrated by repeated measurements at 3-year intervals in 2017 and 2020 (Supplementary Fig. S2).

Culture and experiments on primary human cardiac fibroblasts

Detailed protocols are available in the Supplementary methods.

Statistical analysis

Based on published data[16] and the comparison formula ($n_T > 2\frac{\sigma^2}{\delta_0^2}(1.96 + 0.84)^2$), a sample size of 65 subjects per group has been calculated. Because of the uncertainty about the signal (our study was exploratory), the sample size has been extended in all groups from both cohorts, considering availability of plasma samples.

Statistical analyses were performed using SPSS Version 26 (SPSS Corp., Somers, New York), R Version 4.1.2 software (http://www.r-project.org), and GraphPad Prism 9. Continuous variables were expressed as mean ± 1 standard deviation (SD) if normally distributed or as median (25th and 75th percentiles) if not normally distributed. Normality of a continuous distribution was assessed using histograms, QQ plots, as well as Skewness and Kurtosis tests. Categorical variables were expressed as counts and percentages. Biomarker levels were log-transformed (base 10) to establish normality. Comparison between groups was performed using independent sample t-test, one-way analysis of variance (ANOVA) with homogeneity of variance assessment (Levene's test) and post-hoc Bonferroni adjustment for quantitative variables, and chi-square

test, if the expected frequency count was greater than 5, or Fisher's exact test for categorial variables, as appropriate; $p < 0.05$ was considered statistically significant. If the homogeneity of variance was not respected, non-parametric analysis was conducted. The correlations between *myo*-inositol and biomarkers were assessed using Pearson's correlation coefficient.

The study population was divided into three equal groups according to *myo*-inositol level tertiles. Clinical, biological, and imaging parameters were compared among tertiles. Univariable and multivariable linear regression and logistic regressions were used to determine characteristics associated with high *myo*-inositol values. The assumptions for the linear (linearity independence of residuals, homoscedasticity, normality of residuals, and absence of collinearity among independent variables) and logistic regression (linearity assumption for quantitative predictors) were assessed. The model for linear and logistic regression analysis was constructed using a forward selection method based on the lowest AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) calculated for all significant clinical variables ($p < 0.10$). To assess the overall fit and robustness of the model, we evaluated the AIC and the BIC, along with the R-squared for the linear regression analysis, and the likelihood ratio (LR) for the logistic regression analysis. Receiver operating characteristic (ROC) curves were drawn to calculate the areas under the curve (AUC) and assess the diagnostic value of *myo*-inositol in patients with HF.

Since plasma *myo*-inositol has not yet been evaluated in a healthy elderly population in the literature, we established a concentration cut-off separating the normal from the abnormal *myo*-inositol level. Given the normal distribution of *myo*-inositol across the no HF population (Skewness = 1.4, Kurtosis = 2.3), concentration cut-off of 69.8 μM, for HFpEF, and 67.5 μM, for HFrEF, were defined as the mean of the no HF population matched for age +2 SD. Patients with HFpEF and HFrEF were stratified according to this cut-off and clinical, biological, and imaging parameters were compared. Kaplan–Meier curves and the log-rank test were employed to illustrate event-free survival among patients with HF stratified by *myo*-inositol levels. The study's time origin began at the participants' inclusion until the primary endpoint occurred over 5-year period. Patients with HFpEF were censored in the absence of events, whereas patients with HFrEF were censored for the absence of events, heart transplantation, or ventricular assist device therapy placement. The potential linear association of *myo*-inositol and hazard of primary endpoint in HFpEF and HFrEF was illustrated using separate splines curves with 3 degrees of freedom, corresponding to 10 knots evenly spaced in the range (min–max) of *myo*-inositol values, using the *pspline* function (*survival* R package). To determine independent predictors of the primary endpoint, uni- and multivariable Cox proportional-hazards models were employed, presenting hazard ratios (HRs) and 95% confidence interval (CI) expression. All clinical, biological and imaging parameters were considered for inclusion in the univariable model. Then, a forward selection model including all significant ($p < 0.20$) univariable clinical correlates of survival was used to construct a clinical baseline model predicting the primary endpoint (a composite of 5-year all-cause mortality or hospitalization for HF). The decision to include a covariate with a *p*-value of 0.2 instead of 0.05 aims to mitigate the risk of false discoveries while considering potentially relevant variables that might be overlooked with a stricter threshold. The number of covariates composing the baseline model meets the condition of an event per variable greater than 10.[22] To evaluate the additive prognostic value of *myo*-inositol and estimated glomerular filtration rate (eGFR), the ability of each parameter separately included in the combined model for improving the prediction of the primary endpoint was tested individually using LR tests (χ^2 to enter). Proportional hazards assumption of the covariates and the model was assessed using Schoenfeld residuals, the linearity assumption of continuous covariates was verified with marginal residuals and multicollinearity check of covariates was conducted using the Variance Inflation Factor (VIF).

Role of funders

Funders had no role in study design, data collection, data analysis, interpretation, or writing.

Results

Characteristics of the Belgian and Canadian cohorts

94 no HF, 221 patients with HFrEF and 136 with HFpEF were consecutively included in the Belgian discovery cohort. Baseline demographic, clinical and imaging characteristics of participants are presented in Table 1. Patients with HF were older, had a higher incidence of cardiovascular risks factors and comorbidities compared to no HF subjects. They displayed lower haemoglobin concentration, lower eGFR values, and higher fasting blood glucose levels. Median concentration of the NTproBNP was significantly higher in patients with HF than in no HF individuals (HFrEF: 2662 [1191–4790] pg/mL; HFpEF: 1925 [930–3354] pg/mL; no HF: 57 [38–120] pg/mL), as well as LA volumes, E wave velocities, E/e′ ratios, LV masses, and pulmonary artery pressures. Compared to those with HFrEF, patients with HFpEF were older, more often female, with more comorbidities like atrial fibrillation, hypertension, and diabetes, and had lower haemoglobin concentrations, higher blood glucose, and lower eGFR values. The proportion of chronic kidney disease (CKD) (eGFR < 60 mL/min/1.73 m^2) tended to be higher in HFpEF, although this did not reach statistical significance (Table 1).

