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Sympathetic nervous system activity, tissue iron distribution, and their modulation by empagliflozin in the EMPATROPISM-FE trial

Short title: SNS and tissue iron in the EMPATROPISM-FE trial

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ABSTRACT

In the EMPATROPISM-FE trial, lower myocardial iron content correlated with cardiac remodeling, impaired exercise capacity, reduced hematopoiesis and higher norepinephrine (NE) levels; exploratory causal mediation analysis suggested interrelations between empagliflozin effects on iron homoeostasis and treatment benefits. Using magnetic resonance T2* quantification, this post-hoc analysis explored tissue iron content (TIC), interrelations between TIC and NE levels, and their changes following 6 months' treatment with empagliflozin versus placebo. In all tissues/organs, baseline TIC correlated closely with NE (all $p < 0.001$). NE levels at or above the median (600 pg/mL) were associated with significantly lower TIC in cardiac walls, skeletal muscle and bone marrow, but higher TIC in spleen and liver. Empagliflozin significantly reduced NE, with concomitant inverse changes in TIC. Structural/functional tissue/organ improvements correlated with NE and TIC changes. These findings suggest that sympatholysis with concomitant improvements in tissue iron homoeostasis may help explain the multiple clinical benefits of sodium-glucose cotransporter-2 inhibition.

Clinical Trial Registration: www.clinicaltrials.gov; NCT03485222

Key words: heart failure, empagliflozin, tissue iron, norepinephrine

INTRODUCTION

Sodium-glucose co-transporter-2 (SGLT2) inhibitors are now recommended for all patients with HF.¹ Among other effects, they modulate iron metabolism,^{2,3} enhance erythropoiesis,^{2,4-7} and improve cardiac remodeling,^{2,8-11} functional capacity,^{9,12} and short- and longer-term clinical outcomes.¹³⁻¹⁵ Mechanistic understanding of SGLT2 inhibitor benefits is limited, but in vitro and in vivo studies showed that sympathetic nervous system (SNS) overactivity upregulates SGLT2 expression^{16,17} and promotes translocation of SGLT2 to cell surfaces,¹⁸ induces intracellular iron deficiency and mitochondrial dysfunction in myocytes,¹⁹⁻²² downregulates both mRNA and protein levels of molecules related to cellular iron uptake,¹⁹ and impacts on circulating iron markers.^{2-7,12,23} Conversely, SGLT2 inhibitors attenuate SNS activity,^{11,16,24,25} reduce NE in renal and cardiac tissues,^{18,26} increase mRNA and iron regulatory proteins on cell surfaces,⁶ ameliorate left ventricular (LV) fibrosis and stiffness,¹¹ and augment myocardial iron content and iron utilization.^{2,3,5,25}

In the EMPATROPISM-FE trial, empagliflozin reduced blood pressure, heart rate and plasma norepinephrine (NE) levels compared with placebo in patients with heart failure with reduced ejection fraction, consistent with sympatholysis, and promoted reversal of LV remodeling, irrespective of anemia status.²⁵ Exploratory mediation analysis and close inverse associations between myocardial iron content (assessed by cardiac magnetic resonance [CMR] T2* quantification) and indices of LV structure and function, hematopoiesis, physical capacity, and NE levels (as a surrogate of SNS activity) suggested that modulation of the SNS-SGLT2 crosstalk may contribute to the coordinated cardiovascular and hemato-metabolic effects of SGLT2 inhibition.^{2,16,17,25}

Using EMPATROPISM-FE data and biomaterials, this exploratory analysis examined the possibility of a common regulatory framework for iron homeostasis across organs and tissues. T2* mapping was performed at multiple cardiac and extracardiac sites visualized

during CMR to estimate tissue iron content (TIC). We hypothesized that TIC across these regions would be associated with SNS activity and that the sympatholytic effects of empagliflozin would promote redistribution of iron toward metabolically active tissues, accompanied by structural and functional improvement. Patients were stratified according to baseline NE (below versus at/above the median). We assessed associations between NE and TIC across cardiac and extracardiac tissues, and between NE and indices of organ structure/function, and circulating markers of iron metabolism and erythropoiesis. Finally, we evaluated the effects of 6 months of treatment with empagliflozin versus placebo across NE strata and examined associations between treatment-induced changes in TIC, organ remodeling and function, and systemic iron-related biomarkers.

RESULTS

Study Population

A total of 77 patients were divided into subgroups with lower (n=33) versus higher (n=40) SNS activity that were defined using a baseline NE level of 600 pg/mL as the threshold (reflecting the upper limit of normal for most assays). These subgroups did not differ significantly in age, sex distribution, race, characteristics such as HF etiology, symptoms, hospitalization rates in the year before enrolment and most comorbidities, although patients with higher SNS activity tended to be younger and were more often of Black ethnicity (**Table 1**). Iron deficiency was highly prevalent in both subgroups but patients with higher versus lower NE levels were more likely to have anemia. HF pharmacotherapy did not differ between subgroups, except for higher prescription rates of a mineralocorticoid-receptor antagonist (MRA) and a trend towards more diuretic use in the subgroup with higher versus lower NE levels (**Table 1**).

Efficacy Outcomes

At baseline, vital status parameters were similar in both subgroups, despite the large between-subgroup difference in NE levels. T2* varied between individual tissues/organs, with the highest T2* in right ventricular (RV) and LV walls and the lowest T2* in bone marrow (**Table 2, Figure 1**). Within subgroups, T2* in heart muscle, skeletal muscle and bone marrow was consistently higher (indicating lower TIC) in individuals with higher versus lower NE while T2* values in liver and spleen were lower (higher TIC) (**Table 2, Figure 1**). Furthermore, LV structure and function parameters indicated more advanced adverse remodeling in the subgroup with higher baseline NE; trends toward larger RV volumes and a shorter walking distance in the higher versus lower NE subgroup did not reach statistical significance. Patients with higher NE had also lower peak oxygen consumption, hemoglobin, hematocrit, and red blood cell (RBC) count, but higher erythroferrone levels (**Table 2**). Erythropoietin, RBC distribution width, hepcidin, iron levels, transferrin saturation (TSAT) and hsCRP levels did not differ significantly between subgroups.

Interrelations Between Outcome Variables at Baseline

Plasma norepinephrine: In both subgroups, NE showed a strong positive correlation with T2* in cardiac walls, skeletal muscle and bone marrow, and strong inverse correlations with T2* in the liver and especially the spleen (**Figure 2, Table 3**). NE levels were significantly associated with LV volumes and mass, and inversely associated with LV ejection fraction; associations with RV parameters were weaker but directionally consistent, while peak VO₂ and 6MWD were inversely related to NE (**Figure S1a; Table 3**). NE appeared unrelated to erythropoietin, but showed a strong positive correlation with erythroferrone and inverse correlations with indices of erythropoiesis except for RBC distribution width (**Figure S1b, Table 3**). NE was not correlated with laboratory iron markers, hepcidin (**Figure S1c, Table**

3), or vital status parameters, except for a modest positive association with body mass index (BMI) (**Figure S1d, Table 3**).

T2 in different tissues/organs:* T2* measurements showed distinct correlation patterns, consistent with differential but interrelated iron distribution across tissues/organs (**Table 3**). Myocardial, skeletal muscle, and bone marrow T2* appeared closely intercorrelated, whereas inverse associations were observed with splenic T2* and, to a lesser extent, hepatic T2*. Close correlations between T2* and NE suggest coordinated modulation of iron distribution by SNS activity. Lower T2* (higher TIC) in myocardium, skeletal muscle, and bone marrow were associated with better physical capacity and erythropoietic indices, indicating functional dependence of these organs on tissue iron availability. In contrast, no consistent associations were observed between T2* and circulating systemic iron markers and hepcidin, with the exception of a weak inverse correlation between splenic T2* and ferritin (**Table 3**).

Hepcidin: Hepcidin showed a strong inverse association with hepatic T2*, consistent with increased hepcidin production in response to higher hepatic TIC (reflected by lower T2*) at its primary site of synthesis (**Table 3**). Additional inverse associations were observed between hepcidin and erythropoietin, hemoglobin, red blood cell distribution width, transferrin, and eGFR. In contrast, hepcidin was positively correlated with ferritin levels. There was no association between hepcidin and NE.

Soluble transferrin receptor: STfR concentrations were positively associated with erythroferrone and erythropoietin, transferrin and RBC distribution width, and inversely associated with serum iron and TSAT. sTfR also showed a positive association with hsCRP concentrations (**Table 3**).

Erythropoietin and erythroferrone: Associations of erythropoietin and erythroferrone with other efficacy endpoints were directionally consistent but differed substantially in

magnitude (**Table 3**). Erythropoietin showed no significant associations with NE or T2* across tissues. In contrast, erythroferrone correlated closely with NE and T2* in metabolically active tissues (myocardium, skeletal muscle, and bone marrow) and was inversely associated with T2* in reticuloendothelial organs (spleen and liver). Both erythropoietin and erythroferrone were modestly inversely associated with physical capacity, with erythropoietin more closely linked to 6MWD and erythroferrone to peak VO₂. Both were also positively associated with sTfR and inversely associated with hematopoietic variables. Divergent associations were noted for systemic iron regulators: erythropoietin was selectively correlated with hepcidin, whereas erythroferrone was selectively inversely correlated with plasma iron levels and TSAT (**Table 3**).

Effects of Empagliflozin in Subgroups by Baseline Norepinephrine

NE and vital status parameters (Figure S2, Table S1): After 6 months, NE levels were significantly lower in the empagliflozin group versus the placebo group. Changes were consistent with a sympatholytic effect across subgroups ($p_{\text{interaction}}=0.595$). Concurrently, systolic and diastolic blood pressure and heart rate declined (all $p<0.001$), and there was a modest reduction in BMI ($p=0.011$).

T2 changes in different tissues/organs (Figure 3; Table S1):* Treatment effects on T2* were consistent across subgroups for cardiac and non-cardiac tissues. Empagliflozin induced a significant T2* decline in RV and LV walls, skeletal muscle and bone marrow, whereas T2* increased in the liver and especially the spleen. The effect on splenic T2* was larger in the subgroup with lower baseline NE levels ($p_{\text{interaction}}=0.083$), compatible with greater iron release. Overall, patterns of changes indicated iron redistribution from reticuloendothelial to metabolically active tissues/organs.

Cardiac structure/function and exercise capacity (Figure 4a, Table S1): Concurrent with T2* changes, cardiac volumes and LV mass decreased, while LV and RV ejection fractions (LVEF, RVEF) improved in all patients receiving empagliflozin (but RV improvements were less pronounced and did not reach statistical significance). Maximal and submaximal exercise capacity also increased significantly during treatment with empagliflozin, with no evidence of subgroup-specific differences.

Hepcidin and laboratory iron markers (Figure 4b; Table S1): Empagliflozin treatment reduced hepcidin levels, with a larger decrease in the subgroup with higher baseline NE ($p_{\text{interaction}}=0.102$), suggesting a subgroup-specific effect. Concurrently, sTfR increased, while ferritin declined, with decreases reaching statistical significance mainly due to greater changes in the higher NE subgroup. The between-subgroup difference in change from baseline in sTfR was statistically significant ($p_{\text{interaction}}=0.014$). Iron levels and TSAT declined in the higher NE subgroup, though these changes did not reach statistical significance.

Erythropoietin, erythroferrone and RBC indices (Figure 4c; Table S1): Empagliflozin had no significant overall effect on erythropoietin. Numerically, a small increase was observed in the higher NE subgroup, but the between-subgroup difference was not statistically significant ($p_{\text{interaction}}=0.205$). In contrast, erythroferrone increased markedly in both subgroups ($p<0.001$). Concurrent significant increases in hemoglobin, hematocrit, RBC count, and RBC distribution width indicated consistent activation of hematopoiesis, with no significant subgroup interactions.

Associations Between Changes in Efficacy Outcomes at 6-Month Follow-Up

Despite several differential treatment effects in patients with higher versus lower baseline NE, most correlations were directionally consistent with baseline findings. Changes in NE levels were closely correlated with changes in T2* (except for in the liver), and these

associations were consistent across treatment arms (**Figure 5A; Table 4**). Conversely, changes in erythroferrone showed a diverging association pattern compared with baseline. Mean erythroferrone concentrations further increased in both subgroups during treatment with empagliflozin while no meaningful changes were observed during treatment with placebo. Individual changes showed significant inverse associations with changes in T2* (and in NE levels) in metabolically active tissues, but positive associations with splenic T2*. In both treatment arms, these associations were strongest with bone marrow T2* changes. In the liver, associations differed between study arms and reached significance only in the placebo group, consistent with the possibility of additional modifying factors (**Figure 5B; Table 4**).

In accordance with the otherwise uniform correlation structure observed across variables and time points, organ-specific increases in TIC (decreases in T2*) were associated with favorable changes in cardiac structure and function, physical capacity, and hematopoiesis indices (**Table 4**). Also consistent with baseline correlations, changes in hepcidin, sTfR and erythropoietin showed no associations with NE changes, and their relationships with changes in systemic iron markers and erythropoiesis indices remained mostly directionally consistent (albeit stronger in empagliflozin-treated patients) (**Figure S3; Table 4**).

Correlations between baseline-adjusted 6-month follow-up measurements were directionally consistent with those at study entry, and similar for NE levels, T2* values and indices of physical capacity and hematopoiesis; associations with erythroferrone were generally weaker following treatment (**Table S2**).

DISCUSSION

This exploratory analysis of EMPATROPISM-FE data and biomaterials provides novel insights into tissue-specific versus systemic iron homeostasis and their modulation by

empagliflozin. We identified previously unreported inverse associations between SNS activity (based on baseline NE levels) and iron distribution across metabolically active versus reticuloendothelial organs/tissues. Our observations further support a relationship between SNS overactivity and progressive disruption of iron homeostasis with declining TIC across multiple compartments.^{13,27-29} Furthermore, site-specific iron allocation and TIC, but not systemic iron status markers, were significantly associated with tissue/organ performance. Regardless of whether baseline NE was high or low, empagliflozin modulated TIC and significantly improved tissue/organ structure and function, suggesting that the previously reported close associations between treatment with a SGLT2 inhibitor and reversal of adverse cardiac remodeling,^{2,8-11} improved exercise capacity,^{9,12} and augmented hematopoiesis^{2,4-7} may also be related to sympatholytic effects.

SNS overactivity at baseline was not readily apparent from routine clinical assessment because vital parameters and most baseline characteristics were similar between the subgroups with lower or higher baseline NE levels. Nevertheless, patients with higher baseline NE (≥ 600 pg/mL) had lower hematopoietic indices and a higher prevalence of anemia, supporting an association between SNS overactivity and impaired erythropoiesis. The higher proportion of patients receiving a MRA in this subgroup likely reflects a more advanced state of disease rather than a direct effect of MRA therapy, consistent with the trend toward more frequent diuretic use, suggesting confounding by indication rather than an influence of MRA treatment on SNS activity.