	no HF	HF		*p-value*
	(n = 94)	HFrEF (n = 221)	HFpEF (n = 136)	
Baseline characteristics				
Age (years)	58 ± 18	62 ± 13[a]	79 ± 8[a,b]	<0.0001
Male (*n*, %)	50 (53)	186 (84)[a]	54 (40)[b]	<0.0001
BMI (kg/m^2)	25 ± 4	27 ± 5[a]	29 ± 6[a,b]	<0.0001
Mean blood pressure (mmHg)	99 ± 13	83 ± 13[a]	94 ± 14[a,b]	<0.0001
NYHA class III and IV (*n*, %)	0 (0)	132 (60)[a]	57 (42)[a,b]	<0.0001
Medical history				
Atrial fibrillation (*n*, %)	1 (1)	69 (32)[a]	85 (62)[a,b]	<0.0001
Ischaemic cardiomyopathy (*n*, %)	0 (0)	115 (52)[a]	47 (35)[a,b]	<0.0001
COPD (*n*, %)	0 (0)	33 (15)[a]	127 (10)[a]	0.0088
Sleep apnea (*n*, %)	0 (0)	23 (10)[a]	17 (13)[a]	0.0051
Chronic kidney disease (*n*, %)	15 (16)	107 (48)[a]	81 (59)[a]	<0.0001
Cardiovascular risk factors				
Hypertension (*n*, %)	31 (33)	122 (55)[a]	127 (93)[a,b]	<0.0001
Diabetes (*n*, %)	15 (16)	45 (20)	55 (40)[a,b]	<0.0001
Hypercholesterolemia (*n*, %)	56 (60)	132 (60)	89 (66)	0.49
Smoking (*n*, %)	17 (18)	151 (68)[a]	57 (42)[a,b]	<0.0001
Family history of CV disease (*n*, %)	15 (16)	83 (38)[a]	13 (15)[b]	<0.0001
Medication				
ACE inhibitor–ARBs (*n*, %)	21 (22)	202 (91)[a]	92 (68)[a,b]	<0.0001
Betablocker (*n*, %)	6 (6)	193 (87)[a]	90 (66)[a,b]	<0.0001
Diuretics (*n*, %)	4 (4)	177 (80)[a]	111 (82)[a]	<0.0001
MRA (*n*, %)	0 (0)	168 (76)[a]	28 (21)[a,b]	<0.0001
CCB (*n*, %)	14 (15)	12 (5)[a]	57 (42)[a,b]	<0.0001
Anticoagulants (*n*, %)	1 (1)	85 (39)[a]	75 (55)[a,b]	<0.0001
Antiplatelet agents (*n*, %)	15 (16)	128 (58)[a]	55 (40)[a,b]	<0.0001
Statins (*n*, %)	18 (19)	112 (57)[a]	59 (45)[a,b]	<0.0001
Echocardiography				
LA diameter (mm)	34 ± 5	48 ± 9[a]	46 ± 7[a]	<0.0001
LA indexed volume (mL/m^2)	21 ± 5	46 ± 19[a]	46 ± 18[a]	<0.0001
LV end-diastolic volume indexed (mL)	67 ± 12	125 ± 51[a,c]	67 ± 17[b]	<0.0001
LV ejection fraction (%)	64 ± 5	24 ± 8[a,c]	62 ± 8	<0.0001
LV mass index (g/m^2)	73 ± 17	119 ± 40[a]	96 ± 25[a,b]	<0.0001
E wave velocity (m/s)	63 ± 17	80 ± 23[a]	93 ± 31[a,b]	<0.0001
E/e' septal ratio	9.2 ± 2.4	17.1 ± 9.1[a]	18.9 ± 9.1[a]	<0.0001
E/A ratio	1.1 ± 0.5	1.9 ± 0.7	1.3 ± 0.7[b]	<0.0001
ePASP (mmHg)	17 ± 5	34 ± 14[a]	31 ± 11[a]	<0.0001
Biology				
Haemoglobin (g/dL)	14.3 ± 1.2	13.2 ± 2[a]	11.7 ± 1.9[a,b]	<0.0001
GFR (mL/min/1.73 m^2) by CK-EPI	77 ± 17	62 ± 27[a]	55 ± 21[a,b]	<0.0001
Creatinine (mg/dL)	0.9 (0.8–1.1)	1.2 (0.9–1.6)[a]	1.2 (0.9–1.5)[a]	<0.0001
Glycaemia (mg/dL)	96 ± 16	119 ± 42[a]	134 ± 67[a,b]	<0.0001
NTproBNP (pg/mL)	57 (38–120)	2662 (1191–4790)[a]	1925 (930–3554)[a]	<0.0001
Myo-inositol (µM)	34.5 (30.0–43.5)	44.2 (34.4–61.1)[a]	58.2 (43.6–84)[a,b]	<0.0001
				<0.0010, adjusted for age and sex

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ePASP, estimated systolic pulmonary artery pressure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LA, left atrium; LV, left ventricular; MRA, mineralocorticoid receptor antagonist; NTproBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association. Chronic kidney disease: GFR < 60 mL/min/1.73 m^2. Continuous variables have been described using mean ± standard deviation (SD) or median (IQR P25–P75, where P stands for percentile) and categorical variables as numbers (%). *p*-values are derived from one-way ANOVA, the Bonferroni test or Chi square test when appropriate. For *myo*-inositol, ANCOVA test has been used to adjust for age and sex. [a]$p < 0.05$ individual category *vs.* no HF. [b]$p < 0.05$ individual category *vs.* HFrEF. [c]$p < 0.05$ individual category *vs.* HFpEF.

Table 1: **Characteristics of the discovery Belgian cohort.**

The Canadian biobank-based cohort comprised 105 no HF, as well as 103 HFrEF and 77 HFpEF cases, whose characteristics are depicted in Table 2. Interestingly, median NTproBNP concentration were significantly increased in patients with HF (HFrEF: 754 [362–1547] pg/mL; HFpEF: 718 [379–1624] pg/mL; no HF: 116 [55–178] pg/mL) but globally lower than the values measured in the discovery cohort. As observed in the Belgian cohort, HFpEF cases were older, more often female, and exhibited more comorbidities like atrial fibrillation, hypertension, and diabetes. However, Canadians patients with HFpEF were younger and more often male, had higher body mass index, better eGFR values, and a higher prevalence of diabetes and ischaemic cardiomyopathy than their Belgian counterparts.

Increased *myo*-inositol plasmatic level in patients with HF

Median *myo*-inositol concentration was significantly higher in patients with HF compared to no HF subjects in both discovery (no HF: 34.5 [30.0–43.46]; HF: 48.4 [37.0–75.5]) and confirmation cohort (no HF: 42.0 [36.3–48.9]; HF: 50.9 [39.8–69.7]). Interestingly, patients with HFpEF showed significant, or trend of higher *myo*-inositol levels compared to patients with HFrEF in the Belgian cohort and in the Canadian cohort, respectively (Fig. 1a–b), irrespective of sex (Supplementary Table S1).

Myo-inositol tertiles analysis in the Belgian cohort highlighted that patients with the highest values of *myo*-inositol were almost exclusively HF subjects (96% of all cases), often elderly females, and had more diabetes and prior atrial fibrillation (Table 3). They displayed a higher body mass index, higher diuretic intake, worse renal function, higher prevalence of hypertension and lower haemoglobin concentration. Regarding imaging parameters, they also presented more severe diastolic dysfunction, as demonstrated by higher LA volumes, E/e' ratio and pulmonary artery pressures. In both cohorts, more than 50% of patients with HFpEF were in the highest *myo*-inositol tertile, whereas the proportion of patients with HFpEF in the lowest tertile was less than 20%. The opposite pattern was observed for no HF subjects, while patients with HFrEF were more equally distributed across the different *myo*-inositol tertiles (Fig. 1c and d).

A strong correlation was found between plasma *myo*-inositol level and renal function among all subjects in both the discovery (eGFR: Pearson's r = –0.59, $p < 0.0001$; creatinine: Pearson's r = 0.58, $p < 0.0001$: Fig. 2a and Supplementary Fig. S3a) and the confirmation (eGFR: Pearson's r = –0.71, $p < 0.0001$; creatinine: Pearson's r = 0.71, $p < 0.0001$: Fig. 2b and Supplementary Fig. S3b) cohorts. Consequently, we have demonstrated that *myo*-inositol levels progressively increase as kidney function declines across all groups of both cohorts combined (Supplementary Table S2).