At the tissue level, iron distribution followed a clear hierarchy, with the lowest T2* in bone marrow, intermediate levels in the liver and spleen, and the highest T2* in myocardium and skeletal muscle, reflecting physiological iron allocation across erythropoietic, storage/recycling, and metabolic compartments, respectively.^{30,31}

T2* values were higher in the RV than in the LV, consistent with lower TIC in the RV. Although this difference may partly reflect geometric and susceptibility-related effects associated with the thinner RV wall, post-mortem studies support a biological basis for chamber-specific differences.^{20,32} This interpretation is further supported by evidence linking myocardial TIC to mitochondrial enzyme activity and cardiac energetics, suggesting that lower RV TIC may reflect the lower oxidative demands of the pulmonary relative to the systemic ventricle.^{20,32} Taken together with previous evidence,²⁰ our results suggest that CMR relaxometry can noninvasively capture these chamber-specific biological differences. Nevertheless, our findings should be interpreted with caution, because accurate delineation of the thin RV free wall on CMR images remains challenging and precluded reliable measurements in a subset of our study participants.³³

Superimposed on this organ-specific hierarchy, SNS activity was associated with distinct patterns of iron distribution. Higher NE levels correlated with higher T2* values (lower TIC) in metabolically active tissues, whereas inverse associations were observed in reticuloendothelial organs, consistent with redistribution of iron toward storage compartments during SNS overactivity. Liver T2* correlated closely with splenic T2* and showed inverse associations with NE, T2* levels in metabolically active tissues, erythroferrone, and hepcidin. Consistent with its central role in systemic iron metabolism,^{2,4,34,35} hepcidin correlated inversely with erythropoietin, transferrin and sTfR, and positively with ferritin. Notably, hepcidin levels showed no meaningful association with NE or extrahepatic T2*, and neither NE nor extrahepatic T2* correlated with laboratory markers of systemic iron status.

Beyond its established role in erythropoiesis,³⁶ the close association of erythroferrone with iron distribution across multiple sympathetically innervated organs suggests a broader role of this hormone in the inter-organ regulation of tissue iron allocation, independent of any treatment. This seems consistent with its structural relationship to other members of the

C1q/TNF-related protein family, which mediate endocrine communication across multiple metabolically active organs, including the small intestine and adipose tissue as well as erythropoietic bone marrow, heart and skeletal muscle.³⁶ The modest positive association between circulating NE and BMI observed in our cohort is in keeping with knowledge that adipose tissue participates in reciprocal neurohormonal and metabolic signaling that influences SNS activity.³⁷

Collectively, all these observations point toward a novel model in which SNS overactivity engages a multiorgan regulatory network, with NE and erythroferrone jointly modulating hepatic iron concentration, while hepcidin serves as a mechanistic interface linking tissue-level iron handling with systemic endocrine regulation of iron availability.^{30,38,39} Additional studies are needed to define how ferroportin integrates into this proposed regulatory network⁴⁰ and to better clarify the complementary roles of erythroferrone and erythropoietin in maintaining systemic iron homeostasis.

Across multiple tissues/organs, both SNS activity (based on NE level) and T2* were closely linked with organ-specific structure and function, with higher NE and T2* levels correlating with larger ventricular volumes, lower LVEF, reduced physical capacity, and lower hemoglobin, hematocrit and RBC count. Among reticuloendothelial organs, only splenic T2* showed a strong inverse association with NE, potentially implicating the spleen as a central mediator of reduced systemic iron availability during SNS overactivity. In contrast, circulating markers of systemic iron status showed no meaningful associations with either NE or parameters of tissue/organ structure and function, thus corroborating that organ-level iron availability, rather than systemic iron status underlies tissue-specific structural and functional impairments, as reported previously.^{20-22,27-29,31,41} The selective association of hsCRP with sTfR raises the possibility that low-grade inflammation may influence iron

homeostasis through mechanisms preferentially affecting cellular iron demand rather than tissue iron distribution.

After 6 months, treatment with empagliflozin had a consistent sympatholytic effect across baseline NE strata, with significant reductions in circulating NE, blood pressure and heart rate, together with structural and functional improvements, consistent with previous findings.^{11,13,24} In parallel, treatment was associated with redistribution of iron from reticuloendothelial stores toward metabolically active tissues. This was reflected by concordant reductions in T2* in the myocardium, skeletal muscle and bone marrow, together with increases in T2* in the liver and, most prominently, the spleen. Collectively, these observations are consistent with reports of enhanced iron availability and utilization after starting treatment with an SGLT2 inhibitor.^{2-7,11,16,24,25} Such redistribution may provide a mechanistic explanation for the rapid and sustainable activation of erythropoiesis and other early structural, functional and clinical benefits reported in previous clinical trials^{9,13,14,42} and observed in the current study, including in patients with baseline iron deficiency and in the absence of iron supplementation.

Most T2* changes occurred proportionally across tissues and NE subgroups. However, compared to the subgroup with lower baseline NE, patients with higher baseline NE had a significantly attenuated increase in splenic T2*, consistent with reduced iron mobilization, whereas hepatic T2* increased to a similar extent in both subgroups. This was accompanied by a blunted erythropoietic response, reflected by a significantly greater increase in sTfR and trends toward greater declines in hepcidin, ferritin, TSAT and circulating iron, suggesting that erythropoietic iron demand exceeded systemic iron availability in this subgroup. Together with lower baseline hemoglobin and a higher prevalence of anemia, these findings suggest that patients with greater SNS overactivity entered treatment with more

exhausted iron reserves, which became more apparent once empagliflozin activated erythropoiesis.

Correlations between changes in efficacy outcomes at 6-month follow-up had the same characteristics as the correlations observed at baseline. Changes in both NE and erythroferrone were consistently associated with iron redistribution, characterized by iron release from the spleen and iron replenishment of metabolically active tissues. Notably, these relationships were nearly identical in the empagliflozin and placebo groups, suggesting an underlying physiological regulatory network rather than a treatment-specific effect. Together, our findings suggest that declining SNS activity may represent a key physiological correlate linking tissue iron redistribution, hematopoietic adaptation, and improvements in cardiac structure and physical performance. In addition, changes in both NE and erythroferrone showed a distinct tissue hierarchy, with the strongest associations observed for changes in bone marrow T2*. This distribution pattern suggests that iron uptake during treatment with empagliflozin is most closely aligned with erythropoietic demand, reflecting the dominant role of erythroid tissues in systemic iron utilization.

The main exception to the otherwise preserved correlation structure between baseline and changes at 6 months was erythroferrone. At baseline, higher erythroferrone concentrations were associated with sympathetic overactivity, lower iron availability within metabolically active tissues, and less favorable cardiac structure and physical performance. During treatment, however, declining sympathetic activity (i.e. reduced NE) was accompanied by further increases in erythroferrone that closely tracked iron mobilization from reticuloendothelial stores alongside replenishment of the bone marrow, myocardium, and skeletal muscle. Greater increases in erythroferrone were also associated with enhanced hematopoiesis, reverse cardiac remodeling, and improved exercise capacity. Interestingly, cross-sectional associations between erythroferrone and other physiological variables became

attenuated at 6 months, despite largely preserved relationships among NE, TIC, hematopoiesis and organ structure/function. This apparent dissociation is compatible with progressive homeostatic feedback regulation, whereby erythroferrone remains part of the adaptive response while becoming less predictive of TIC.

Our findings complement observations from the EMPEROR program, in which elevated baseline erythroferrone levels were strongly associated with subsequent cardiovascular death or HF hospitalization risk in placebo-treated patients, whereas this association was markedly attenuated when patients were treated with empagliflozin for 52 weeks.⁴ Similarly, in Empire HF, erythroferrone remained persistently elevated despite partial normalization of several other iron-related biomarkers during empagliflozin therapy.⁵ As intracellular iron stores are progressively replenished, the drive for further tissue iron restoration declines, weakening the association between erythroferrone concentrations and TIC as a new steady state is established.

Although causality cannot be inferred from this post hoc analysis, the consistency of the baseline, longitudinal and prognostic observations across the present study and previous SGLT2 inhibitor trials raises the possibility that erythroferrone has a broader physiological role than currently appreciated. Rather than acting solely as an erythroid regulator of systemic iron availability, erythroferrone could represent an adaptive endocrine signal within a homeostatic network that contributes to restoration of intracellular iron availability across erythroid tissues and other tissues with high metabolic requirements. Within this model, erythroferrone does not reflect the severity of intracellular iron deficiency itself, but the magnitude of the adaptive physiological response directed toward its correction. Within this proposed SNS-erythroferrone-tissue iron axis, sympathetic overactivity is associated with maladaptive iron partitioning and reduced intracellular iron availability, whereas erythroferrone reflects an adaptive response directed toward restoring tissue iron

homeostasis. This working model requires prospective mechanistic validation but provides a coherent conceptual basis that reconciles the apparently divergent cross-sectional, longitudinal and prognostic associations of erythroferrone observed in the present study and previous SGLT2 inhibitor trials.

Strengths and Limitations

This exploratory analysis revealed a previously unreported TIC hierarchy across cardiac and extracardiac organs, including an inter-organ gradient related to iron utilization and a within-organ gradient associated with SNS activity indexed by NE levels. Integration of quantitative multi-organ CMR measures of cardiac structure and function, circulating biomarkers, and maximal and submaximal exercise testing enabled comprehensive characterization of associations linking autonomic status, erythroferrone, tissue iron distribution, and metabolic adaptation. The observed modulation of several components of this network following treatment with empagliflozin provides mechanistic hypotheses regarding how SGLT2 inhibition may influence inter-organ iron trafficking, erythropoietic adaptation, and tissue/organ structure and function.

Several limitations should be acknowledged. First, plasma NE levels represent an indirect index of SNS activity and are influenced by neuronal reuptake, spillover, metabolic degradation and clearance dynamics,⁴³ limiting their ability to capture more subtle, tissue-specific or organ-level variations in sympathetic activity. Nevertheless, NE quantification has established prognostic relevance in HF. Previous clinical studies have linked elevated NE to impaired systemic iron status,^{23,25,26} and experimental data support interactions between neurohormonal activation, intracellular iron depletion, and mitochondrial dysfunction.^{11,16,17,19-22} Second, although myocardial T2* assessment is becoming established for the non-invasive evaluation of TIC and treatment-associated TIC changes,^{20,41,44-46}

extracardiac relaxometry remains less extensively validated. Moreover, T2* does not directly quantify tissue iron, and interorgan comparisons assume comparable relaxivity properties despite substantial structural differences between tissues. However, previous human and experimental studies reported consistent associations between relaxometry measures and TIC across different organs,^{20,46,47} supporting the biological plausibility of the observed inter-organ differences and longitudinal changes. Furthermore, the modest sample size, single-center design, and specific characteristics of the study population limit statistical power and generalizability, particularly for subgroup and correlation analyses. The cohort was recruited from a single urban catchment area and was characterized by severely impaired iron status and a high prevalence of anemia at baseline. This distinct clinical phenotype may have amplified treatment-associated changes in iron regulation and tissue iron redistribution compared to populations with less severe iron impairment or earlier-stage HF. Therefore, validation in broader and more diverse HF populations is required to determine the generalizability of our findings. Finally, the exploratory and post-hoc nature of all analyses precludes causal inferences. The observed associations should therefore be considered hypothesis-generating and require confirmation in prospective mechanistic studies designed to directly test the proposed SNS-erythroferrone-tissue iron axis.

Conclusions

Post-hoc analyses of the EMPATROPISM-FE trial revealed a two-level organization of TIC, characterized by an organ-specific hierarchy and an SNS-associated redistribution pattern. The integrated pattern of associations among NE, TIC, erythroferrone, and tissue-specific structural/functional parameters support the hypothesis of an SNS-erythroferrone-tissue iron axis that may be modulated by empagliflozin. Treatment with empagliflozin was associated with sympatholysis and redistribution of iron toward tissues with high metabolic

requirements, accompanied by improvements in organ function and erythropoietic markers. If validated, this model may provide mechanistic insights into SGLT2 inhibition and inform biomarker-guided approaches for HF phenotyping, risk stratification, treatment optimization, and future drug development. Given the exploratory nature of the analyses, causal inferences cannot be made and the findings require confirmation in prospective mechanistic trials.

ONLINE METHODS

Study Design and Patient Population

The design and primary results of the EMPATROPISM-FE study have been published previously.^{2,25} Briefly, EMPA-TROPISM (ATRU-4) was an investigator-initiated, single-center, double-blind, placebo-controlled trial (NCT03485222) in which non-diabetic ambulatory patients with stable New York Heart Association (NYHA) class II–IV HF and a left ventricular ejection fraction <50% were randomized 1:1 to empagliflozin 10 mg/day or matching placebo in addition to stable guideline-directed HF therapy.⁹ The protocol was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai, and all participants provided written informed consent. Baseline and 6-month assessments included physical examination, CMR, exercise testing, standard laboratory analyses, and biomaterial collection.

Among 84 EMPATROPISM participants, 77 with 6-month CMR follow-up, no iron supplementation before or during the trial, and available baseline NE measurements were included in this exploratory EMPATROPISM-FE analysis.

Endpoints

The main outcomes were CMR-derived T2* values (a surrogate marker of TIC⁴⁴) in the left and right ventricular myocardium, skeletal muscle, bone marrow, liver, and spleen according

to baseline NE level (below vs. ≥ 600 pg/mL), and their changes after 6 months of empagliflozin versus placebo. Secondary outcomes included subgroup differences in vital signs, organ and tissue structure and function, exercise capacity, hematopoiesis, and systemic iron homeostasis/iron status, as well as their 6-month changes. All longitudinal changes were adjusted for baseline values.

Procedures and assessments

To minimize procedural variability, all participants underwent the same sequence of assessments, preferably supervised by the same personnel. Study visits began at 8:00 am. Following venous cannulation, blood samples were collected after ≥ 30 minutes of supine rest in a quiet room. Vital parameters and a 12-lead electrocardiogram were obtained towards the end of the resting period.

CMR Imaging and T2 Quantification*

CMR image acquisition and analysis have been described previously.^{2,4,24} Briefly, gated end-expiratory CMR data were obtained with a 1.5-T scanner (Magnetom Avanto FIT, Siemens HealthCare, Erlangen, Germany) using phased-array coils. Cine images were acquired from base to apex using electrocardiographically gated steady-state free precession sequences. Cardiac T2* quantification employed multi-echo gradient-echo sequences with at least 8 echo times (2.59–21 ms). As extracardiac T2* assessment was not prespecified, no dedicated image sequences were acquired for the assessment of non-cardiac tissues. Commercial software (cvi42, Circle Cardiovascular Imaging, Calgary, Alberta, Canada) was used for image analysis as previously described.^{2,9} T2* was measured in the ventricular septum and (where possible) RV free wall on mid-ventricular short axis images; additional regions were placed in the paravertebral skeletal muscles, thoracic vertebral bone marrow, and captured

areas of the liver and spleen while avoiding organ boundaries, bone, fibrous septa, or vascular cross sections. Mean signal intensities across increasing echo times were fitted using mono-exponential decay models with nonlinear curve-fitting algorithms (**Figure S4**).

LV and RV volumes, LV mass, LVEF, and RV ejection fraction were quantified by tracing endocardial and epicardial contours from base to apex in all images as previously described.^{2,9} Reported values represent the mean measurements of two independent blinded investigators.

Physical Capacity

Cardiopulmonary exercise testing (CPET) and the 6-minute walk test (6MWT) were used to assess maximal and submaximal exercise capacity.^{2,24} Patients were examined in the fasting state, and identical testing sequences were used at baseline and follow-up to minimize inter-test variability.