	no HF	HF		*p-value*
	(n = 105)	HFrEF (n = 103)	HFpEF (n = 77)	
Baseline characteristics				
Age (years)	68 ± 8	66 ± 8	70 ± 7[a,b]	<0.0001
Male (*n*, %)	51 (51)	90 (92)[a]	45 (62)[b]	<0.0001
BMI (kg/m^2)	29 ± 6	30 ± 6	33 ± 6[a]	<0.0001
Mean blood pressure (mmHg)	94 ± 10	83 ± 10	88 ± 10	<0.0001
Medical history				
Atrial fibrillation (*n*, %)	21 (20)	42 (41)[a]	50 (65)[a]	<0.0001
Ischaemic cardiomyopathy (*n*, %)	35 (33)	100 (97)[a]	60 (78)[a,b]	<0.0001
Chronic kidney disease (*n*, %)	14 (14)	36 (37)[a]	31 (43)[a]	<0.0001
Cardiovascular risk factors				
Hypertension (*n*, %)	64 (61)	86 (84)[a]	74 (96)[a]	<0.0001
Diabetes (*n*, %)	18 (17)	46 (45)[a]	43 (56)[a]	<0.0001
Hypercholesterolemia (*n*, %)	73 (70)	99 (96)[a]	64 (83)[a]	<0.0001
Smoking (*n*, %)	7 (7)	25 (25)[a]	2 (3)[b]	<0.0001
Family history of CV disease (*n*, %)	90 (86)	92 (90)	67 (87)	0.73
Medication				
ACE inhibitor–ARBs (*n*, %)	41 (39)	98 (95)[a]	51 (66)[a,b]	<0.0001
Betablocker (*n*, %)	35 (33)	98 (95)[a]	64 (83)[a]	<0.0001
Diuretics (*n*, %)	12 (11)	73 (69)[a]	58 (75)[a]	<0.0001
MRA (*n*, %)	1 (1)	44 (43)[a]	14 (18)[a]	<0.0001
CCB (*n*, %)	17 (16)	14 (14)	38 (49)[a,b]	<0.0001
Antiplatelet agents (*n*, %)	2 (2)	25 (24)[a]	13 (17)[a]	<0.0001
Statins (*n*, %)	66 (63)	95 (92)[a]	64 (83)[a]	<0.0001
Echocardiography				
LV ejection fraction (%)	NA	29 ± 6	59 ± 6	<0.0001
Biology				
	(n = 103)	(n = 99)	(n = 74)	
GFR (mL/min/1.73 m^2) by CK-EPI	80 ± 14	67 ± 20[a]	64 ± 22[a]	<0.0001
Creatinine (mg/dL)	0.85 [0.72–0.95]	1.14 [0.97–1.32][a]	1.02 [0.87–1.36][a]	<0.0001
Glycaemia (mg/dL)	117 ± 34	150 ± 77[a]	141 ± 55[a]	<0.0001
NTproBNP (pg/mL)	116 (55–178)	754 (362–1547)[a]	718 (379–1624)[a]	<0.0001
Myo-inositol (µM)	42.4 (36.4–48.5)	50.0 (38.8–67.9)[a]	53.4 (42.0–72.5)[a]	<0.0001

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; CV, cardiovascular; eGFR, estimated glomerular filtration rate; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricular; MHI, Montreal Heart Institute; MRA, mineralocorticoid receptor antagonist; NTproBNP, N-terminal pro B-type natriuretic peptide; ns, non significant. Continuous variables have been described using mean ± standard deviation (SD) or median (IQR P25–P75, where P stands for percentile) and categorical variables as numbers (%). *p*-values are derived from one-way ANOVA, the Bonferroni test or Chi square test when appropriate. [a]$p < 0.05$ individual category *vs.* no HF. [b]$p < 0.05$ individual category *vs.* HFrEF.

Table 2: **Characteristics of the confirmation Canadian cohort (MHI biobank).**

Interestingly, we did not observe a significant difference in *myo*-inositol content between the patients with HFpEF of both cohorts, despite their phenotypic singularities.

Clinical parameters associated with elevated plasma *myo*-inositol levels

We further investigated clinical characteristics associated with increased plasma *myo*-inositol levels, based on our *myo*-inositol tertile analysis, in the

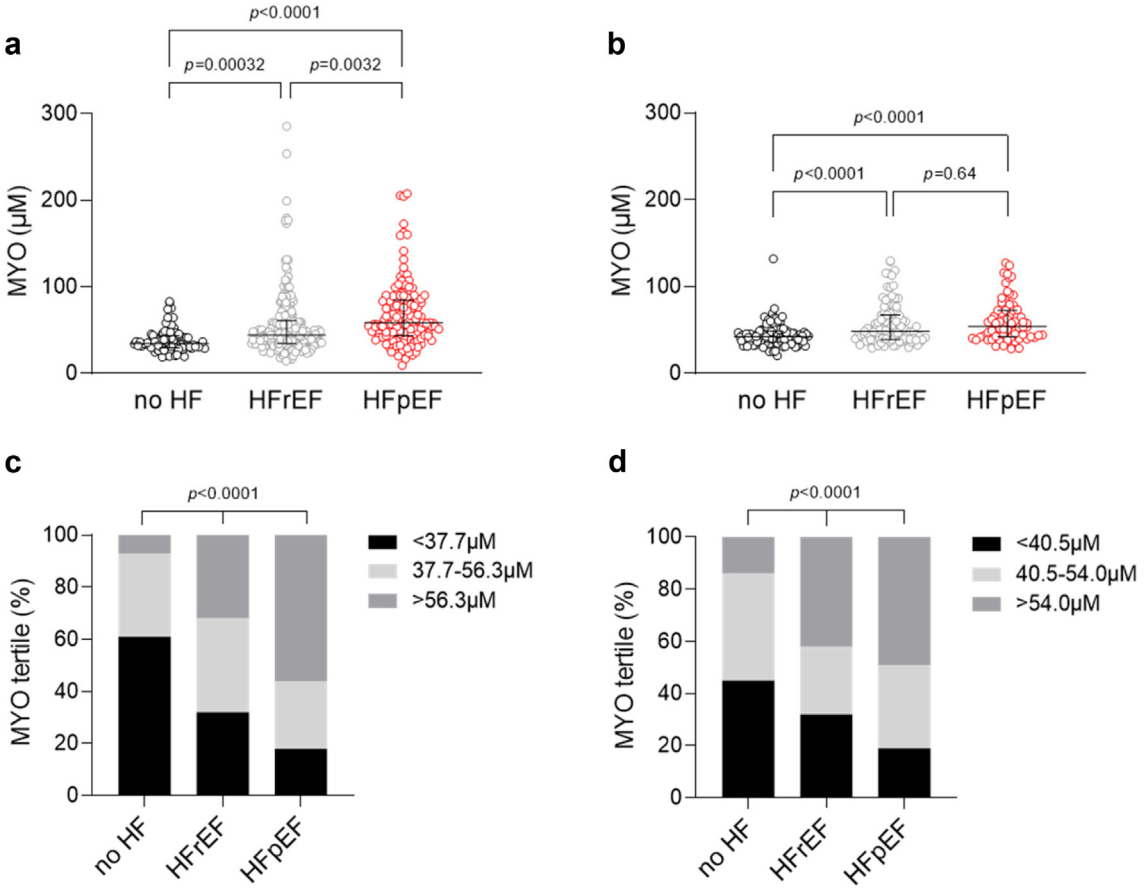

Fig. 1: Plasma *myo*-inositol (MYO) levels in no HF individuals and patients with HF from the Belgian discovery (a, c) and Canadian confirmation (b, d) cohorts. a and b, *myo*-inositol concentrations in no HF individuals, patients with HFrEF or HFpEF. Scatterplot of *myo*-inositol concentrations in no HF individuals, patients with HFrEF or HFpEF. Black line represents the median with interquartile range (IQR, P25–P75, where P stands for percentile). Statistical analysis by one-way ANOVA with Bonferroni's *post-hoc* test; c and d, *myo*-inositol concentrations among the different patient subgroups (no HF individuals, HFrEF, and HFpEF). Plasma *myo*-inositol level groups (tertiles): black square: low *myo*-inositol level, corresponding to 1st tertile; light grey square: intermediate *myo*-inositol level, 2nd tertile; dark grey square: high *myo*-inositol level, 3rd tertile. Statistical analysis by global subgroup Chi-square test, (no HF *vs.* HFrEF *vs.* HFpEF) is presented. MYO, *myo*-inositol; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction.

well-characterised prospective Belgian cohort. Logistic regression showed that elevated levels of plasma *myo*-inositol were associated with HF sub-group (HFrEF *vs.* no HF, HFpEF *vs.* no HF), sex, body mass index, atrial fibrillation, diabetes, hypertension, diuretics, LA volume, E/e' ratio, estimated pulmonary artery systolic pressure (ePASP), haemoglobin concentration, glycaemia, NT-proBNP, and eGFR values (Supplementary Table S3). After adjusting for sex, body mass index, hypertension, diabetes, and atrial fibrillation, logistic regression analysis revealed that only HFpEF status (Odds Ratio (OR): 4.39 [1.61–13.1]) and eGFR (OR: 0.94 [0.93–0.96]) were associated with high plasma *myo*-inositol levels (Supplementary Table S3). Univariable and multivariable linear regression between *myo*-inositol levels and clinical characteristics yielded to similar results (Supplementary Table S4, HFpEF status ($\beta = 0.10$ [0.038–0.17], $p = 0.0018$), and eGFR ($\beta = -0.005$ [–0.006 to 0.004], $p < 0.0001$). Accordingly, *myo*-inositol levels remained higher in patients with HFpEF, compared to no HF or patients with HFrEF, regardless of renal function, being normal (eGFR $\geq$ 60 mL/min/1.73 m^2) or impaired (eGFR < 60 mL/min/1.73 m^2) (Fig. 2c).

Myo-inositol levels were stratified based on age categories (tertiles) in the Belgian cohort. *Myo*-inositol exhibited a gradual but statistically significant increase with advancing age in no HF group: 31.3 [26.8–36.5] in the first tertile (<60 years), 37.2 [31.8–42.6] in the second tertile (60–74 years), and 43.0 [34.7–74.6] in the third tertile (≥75 years) ($p < 0.0001$). However, even after adjusting for age and sex, *myo*-inositol levels remained

	Plasma *myo*-inositol level groups			*p-value*
	Low (1st tertile)	Intermediate (2^{sd} tertile)	High (3rd tertile)	
Myo-inositol (μM)	<37	37.7–56.3	>56.3	
Study groups (*n*)	153	145	153	
no HF (*n*, %)	57 (37)	30 (21)	7 (4)	<0.0001
HFrEF (*n*, %)	72 (47)	79 (54)	70 (46)	
HFpEF (*n*, %)	24 (16)	36 (25)	76 (50)	
Baseline characteristics				
Age (years)	60 ± 16	67 ± 15	71 ± 12[a,b]	<0.0001
Male (*n*, %)	102 (67)	104 (72)	84 (55)[a,b]	0.0077
BMI (kg/m^2)	26 ± 4	27 ± 5	28 ± 6[a,b]	<0.0001
Mean blood pressure (mmHg)	90 ± 14	90 ± 15	90 ± 15	0.34
NYHA class III and IV (*n*, %)	54 (35)	65 (45)	70 (46)	0.21
Medical history				
Atrial fibrillation (*n*, %)	40 (26)	41 (28)	74 (48)[a,b]	<0.0001
Ischaemic cardiomyopathy (*n*, %)	45 (29)	56 (39)	61 (40)	0.23
COPD (*n*, %)	15 (10)	16 (11)	16 (11)	0.98
Sleep apnea (*n*, %)	11 (7)	13 (9)	15 (10)	0.76
Cardiovascular risk factors				
Hypertension (*n*, %)	73 (48)	91 (63)[a]	116 (76)[a,b]	<0.0001
Diabetes (*n*, %)	29 (19)	37 (26)	49 (32)[a]	<0.0001
Hypercholesterolemia (*n*, %)	83 (54)	91 (63)	104 (68)[a]	0.05
Smoking (*n*, %)	81 (53)	67 (26)	77 (40)[b]	0.63
Family history of CV disease (*n*, %)	39 (26)	41 (28)	45 (29)	0.78
Medication				
ACE inhibitor–ARBs (*n*, %)	92 (60)	106 (73)[a]	117 (77)	<0.0046
Betablocker (*n*, %)	88 (58)	95 (66)	106 (69)[a]	0.091
Diuretics (*n*, %)	66 (43)	99 (68)[a]	127 (83)[a,b]	<0.0001
MRA (*n*, %)	63 (41)	71 (49)	62 (41)	0.27
CCB (*n*, %)	19 (12)	30 (21)	34 (22)[a]	0.060
Anticoagulants (*n*, %)	40 (26)	50 (35)	41 (46)[a,b]	0.0033
Antiplatelet agents (*n*, %)	52 (37)	67 (46)	79 (52)[a]	0.030
Statins (*n*, %)	46 (30)	70 (48)	73 (48)	0.0022
Echocardiography				
LA diameter (mm)	43 ± 10	44 ± 9	46 ± 7[a]	<0.0001
LA indexed volume (ml/m^2)	35 ± 20	42 ± 22[a]	46 ± 18[a]	0.0032
LV end-diastolic volume indexed (ml)	91 ± 36	101 ± 57	95 ± 46	0.20
LV ejection fraction (%)	44 ± 20	42 ± 21	46 ± 21	0.30
LV mass index (g/m^2)	100 ± 33	110 ± 48	107 ± 32	0.059
E wave velocity (m/s)	75 ± 25	80 ± 26	86 ± 29[a]	0.0008
E/e' septal ratio	13 ± 7	16 ± 8[a]	19 ± 10[a,b]	<0.0001
E/A ratio	1.17 (0.81– 1.78)	1.31 (0.77– 2.05)	1.26 (0.79–2.20)	0.33
ePASP (mmHg)	26 ± 2	32 ± 14	35 ± 13[a,b]	<0.0001
Biology				
Haemoglobin (g/dL)	14 ± 2	13 ± 2	12 ± 2[a,b]	<0.0001
GFR (mL/min/1.73 m^2) by CK-EPI	78 ± 23	64 ± 17[a]	46 ± 21[a,b]	<0.0001
Creatinine (mg/dL)	0.9 (0.8–1.1)	1.1 (0.9–1.3)[a]	1.4 (1.1–1.9)[a,b]	<0.0001
Glycaemia (mg/dL)	108 ± 31	118 ± 40	129 ± 67[a]	0.0057
NTproBNP (pg/mL)	939 (116–2832)	1958 (706–4095)[a]	2204 (929–4154)[a]	<0.0001

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ePASP, estimated systolic pulmonary artery pressure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LA, left atrium; LV, left ventricular; MRA, mineralocorticoid receptor antagonist; NTproBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association. Continuous variables have been described using mean ± standard deviation (SD) or median (IQR P25–P75, where P stands for percentile) and categorical variables as numbers (%). *p*-values are derived from one-way ANOVA, the Bonferroni test or Chi square test when appropriate. [a]$p < 0.05$ individual category *vs.* tertile 1. [b]$p < 0.05$ individual category *vs.* tertile 2.