CPET consisted of upright incremental bicycle ergometry (Lode, Groningen, The Netherlands) with respiratory gas analysis (Med Graphics Ultima O2, MGC Diagnostics Corporation, Saint Paul, Minnesota). Peak oxygen consumption (VO_2) was defined as the highest 30-second average oxygen uptake. The 6MWT was performed according to American Thoracic Society guidelines.⁴⁸ Patients were instructed to walk as fast as possible over a 6-minute period, without encouragement.

Laboratory Markers

Whole blood was collected in EDTA tubes, kept on ice until centrifugation, and plasma stored at -80°C . Analyses were performed after study completion at the Cardiovascular Institute, Icahn School of Medicine at Mount Sinai Health System. Plasma NE and erythroferrone were measured using commercial ELISA kits (Fast Track ELISA, Rocky Mountain Diagnostics,

93–33,333 pg/mL; MyBioSource, 15.625–1000 pg/mL). Laboratory personnel were blinded to patient identity, treatment allocation, and study time points. Systemic iron markers (iron, transferrin, TSAT, sTfR, ferritin), hsCRP, hepcidin, and erythropoietin were assessed at the Biomarker Laboratory, University Heart & Vascular Center Hamburg, Germany, as previously described.⁵ Red blood cell indices and estimated glomerular filtration rate (eGFR) were measured as part of routine laboratory assessments on examination days.

Statistical analysis

Continuous variables are reported as mean $\pm$ standard deviation or median (interquartile range), and categorical variables as counts (percentages). Continuous variables with skewed distribution were log-transformed to fulfil the sufficient condition of symmetric distribution of the residuals of the regression model and for the Pearson correlation coefficient. Analyses were performed on available cases without imputation. Pearson correlation coefficients were used to assess associations between baseline NE and T2* values across tissues, and other clinical outcomes, in the overall cohort and in subgroups defined by baseline NE level (below versus at or above the median). The same approach was applied to changes from baseline to 6 months.

Treatment effects on clinical outcomes were assessed using baseline-adjusted linear regression models including a treatment-by-subgroup interaction term. To account for the hierarchical structure of the data, with six T2* measurements nested within each individual, a linear mixed-effects model was applied. A patient-level random effect was specified to capture intra-individual correlation across tissues. In line with the linear regression approach, the model was baseline-adjusted and included treatment arm, norepinephrine subgroup, and tissue/organ type as fixed effects. To assess the consistency of the treatment effect across

subgroups and tissues/organs, a three-way interaction term (treatment $\times$ subgroup $\times$ tissue/organ) was modeled.

Results are presented as adjusted differences (or ratios for log-transformed variables) with 95% confidence intervals and visualized using forest plots. Following a hierarchical testing strategy, subgroup-specific p-values are reported when the interaction term reaches $p < 0.1$; otherwise, the overall treatment effect p-value is reported.

All tests were two-sided with $\alpha = 0.05$. Given the exploratory nature of the analyses, p-values were not adjusted for multiple testing. All analyses were performed using Stata version 19.5 (StataCorp).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data Availability

De-identified data supporting the study findings are available from the corresponding author upon reasonable request. Owing to the study's modest sample size and single-center design, which increase re-identification risk, access is restricted to researchers with appropriate human-subject confidentiality training. Statistical source data are provided.

Sources of Funding

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Author Contributions

C.E.A.: research conception and study design, acquisition of funding, study coordination, data analysis and interpretation, manuscript drafting and revision of the manuscript. S.S.: statistical analysis plan, data analysis and interpretation, manuscript drafting and critical revision of the manuscript. L.M.S.G.: data analysis and interpretation, manuscript and figure drafting and critical revision of the manuscript. C.G.S.-G. and J.A.R.-I.: data acquisition, analysis and interpretation and critical revision of the manuscript. T.Z.: laboratory biomarker analysis and quality control and critical revision of the manuscript. C.M. and G.E.: research conception and study design and critical revision of the manuscript. U.D. and. S.F.: critical revision of the manuscript. J.J.B.: research conception and study design, acquisition of funding and critical revision of the manuscript.

Competing Interests Statement

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TABLES

Table 1. Patient characteristics overall and in subgroups by baseline norepinephrine levels in the EMPATROPISM-FE trial

	Total (n=77)	Norepinephrine <600 pg/mL (n=37; 48.1%)	Norepinephrine ≥600 pg/mL (n=40; 51.9%)	p-value
Demographics				
Age, years	62.3 ± 12.1	64.4 ± 12.0	60.4 ± 12.1	0.197
Male sex	48 (62.3)	24 (64.9)	24 (60.0)	0.814
Race				0.113
White	21 (27.3)	11 (29.7)	10 (25.0)	
Black	15 (19.5)	4 (10.8)	11 (27.5)	
Latino/Hispanic	39 (50.6)	22 (59.5)	17 (42.5)	
Asian	2 (2.6)	0 (0.0)	2 (5.0)	
Heart failure features and comorbidities				
Ischemic HF	41 (53.9)	22 (59.5)	19 (48.7)	0.368
NYHA III/IV	13 (16.9)	4 (10.8)	9 (22.5)	0.228
Hypertension	56 (73.7)	28 (75.7)	28 (71.8)	0.797
Atrial fibrillation (ECG)	15 (19.5)	9 (24.3)	6 (15.0)	0.391
Chronic kidney disease (CKD-EPI)	14 (18.2)	7 (18.9)	7 (17.5)	1.000
Iron deficiency ^a	69 (93.2)	33 (91.7)	36 (94.7)	0.670
Anemia ^b	22 (28.6)	5 (13.5)	17 (42.5)	0.006
Heart failure therapies				
ACEi/ARB/ARNi	63 (82.9)	30 (81.1)	33 (84.6)	0.766
Beta-blocker	67 (88.2)	34 (91.9)	33 (84.6)	0.481
Mineralocorticoid antagonist	21 (27.6)	4 (10.8)	17 (43.6)	0.002
Diuretic	51 (67.1)	21 (56.8)	30 (76.9)	0.087

Values are mean ± standard deviation (p-values from Kruskal-Wallis rank test) or number of patients (%) (p-values from Fisher's exact test). The number of missing values is reported if values were missing for >5% of patients.

^aIron deficiency was defined as plasma iron level ≤13 µmol/L or transferrin saturation <20%.

^bAnemia was defined according to World Health Organization criteria as hemoglobin <13 g/dL (<12 g/dL) in men (women).

ARB indicates angiotensin receptor blocker; ACEi, angiotensin converting enzyme inhibitor; ARNi, angiotensin receptor neprilysin inhibitor; CKD-EPI, estimated glomerular filtration rate <60 mL/min/1.73 m² assessed by the Chronic Kidney Disease Epidemiology Collaboration formula); ECG, electrocardiogram; HF, heart failure; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association.