Table 3: **Clinical, biological and echocardiographic characteristics among plasma *myo*-inositol tertiles in the discovery Belgian cohort.**

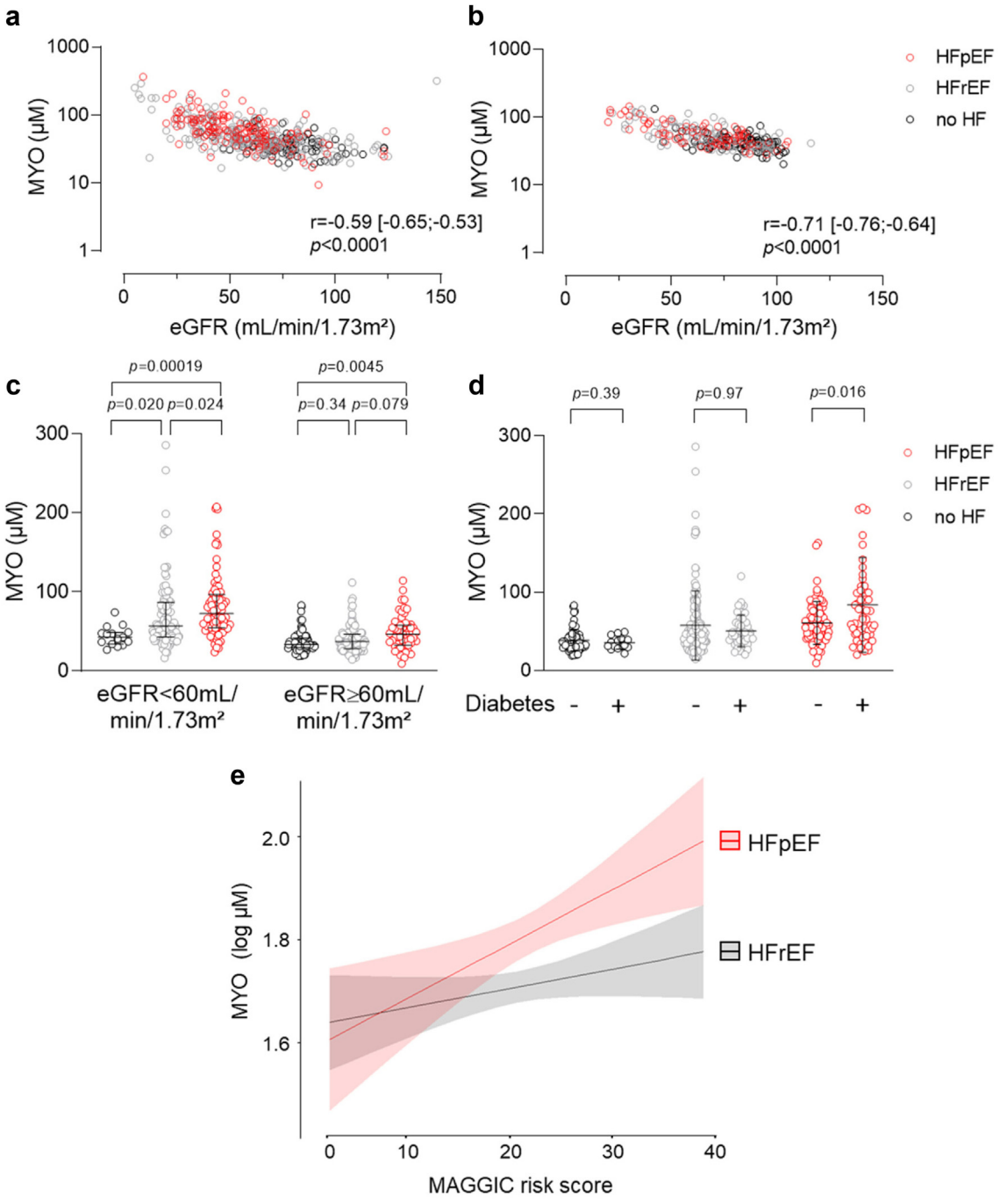

Fig. 2: Clinical parameters associated with *myo*-inositol. a, b correlation between eGFR and *myo*-inositol concentrations in the Belgian and Canadian cohorts; c, plasmatic *myo*-inositol level in no HF individuals and patients with HF, with or without renal dysfunction. no HF individuals and patients with HF were sorted according to eGFR. Statistical analysis by one-way ANOVA with Bonferroni's *post-hoc* test; d, Plasmatic *myo*-inositol level in no HF individuals and patients with HF, with or without diabetes. c and d, Black line represents the median with interquartile range (IQR, P25–P75, where P stands for percentile). Statistical analyses by Wilcoxon rank-sum test; e, Linear regression between and MAGGIC risk score according to HF subgroup. Shaded area represents 95% confidence band. eGFR, estimated glomerular filtration rate; MYO, *myo*-inositol; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction.

higher in patients with HF compared to those with no HF (Table 1). Importantly, linear multivariable analysis revealed that neither age nor sex independently predicted high *myo*-inositol levels (Supplementary Table S4).

Of note, among patients with HFpEF, those with diabetes exhibited elevated levels of *myo*-inositol when compared to their non-diabetic HFpEF counterparts (Fig. 2d). However, diabetic patients without HF or with HFrEF did not show higher levels of *myo*-inositol compared to non-diabetic individuals, confirming that, among co-morbidities, diabetes itself was not a predictor of elevated *myo*-inositol levels. Furthermore, the difference in *myo*-inositol levels between patients with HFpEF and HFrEF was not due to co-morbidities and HF severity. Indeed, *myo*-inositol levels were more correlated to MAGGIC score in patients with HFpEF ($\beta = 0.01$, $p = 0.0035$) compared to those with HFrEF ($\beta = 0.0037$, $p = 0.12$), despite no interaction between MAGGIC score and HF status ($p = 0.11$) (Fig. 2e). Finally, the area under the ROC curves (AUC) of plasma *myo*-inositol concentration was able to distinguish patients with HFpEF from no HF subjects or patients with HFrEF. Notably, for the discrimination between HFpEF and no HF (AUC = 0.81, 95% CI: [0.75–0.86], $p < 0.0001$), with a threshold of 47.3 μM, the specificity was 0.85, and the sensitivity 0.70, and for the discrimination of HFpEF and HFrEF (AUC = 0.64, 95% CI: [0.58–0.70], $p < 0.0001$), with a threshold of 52.8 μM, the specificity is 0.67, and the sensitivity is 0.64 (Supplementary Fig. S4a and b). Altogether, these data suggest that the association between HFpEF status and plasmatic *myo*-inositol level does not entirely rely on co-morbidities.

Prognostic value of high plasma *myo*-inositol level in patients with HF

Patients from the Belgian prospective cohort have been followed overtime. Based on a control group matched for age with the HFpEF and HFrEF populations of the discovery cohort, 69.8 μM and 67.5 were respectively defined as the upper normal limit of plasma *myo*-inositol concentration (mean of control population matched for age +2 SD (Supplementary Tables S5 and S6). Over a median follow-up of 22 [5–54] months, 56 patients with HFpEF (41%) died, and 70 patients (52%) were hospitalized for HF. A total of 92 patients reached the primary composite endpoint of all-cause death or HF hospitalization, 43 patients (32%) were censored at 5 years in the absence of events, and 1 patient with HFpEF (1%) was lost to follow-up. The median follow-up of patients with HFrEF was 20 [7–50] months. 101 patients died (46%), 60 patients were hospitalized for HF (27%), 124 patients met the composite primary endpoint, and 97 patients (44%) were censored at 5 years, including those without events (21%) and those undergoing heart transplantation or ventricular assist device therapy (23%). Unadjusted Kaplan–Meier event-free survival curves stratified by abnormal *myo*-inositol cut-off demonstrated that event-free survival significantly declined with elevated *myo*-inositol levels only in patients with HFpEF (Fig. 3a). Moreover, spline curve analysis highlighted that continuous *myo*-inositol was predictive of poor clinical outcome in patients with HFpEF (HR = 2.86 [1.15–7.09]) but not in patients with HFrEF (HR = 1.38 [0.73–2.84]) (Fig. 3b). Of note, renal dysfunction (eGFR) had a similar impact on prognosis in both patients with HFrEF and HFpEF (HR = 0.99 [0.98–0.99] and HR = 0.98 [0.97–0.99], respectively). Thus, the prognostic link of *myo*-inositol in HFpEF cannot be explained solely by the deterioration of renal function. In univariable Cox regression analysis, male, body mass index, NYHA Class III or IV, diabetes, chronic obstructive pulmonary disease (COPD), diuretics, high E/e ratio, LV end-diastolic volume, high ePASP value, high NTproBNP concentration, low haemoglobin concentration, low eGFR value, high *myo*-inositol concentration, and *myo*-inositol concentration ≥69.8 μM were predictors of the primary composite endpoint in patients with HFpEF (Supplementary Table S7). To test the additive value of *myo*-inositol levels, we constructed a multivariable Cox regression baseline model with body mass index, NYHA Class III or IV, diuretics, diabetes, and COPD as independent predictors of the primary composite endpoint of mortality or HF hospitalization; eGFR was not included into the baseline model to avoid collinearity between eGFR value and *myo*-inositol concentrations. After adjustment for baseline model's variables, eGFR (HR: 0.98 [0.97–0.99]), *myo*-inositol ≥69.8 μM (HR: 1.88 [1.20–2.91]), and continuous *myo*-inositol concentrations (HR: 3.30 [1.22–8.97]) provided additional prognostic values for predicting the primary endpoint, adjusted for the baseline model (Fig. 3c, Supplementary Tables S7 and S8).