Table 2. Efficacy outcomes in patient subgroups by baseline norepinephrine levels in the EMPATROPISM-FE trial

	Total (n=77)	Norepinephrine <600 pg/mL (n=37; 48.1%)	Norepinephrine ≥600 pg/mL (n=40; 51.9%)	p-value
Norepinephrine and vital status parameters				
Norepinephrine (pg/mL)	601.3 (391.2–834.7)	386.3 (256.7–429.4)	832.9 (684.5–1128.2)	<0.001
Systolic blood pressure (mmHg)	123.7 ± 18.9	124.2 ± 18.0	123.2 ± 19.9	0.544
Diastolic blood pressure (mmHg)	77.2 ± 10.9	77.9 ± 11.5	76.7 ± 10.5	0.729
Heart rate (ECG, beats/min)	75.7 ± 16.0	75.0 ± 17.1	76.4 ± 15.1	0.524
BMI (kg/m ²)	29.7 ± 6.0	28.8 ± 5.1	30.6 ± 6.6	0.148
CMR-derived T2*(ms) ^a				
T2* LV wall	41.5 ± 4.8	38.1 ± 3.6	44.7 ± 3.4	<0.001
T2* RV wall	53.1 ± 7.2	47.0 ± 3.3	58.1 ± 5.3	<0.001
Missing, n	44	22	22	
T2* skeletal muscle	32.2 ± 4.5	29.6 ± 3.3	34.5 ± 4.1	<0.001
T2* bone marrow	10.1 ± 1.5	9.2 ± 0.9	10.9 ± 1.5	<0.001
T2* liver	25.6 ± 3.9	27.5 ± 3.5	23.8 ± 3.5	<0.001
T2* spleen	29.5 ± 4.8	32.8 ± 3.3	26.5 ± 4.0	<0.001
Left ventricular structure and function				
LV end-diastolic volume (mL)	215.4 ± 72.3	185.7 ± 47.1	243.5 ± 80.9	<0.001
LV end-systolic volume (mL)	139.6 ± 60.9	112.1 ± 33.5	164.2 ± 69.3	<0.001
LV ejection fraction (%)	36.3 ± 8.1	39.0 ± 7.5	33.9 ± 7.9	0.006
LV mass (g)	133.6 ± 49.5	119.1 ± 37.2	146.9 ± 55.9	0.028
Right ventricular structure and function				
RV end-diastolic volume (mL)	155.4 ± 51.0	146.5 ± 50.1	163.7 ± 51.1	0.106
RV end-systolic volume (mL)	82.1 ± 34.1	75.7 ± 31.0	87.8 ± 36.0	0.145
RV ejection fraction (%)	48.2 ± 8.5	49.1 ± 7.1	47.4 ± 9.6	0.343
Physical capacity				
Peak VO ₂ (mL/min/kg)	15.0 ± 4.1	16.5 ± 4.8	13.6 ± 2.8	0.023
Missing, n	24	12	12	
Six-minute walking distance (m)	434.2 ± 98.4	446.0 ± 88.2	423.2 ± 107.0	0.269
Hematopoiesis and RBC indices				
Erythropoietin (mIU/mL)	10.7 (7.8–15.6)	10.6 (7.5–15.4)	11.0 (8.3–15.7)	0.596
Erythroferrone (pg/mL)	1.4 (1.2–1.8)	1.2 (1.0–1.4)	1.7 (1.4–2.0)	<0.001
Hemoglobin (g/dL)	13.4 (12.3–14.4)	14.0 (13.2–14.8)	12.8 (12.1–13.7)	0.001
Hematocrit (%)	40.6 (37.6–43.2)	42.5 (39.7–45.5)	39.2 (36.7–41.8)	<0.001
RBC count (×10 ¹² /L)	4.6 (4.2–5.0)	4.8 (4.4–5.2)	4.4 (4.0–4.8)	0.002
RBC distribution width (%)	14.4 (13.6–15.9)	14.0 (13.6–16.4)	14.4 (13.6–15.4)	0.992

Systemic iron markers and hepcidin

Iron (μmol/L)	7.4 (5.0–9.9)	7.5 (5.6–10.8)	7.0 (4.8–8.4)	0.161
Transferrin (mg/dL)	256.8 (231.0–284.5)	261.5 (233.2–290.0)	251.8 (229.0–273.5)	0.226
Transferrin saturation (%)	11.5 (8.0–14.4)	12.1 (9.0–15.9)	10.5 (7.1–13.4)	0.226
Soluble transferrin receptor (μg/L)	1410.4 (1181.8–1745.8)	1383.0 (1164.8–1736.5)	1474.6 (1185.8–1745.8)	0.817
Missing, n	4	1	3	
Ferritin (μg/L)	91.2 (52.9–145.8)	74.9 (42.5–135.6)	110.4 (62.7–148.8)	0.084
Hepcidin (μg/L)	8.5 (4.3–17.2)	7.2 (3.4–15.8)	10.0 (5.1–19.7)	0.249
Other biomarkers				
eGFR (CKD-EPI, ml/min/1.73 m ²)	81.2 (64.0–97.1)	81.2 (65.8–96.4)	80.8 (63.5–97.2)	0.799
hsCRP (mg/L)	2.3 (1.1–5.2)	1.6 (1.0–4.4)	2.7 (1.3–7.6)	0.158
Missing, n	11	4	7	

Values are mean ± standard deviation or median (interquartile range) (p-values from Kruskal-Wallis rank test) or number of patients (%) (p-value from Fisher's exact test).

The number of missing values reported if values were missing for >5% of patients.

^aT2* (quantified using CMR imaging) is used to estimate tissue iron content; higher values indicate lower iron content.

CKD-EPI indicates estimated glomerular filtration rate <60 mL/min/1.73 m² assessed by the Chronic Kidney Disease Epidemiology Collaboration formula); CMR, cardiac magnetic resonance imaging; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; hsCRP, high sensitive C-reactive protein; IQR, interquartile range; LV, left ventricular; peak VO₂, peak oxygen consumption; RBC, red blood cell; RV, right ventricular.

Table 3. Heat map representation of Pearson correlation coefficients (ρ) between efficacy variables and plasma norepinephrine levels, T2* in various tissues and markers of iron metabolism and erythropoiesis at baseline in the EMPATROPISM-FE trial.

Color scheme of ρ values (direction & strength of associations)								
-1.00	-0.75	-0.50	-0.25	0.00	0.25	0.50	0.75	1.00