From a mechanistic perspective, patients with abnormal *myo*-inositol levels (≥69.8 μM) presented higher high sensitivity troponin T (hsTnT), NTproBNP, fibroblast growth factor 23 (FGF-23), a marker of interstitial fibrosis in patients with HFpEF[23] (Supplementary Table S9). Accordingly, positive correlations were found between circulant *myo*-inositol and hsTnT (Pearson's r = 0.45, $p < 0.0001$), NT-proBNP (Pearson's r = 0.59, $p < 0.0001$), FGF-23 (Pearson's r = 0.44, $p < 0.0001$), and extracellular volume (Pearson's r = 0.24, $p = 0.001$) (Fig. 4a, b, c, d respectively), indicating that *myo*-inositol could reflect adverse myocardial remodelling and especially fibrosis.

Biological effect of *myo*-inositol on human cardiac fibroblast properties

To strengthen our clinical findings on the connection between *myo*-inositol and fibrosis, we have investigated its impact on isolated human cardiac fibroblasts (HCF)

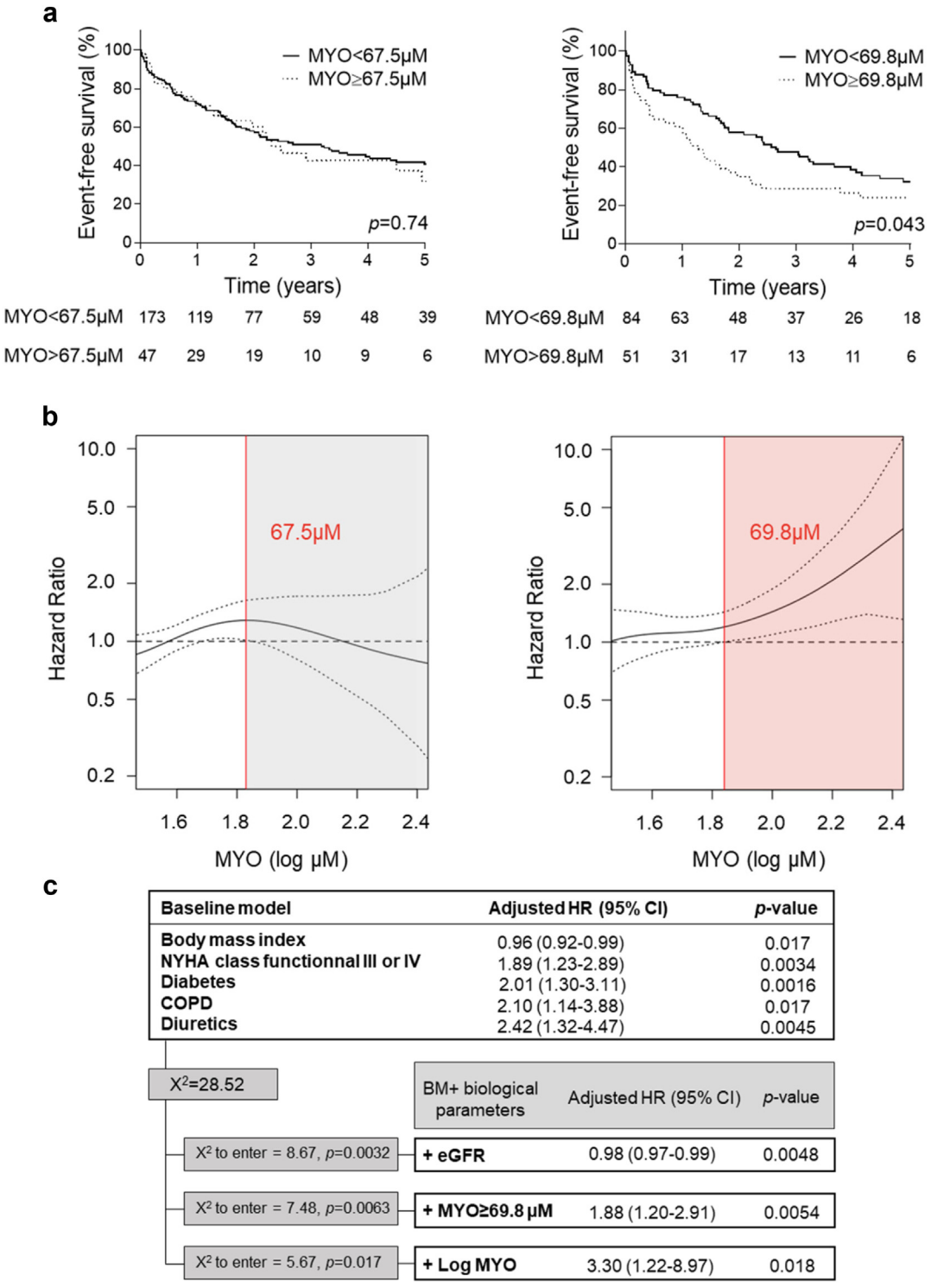

Fig. 3: Prognostic impact of high *myo*-inositol (MYO) levels in HF. a, Kaplan–Meier curves for the 5-year composite endpoint in patients with HFrEF (left) or HFpEF (right) according to abnormal cut-off of plasma *myo*-inositol defined in no HF individuals from the Belgian discovery cohort; b, Spline curves between continuous MYO levels and the hazard ratio of composite endpoint (composite of HF hospitalization and all-cause mortality) in patients with HFrEF (left) or HFpEF (right). Dotted lines areas represent 95% confidence band; c, multivariable Cox regression, model for predicting the composite endpoint. BM, baseline model; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HR, hazard ratio. HFrEF, heart failure with reduced ejection fraction; eGFR, estimated glomerular filtration rate; MYO, *myo*-inositol.