Observed baseline measurement	Norepinephrine	T2* Spleen (ms)	T2* LV (ms)	T2* skeletal muscle (ms)	T2* bone marrow (ms)	Hepcidin ($\mu\text{g/L}$)	Soluble transferrin receptor ($\mu\text{g/L}$)	Erythropoietin (mIU/mL)	Erythroferrone (ng/mL)
	ρ	ρ	ρ	ρ	ρ	ρ	ρ	ρ	ρ
T2* LV wall (ms)	0.80	-0.80	-	0.85	0.64	0.00	0.09	0.13	0.66
T2* RV wall (ms)	0.84	-0.87	0.94	0.90	0.73	0.16	0.35	0.16	0.77
T2* skeletal muscle (ms)	0.75	-0.76	0.85	-	0.52	-0.17	0.15	0.19	0.65
T2* bone marrow (ms)	0.59	-0.55	0.64	0.52	-	0.00	-0.01	0.13	0.76
T2* liver (ms)	-0.47	0.79	-0.53	-0.48	-0.30	-0.56	0.00	0.11	-0.34
T2* spleen (ms)	-0.76	-	-0.80	-0.76	-0.55	-0.17	-0.21	-0.09	-0.62
LV EDV (mL)	0.47	-0.38	0.30	0.33	0.17	-0.08	0.07	0.00	0.17
LV EsV (mL)	0.46	-0.38	0.35	0.33	0.20	-0.05	0.10	-0.04	0.19
LV ejection fraction (%)	-0.27	0.27	-0.32	-0.22	-0.14	-0.06	-0.10	0.07	-0.10
LV mass (g)	0.32	-0.29	0.19	0.23	0.03	-0.05	0.18	0.00	0.06
RV EDV (mL)	0.15	-0.16	-0.02	0.06	-0.03	0.08	0.05	0.03	-0.06
RV ESV (mL)	0.14	-0.17	0.02	0.10	-0.03	0.14	0.06	0.02	-0.06
RV ejection fraction (%)	-0.04	0.14	-0.05	-0.11	0.02	-0.20	-0.09	0.01	0.06
Peak VO ₂ (mL/min/kg)	-0.52	0.50	-0.70	-0.60	-0.39	0.05	-0.21	-0.08	-0.54
6MWD (m)	-0.19	0.21	-0.26	-0.23	-0.13	-0.01	-0.22	-0.33	-0.20
Erythropoietin (mIU/mL)	0.08	-0.09	0.13	0.19	0.13	-0.35	0.38	-	0.20
Erythroferrone (ng/mL)	0.65	-0.62	0.66	0.65	0.76	-0.10	0.24	0.20	-
Hemoglobin (g/dL)	-0.33	0.37	-0.49	-0.43	-0.66	0.23	-0.22	-0.46	-0.70
Hematocrit (%)	-0.34	0.36	-0.50	-0.44	-0.67	0.19	-0.13	-0.42	-0.70
RBC count ($\times 10^{12}/\text{L}$)	-0.36	0.32	-0.44	-0.36	-0.65	0.08	0.08	-0.36	-0.62
RBCDW (%)	-0.01	-0.06	0.09	0.04	0.01	-0.25	0.38	0.27	0.13
Iron ($\mu\text{mol/L}$)	-0.12	0.07	-0.02	-0.06	-0.12	-0.03	-0.26	-0.11	-0.26
Transferrin (mg/dL)	-0.02	0.07	0.00	0.03	-0.10	-0.40	0.36	-0.02	0.08
Transferrin saturation (%)	-0.12	0.05	-0.04	-0.06	-0.08	0.07	-0.36	-0.05	-0.26
STfR ($\mu\text{g/L}$)	0.10	-0.21	0.09	0.15	-0.01	-0.21	-	0.38	0.24
Ferritin ($\mu\text{g/L}$)	0.07	-0.24	0.08	-0.06	-0.03	0.73	-0.19	-0.19	-0.04
Hepcidin ($\mu\text{g/L}$)	-0.05	-0.17	0.00	-0.17	0.00	-	-0.21	-0.35	-0.10
eGFR (mL/min/1.73 m ²)	0.00	0.06	-0.14	-0.03	-0.18	-0.32	0.04	-0.10	-0.18
hsCRP (mg/L)	0.18	-0.25	0.04	0.06	0.01	0.03	0.40	-0.02	0.08

EDV indicates end-diastolic volume; eGFR, estimated glomerular filtration rate; ESV, end-systolic volume; hsCRP, high-sensitive C-reactive protein; LV, left ventricular; peak VO₂, peak oxygen consumption; RBC, red blood cell; RBCDW, RBC

distribution width; RV right ventricular; 6MWD 6-minute walking distance; sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

Table 4. Heat map representation of Pearson correlation coefficients (ρ) between changes in efficacy variables and plasma norepinephrine, T2* in various tissues and markers of iron metabolism and erythropoiesis at the 6-month follow-up visit in the EMPATROPISM-FE trial.

Color scheme of ρ values (direction & strength of associations)									
	-1.00	-0.75	-0.50	-0.25	0.00	0.25	0.50	0.75	1.00
Baseline adjusted change during treatment	Norepinephrine	T2* Spleen (ms)	T2* LV (ms)	T2* skeletal muscle (ms)	T2* bone marrow (ms)	Hepcidin ($\mu\text{g/L}$)	Soluble transferrin receptor ($\mu\text{g/L}$)	Erythropoietin (mIU/mL)	Erythroferrone (ng/mL)
	ρ	ρ	ρ	ρ	ρ	ρ	ρ	ρ	ρ
T2* LV wall (ms)	0.68	-0.85	-	0.87	0.58	-0.12	0.17	0.17	-0.50
T2* RV wall (ms)	0.45	-0.47	0.57	0.57	0.44	0.21	-0.31	-0.06	-0.22
T2* skeletal muscle (ms)	0.65	-0.87	0.87	-	0.52	-0.16	0.11	0.19	-0.52
T2* bone marrow (ms)	0.55	-0.50	0.58	0.52	-	0.13	-0.09	0.16	-0.73
T2* liver (ms)	-0.14	0.21	-0.16	-0.13	-0.12	-0.38	0.04	0.01	0.07
T2* spleen (ms)	-0.65	-	-0.85	-0.87	-0.50	0.19	-0.15	-0.17	0.47
LV EDV (mL)	0.47	-0.53	0.53	0.53	0.50	0.11	-0.28	-0.08	-0.39
LV ESV (mL)	0.46	-0.56	0.50	0.54	0.47	0.13	-0.34	-0.15	-0.36
LV ejection fraction (%)	-0.37	0.42	-0.36	-0.37	-0.33	-0.20	0.32	0.21	0.19
LV mass (g)	0.30	-0.34	0.28	0.31	0.34	0.21	-0.30	-0.20	-0.20
RV EDV (mL)	0.14	-0.20	0.25	0.21	0.05	-0.08	-0.08	0.09	-0.07
RV ESV (mL)	0.20	-0.36	0.33	0.32	0.29	0.10	-0.18	0.11	-0.23
RV ejection fraction (%)	-0.20	0.36	-0.29	-0.28	-0.33	-0.17	0.12	-0.02	0.26
Peak VO ₂ (mL/min/kg)	-0.31	0.46	-0.64	-0.47	-0.40	0.02	-0.04	-0.05	0.33
6-MWD (m)	-0.20	0.34	-0.27	-0.27	-0.47	-0.11	0.09	-0.03	0.43
Erythropoietin (mIU/mL)	0.03	-0.17	0.17	0.19	0.16	-0.30	0.28	-	-0.32
Erythroferrone (ng/mL)	-0.43	0.47	-0.50	-0.52	-0.73	-0.01	0.07	-0.32	-
Hemoglobin (g/dL)	-0.22	0.20	-0.32	-0.24	-0.67	0.00	0.00	-0.37	0.66
Hematocrit (%)	-0.21	0.23	-0.31	-0.26	-0.68	-0.03	0.03	-0.36	0.69
RBC count ($\times 10^{12}/\text{L}$)	-0.08	0.18	-0.27	-0.20	-0.60	-0.04	0.09	-0.25	0.62
RBCDW (%)	0.11	-0.13	0.27	0.17	0.17	-0.25	0.44	0.09	-0.09
Iron ($\mu\text{mol/L}$)	-0.23	0.09	-0.15	-0.04	-0.15	0.40	-0.32	-0.15	0.02
Transferrin (mg/dL)	0.00	-0.04	-0.01	0.04	-0.26	-0.26	0.39	0.01	0.25
TSAT (%)	-0.19	0.06	-0.09	-0.01	-0.04	0.44	-0.36	-0.12	-0.06
STfR ($\mu\text{g/L}$)	0.14	-0.15	0.17	0.11	-0.09	-0.49	-	0.28	0.07
Ferritin ($\mu\text{g/L}$)	-0.15	0.03	-0.08	-0.04	0.18	0.60	-0.45	-0.20	-0.15
Hepcidin ($\mu\text{g/L}$)	-0.07	0.19	-0.12	-0.16	0.13	-	-0.49	-0.30	-0.01
eGFR (ml/min/1.73 m ²)	0.06	-0.23	0.19	0.15	0.20	0.17	-0.30	-0.10	-0.30
hsCRP (mg/L)	0.13	-0.01	0.05	-0.02	0.19	-0.02	0.16	0.24	-0.09

EDV indicates end-diastolic volume; eGFR, estimated glomerular filtration rate; ESV, end-systolic volume; hsCRP, high-sensitive C-reactive protein; LV, left ventricular; peak VO₂, peak oxygen consumption; RBC, red blood cell; RBCDW,

RBC distribution width; RV right ventricular; 6MWD 6-minute walking distance; sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

FIGURE LEGENDS/CAPTIONS

Figure 1. Violin plots showing distributions of baseline T2* in different tissues/organs stratified by baseline norepinephrine level (<600 vs. ≥600 pg/mL) in the EMPATROPISM-FE trial.