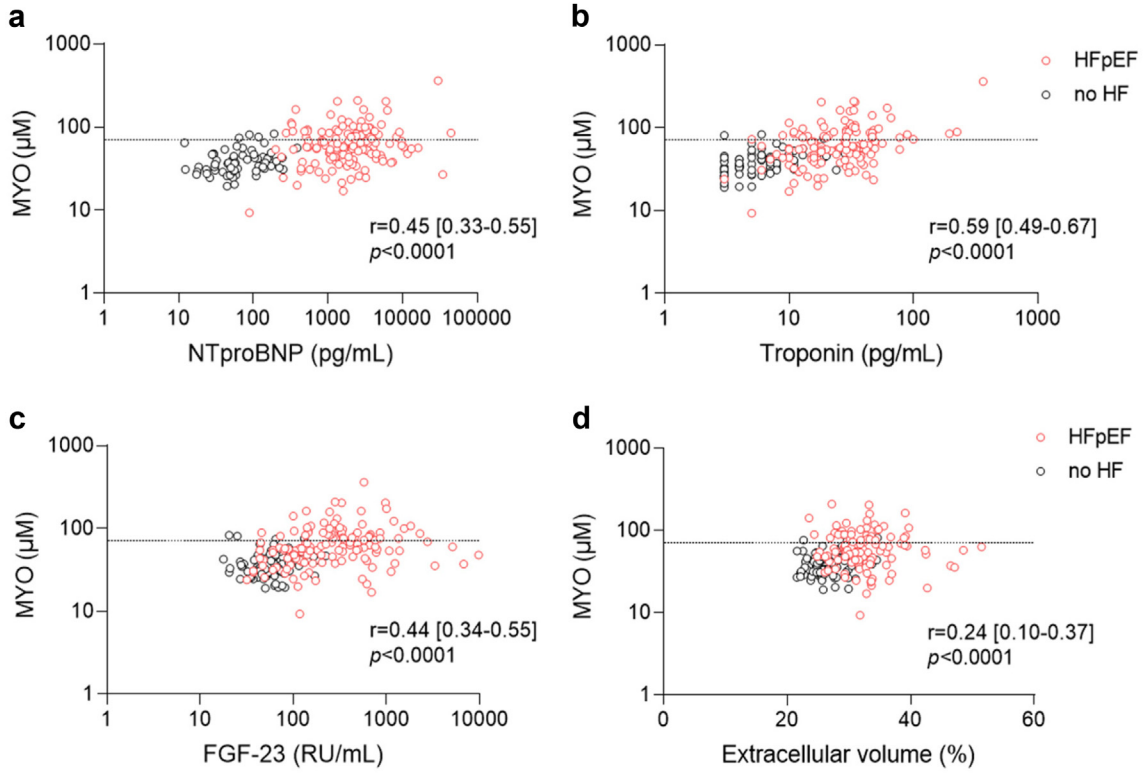

Fig. 4: Correlation between plasma *myo*-inositol and cardiac biomarkers in no HF and HFpEF individuals. Simple linear regression of MYO and: a, NTproBNP (no HF individuals n = 67, HFpEF n = 132); b, Troponin (no HF individuals n = 83, HFpEF n = 130); c, FGF-23 (no HF individuals n = 94, HFpEF n = 136); d, Extracellular volume (no HF individuals n = 79, HFpEF n = 112). 69.8 µM MYO level cut-off is highlighted with a dotted line. MYO, *myo*-inositol; HFpEF, heart failure with preserved ejection fraction; NTproBNP, N-terminal pro b-type natriuretic peptide; FGF-23, fibroblast growth factor 23.

activity. *Myo*-inositol in the culture medium increased the proliferation rate of HCF under foetal bovine serum (FBS) stimulation (Supplementary Fig. S5a) and enhanced their migration (Supplementary Fig. S5b and c). Moreover, we observed that myodifferentiation triggered by transforming growth factor-β (TGF-β) was compromised in cells deprived of *myo*-inositol. This was evidenced by a notable decrease in both alpha-smooth muscle actin (αSMA) mRNA and protein levels (Supplementary Fig. S5d–f). Consistent with previous findings, we demonstrated that SMIT1, but not SMIT2, was highly expressed in both whole heart tissue and in isolated HCF (Supplementary Fig. S5g). Pharmacological and genetic invalidation of SMIT1 abrogated *myo*-inositol accumulation in HCF, demonstrating its central role in *myo*-inositol handling (Supplementary Fig. S5h and i).

Discussion

The current study reveals that *myo*-inositol, a crucial metabolite of cellular function, is elevated in the plasma of patients with HF, and even more in HFpEF cases. Elevated *myo*-inositol is associated with renal dysfunction, HFpEF status, and predicts poor prognosis and clinical outcome only in HFpEF individuals, in whom *myo*-inositol correlates positively with biomarkers of congestion (NT-proBNP), myocardial injury (cardiac troponin T), and cardiac fibrosis (FGF-23 and ECV), suggesting its involvement in myocardial remodelling. The *myo*-inositol/SMIT1 axis might therefore become a prognostic tool or even a potential therapeutic target in patients with HFpEF.

In this study, we evaluated circulant *myo*-inositol level, a rarely investigated metabolite in diseases. *Myo*-inositol is a cyclic polyol with diary and biosynthetic origins[24] with an average plasma level of 30 µM in healthy controls.[25] Few studies have reported elevated *myo*-inositol in Alzheimer's disease, COVID-19, and HF with impaired LVEF.[6,26,27] Here, we demonstrated that *myo*-inositol plasma level was significantly increased in patients with HF compared to no HF subjects. Across all groups, patients with HFpEF consistently showed the highest levels of *myo*-inositol. However, the difference in *myo*-inositol content between patients with HFpEF and HFrEF only reached significance in the Belgian

cohort. Several factors may explain this observation. Although the Belgian HFrEF cohort exhibit similar clinical features than the Canadian cohort, and other cohorts from large registries,[28] HFpEF cohorts substantially differ around the world, as well as in our study. PARAGON-HF, one of the largest world-wide cohorts, presented regional differences in clinical characteristics and outcomes, such as age, sex, body mass index, and comorbidities, which likely influence data interpretation. We previously highlighted such differences between our Belgian cohort and the PARAGON-HF or the ASIAN-HF cohort.[29] Differences between Western European *vs.* North American cohorts reported in PARAGON-HF were comparable to those observed in the present study between patients with HFpEF from Belgium and from Canada.[30] Specifically, Belgian HFpEF individuals were predominantly elderly females with lower body mass index, fewer instances of ischaemic cardiomyopathy history, markedly elevated NTproBNP levels, and poorer renal function compared to Canadian patients with HFpEF. The prevalence of ischaemic cardiomyopathy was lower in the Belgian cohort compared to the Canadian cohort, reflecting once again the specificity of the European *vs.* the North American phenotypes.[29] Interestingly, despite significant phenotypic differences, there was no discrepancy in *myo*-inositol levels among patients with HFpEF from both cohorts, suggesting its association with HFpEF *per se*.

The most striking correlation we observed was between *myo*-inositol level and kidney function. We found that plasmatic *myo*-inositol was positively correlated with creatinine, and inversely correlated with eGFR, concurring with previous findings,[31] and suggesting its association with renal dysfunction. *Myo*-inositol blood concentration is mostly regulated by its catabolism and clearance organized in the proximal tubules, which almost exclusively express the MIOX enzyme.[10] In patients, serum MIOX levels are increased after acute kidney injury and predict chronic kidney disease development,[32] but we lack information about renal MIOX activity or its expression in chronic kidney disease patients. Since approximately 50% of patients with HFpEF present chronic kidney disease,[33] we speculate that the decline in renal function is likely to have negative impact on renal MIOX expression or activity, thereby resulting in elevated circulant *myo*-inositol levels. This hypothesis is supported by the fact that mice lacking MIOX exhibit higher plasma *myo*-inositol levels compared to wild-type mice.[10] Interestingly, *Weston. E and colleagues* demonstrated recently that plasma *myo*-inositol level was not associated with single nucleotide polymorphism (SNP) of genes involved in its transport or metabolism,[34] foreshadowing that *myo*-inositol concentration is settled by a systemic response more than a specific genetic variation. In this regard, we observed that HFpEF status was also associated with *myo*-inositol level independently of kidney function, comorbidities, or HF severity. Of note, diabetic patients with HFpEF had more *myo*-inositol than their non-diabetic counterpart, but diabetes and hyperglycaemia alone were not able to induce *myo*-inositol elevation. Similarly, hypertension and AF were predominant in patients with high *myo*-inositol, but we demonstrated that none of them were able to solely explain plasmatic *myo*-inositol concentration. We do not exclude that additional HFpEF phenotypic characteristics, yet to be determined, might explain its impact on plasma *myo*-inositol levels.

Abnormal *myo*-inositol (>69.8 μM) was associated with poor clinical outcome, namely hospitalization for HF or death, only in patient with HFpEF. Moreover, *myo*-inositol was a great predictor of primary composite endpoint with significant additive value to our baseline model comprising NYHA class, diabetes, COPD, and diuretics intake. Interestingly, *myo*-inositol was correlated to markers of congestion (NTproBNP), cardiac injury (Troponin) and interstitial fibrosis (FGF-23 and ECV). *In vitro* experiments in human isolated cardiac fibroblasts suggest that the *myo*-inositol/SMIT1 axis could have biological effects, potentially impacting the pathophysiology of HFpEF. In addition, our group demonstrated previously that *myo*-inositol, through its transporter SMIT1, was able to induce NOX2 activation and subsequent ROS production in isolated cardiomyocytes, leading to their death.[14] Recent single cells RNA sequencing studies made SMIT1 expression investigation possible in different myocardial cell type, revealing that not only cardiomyocytes and cardiac fibroblasts but also endothelial cells and immune cells, such as macrophages, all express SMIT1 to a certain level, both in human and mouse.[35–38] We do not exclude that the harmful effect of elevated *myo*-inositol also impairs other cells within the heart. Therefore, SMIT1 expression/activity could play a role in HFpEF pathophysiology. Another argument supporting this hypothesis is that the *SLC5A3* locus (SMIT1 gene) comprehend two SNPs, rs9982601 and rs28451064, which both increase the expression of SMIT1 and are associated with coronary artery disease and chronic ischaemic heart disease respectively.[39] One could speculate that the cardiac expression of SMIT1 differs between HFrEF and HFpEF, potentially elucidating the varying impact of *myo*-inositol in both diseases.

SGLT2i are the only available evidence-based drugs that have beneficial clinical outcomes for patients with HFpEF.[1,2] The latter has dramatically changed the clinical practice.[40] Many authors postulated that SGLT2i directly impacts HF pathogenesis, without reaching conclusive mechanism. One hypothesis is that SGLT2i reduce cardiac fibrosis. Several studies revealed that empagliflozin and dapagliflozin were both able to reduce the activation of isolated human cardiac fibroblasts.[41,42] However, our team and others have not been able to highlight SGLT2 expression in the heart or isolated cardiac cells,

illustrating the complexity of SGLT2i action and raising concern for off-target inhibition.[14,41] We recently reported that SMIT1 was the only SGLT fully expressed in human and murine hearts, questioning whether SGLT2i could interact with SMIT1.[15] In this regard, the IC_{50} of empagliflozin, and dapagliflozin for SMIT1 are 29.9, and 28,6 μM, respectively.[43] Although these concentrations exceed what is typically found in the blood of SGLT2i-treated patients,[44] one cannot exclude a direct effect of those inhibitors on SMIT1, which would thereby alleviate the deleterious effect of elevated *myo*-inositol in the myocardium. Alternatively, if SGLT2i do not interfere with SMIT1, it could be argued that a dual inhibition of SGLT2 and SMIT1 might further improve clinical outcomes of patients with HFpEF.

There are several limitations in this study. Heart Failure with mid-range Ejection Fraction (HFmEF, $40 < EF \leq 49\%$) was introduced in the 2016 European Society of Cardiology (ESC) guidelines in response to the TOPCAT trial.[45] Since our study began in 2015, this newly described category of HF was not included, and plasma *myo*-inositol level and impact on HFmEF couldn't be described.

The age and sex difference between patients with HF and no HF subjects, predominantly observed in the Belgian cohort, also poses a potential limitation to our study, even though age or sex themselves were not identified as independent predictors of plasma *myo*-inositol elevation. Furthermore, the differences in characteristics observed between the Belgian and Canadian patients with HFpEF should be interpreted cautiously when considering the specific elevation of *myo*-inositol in Belgian HFpEF individuals.

It is important to acknowledge potential limitations due to our modest sample size, especially in developing a predictive model. Despite efforts to mitigate biases through careful variable selection and collinearity testing, the smaller sample size may limit the model's generalizability. In addition, the built-in selection bias in HRs should be acknowledged, as it can distort results based on follow-up duration and subject selection. It is crucial to consider this bias when interpreting HRs to ensure accurate interpretations. Furthermore, the study of *myo*-inositol's associations and prognosis may encounter unmeasured confounding due to its novelty, necessitating further exploration of related factors.

Additionally, we did not investigate renal MIOX activity or its expression in patients, thereby lacking data to potentially explain how impaired renal function, and hypothetically reduced MIOX activity/expression, impact plasma myo-inositol levels. Furthermore, as with any study involving circulating markers, the increase in plasma *myo*-inositol concentration does not allow us to draw conclusions about intracellular cardiac cell concentration or its impact on cardiac remodelling.

A last limitation is the absence of patients treated with SGLT2i in our cohorts. SGLT2i have been incorporated in the ESC guidelines for patients with HF in 2021, and became standard practice in 2023 in Belgium, when the treatment started being reimbursed for patients with HFpEF or HFrEF. Consequently, we were unable to draw conclusions on SGLT2i's effect on plasma *myo*-inositol levels.

Therefore, it is of great interest to thoroughly explore the *myo*-inositol/SMIT1 axis at the cellular level in future studies, as well as investigate the influence of SGLT2i on *myo*-inositol transport and their potential role in HF development.

Conclusion

Myo-inositol is a metabolite elevated in patients with HF, particularly in HFpEF individuals, and strongly correlated to kidney failure. *Myo*-inositol predicts poor clinical outcome in HFpEF pathology specifically, where it is associated with cardiac remodelling markers such as FGF-23. SMIT1 transports *myo*-inositol within the heart, representing a promising target for future therapies.

Contributors

Anne-Catherine Pouleur, Nassiba Menghoum and Julien Cumps participated in conceptualization, data curation, formal analysis, investigation, validation, visualization and writing of the manuscript and are joined as co-first authors. Alice Marino, Sylvain Battault, David Rhainds, Jean-Claude Tardif, Luc Bertrand contributed to conceptualization of the study. Maria Badii, Sibille Lejeune, Clotilde Roy, enrolled patients from the Belgian cohort. Alexandra Furtos, and Louiza Mahrouche, measured *myo*-inositol in blood samples from both cohorts. Julie Thompson Legault contributed to data collection. Valérie Pedneault-Gagnon, Daniel Charpentier, recruited patients from the Montreal Heart Institute (MHI). Julie Hussin, and Gabrielle Boucher contributed to data interpretation. Damien Gruson, took part in the investigation, measuring biomarkers in the Belgian cohort. Christine Des Rosiers participated in conceptualization, funding acquisition, supervision and writing. Sandrine Horman and Christophe Beauloye conceptualization, funding acquisition, project administration, supervision, visualization and writing of the manuscript, and are joined last authors. Anne-Catherine Pouleur, Christine Des Rosiers, Sandrine Horman and Christophe Beauloye have verified the underlying data.

All authors read and approved the final version of the manuscript.

Data sharing statement

Dataset will be made available upon request through contact with the corresponding author. For sharing of data within a scientific collaboration any proposal should be directed to the corresponding author. Data will only be shared in accordance with legal frameworks, and when the integrity of the individual study participant can be guaranteed. This will be decided by the corresponding author on a case-by-case basis.

Declaration of interests

Jean-Claude Tardif reports grants from Amarin, AstraZeneca, Ceapro, DalCor Pharmaceuticals, Esperion, Ionis, Merck, Novartis, Pfizer and RegenXBio; honoraria from AstraZeneca, DalCor Pharmaceuticals, HLS Pharmaceuticals, Pendopharm and Pfizer; minor equity interest in DalCor Pharmaceuticals; and authorship of patents on pharmacogenomics-guided CETP inhibition and use of colchicine after myocardial infarction. No other conflict of interest was declared for the present study.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105264.

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