Kernel density estimates (colored areas) with embedded boxplots, median (solid lines) and interquartile range (dashed lines). ¹⁾ p-value for within-location comparison of T2* between norepinephrine subgroups <0.001. ²⁾ p-value for within-location comparison <0.05.

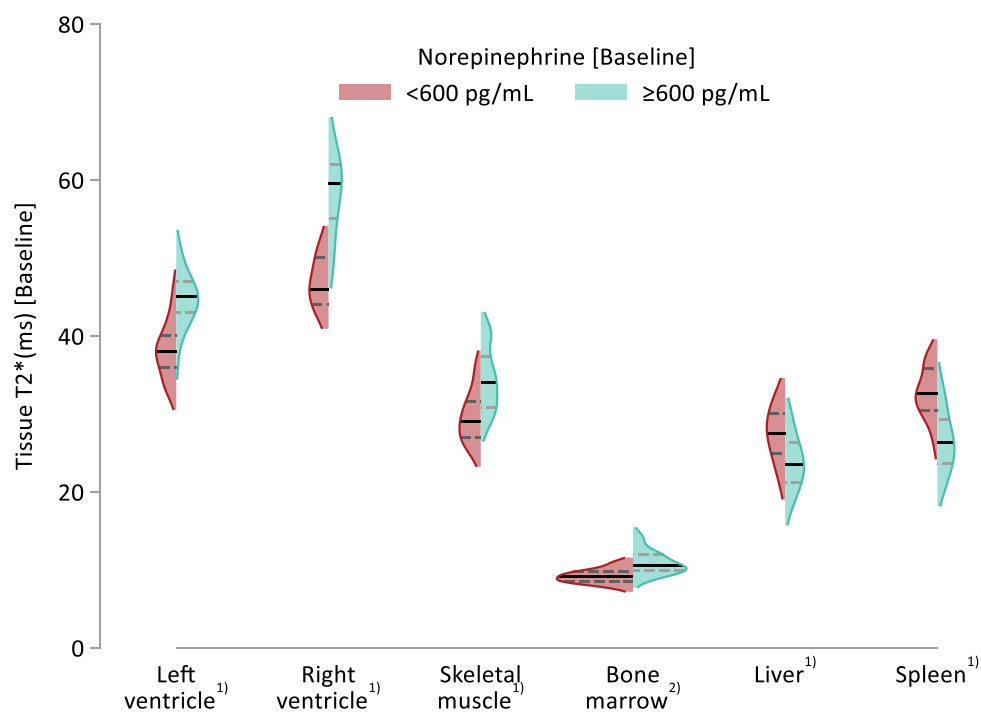


Figure 2. Correlations between baseline norepinephrine level and T2* in different tissues/organs in the EMPATROPISM-FE trial.

Individual data points are color-coded by norepinephrine subgroup (≥ 600 vs. <600 pg/mL).

Pearson correlations were calculated for the full cohort.

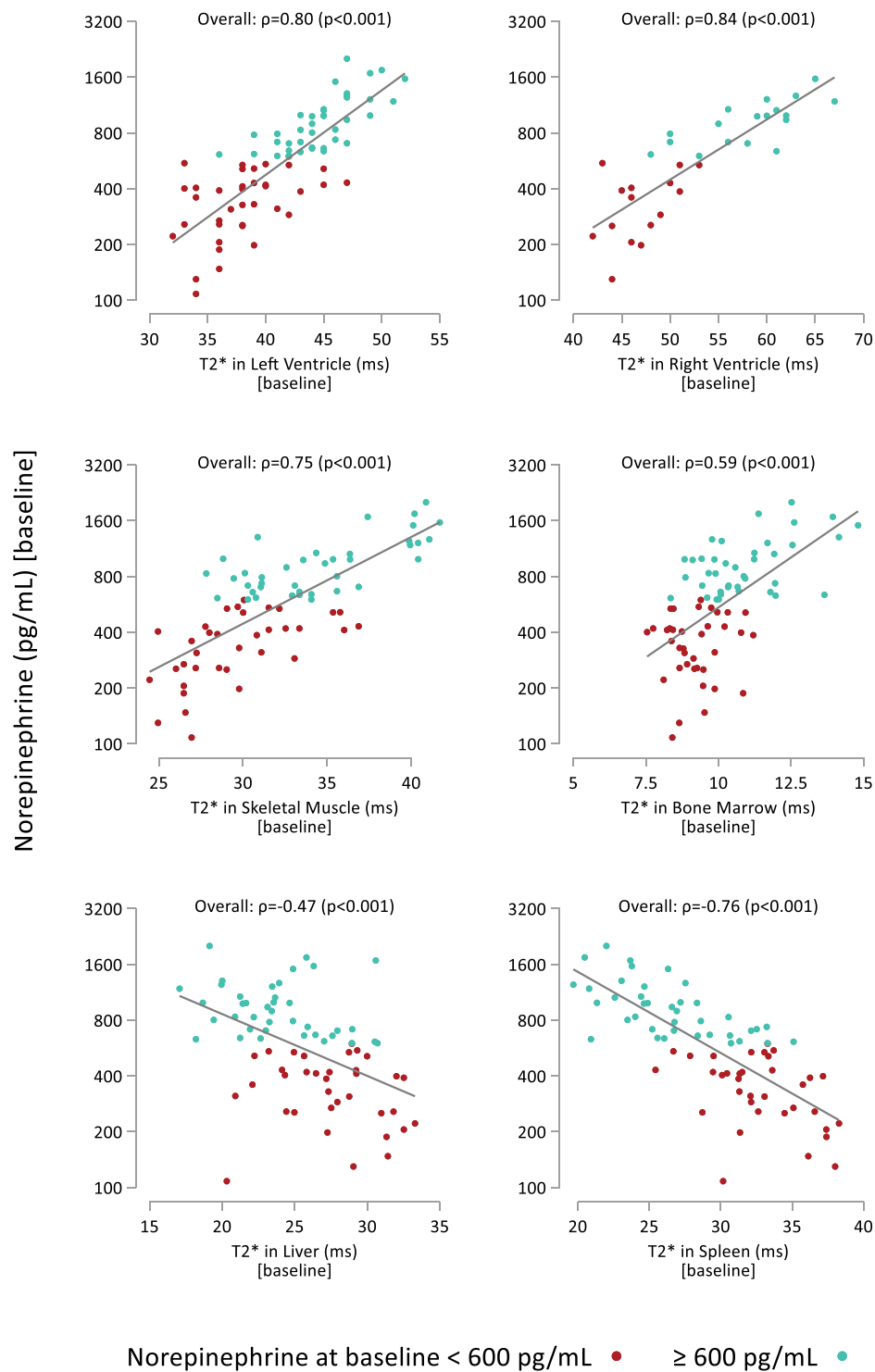


Figure 3. Change in T2* in different tissues/organs after 6-month treatment with empagliflozin or placebo by norepinephrine level subgroup (≥ 600 vs < 600 pg/mL) in the EMPATROPISM-FE trial.

Results are reported as subgroup- and treatment-specific adjusted change from baseline values with corresponding 95% confidence interval (CI). P -values for the interaction term ($P_{\text{interaction}}$); $P_{\text{interaction}} > 0.1$: $P_{\text{treatment}}$ -values refer to the effects of empagliflozin vs. placebo in the entire cohort (regular font), $P_{\text{interaction}} \leq 0.1$: $P_{\text{treatment}}$ -values refer to the effects of empagliflozin vs. placebo within subgroup by norepinephrine level ($P_{\text{treatment}}$, *italic font*).

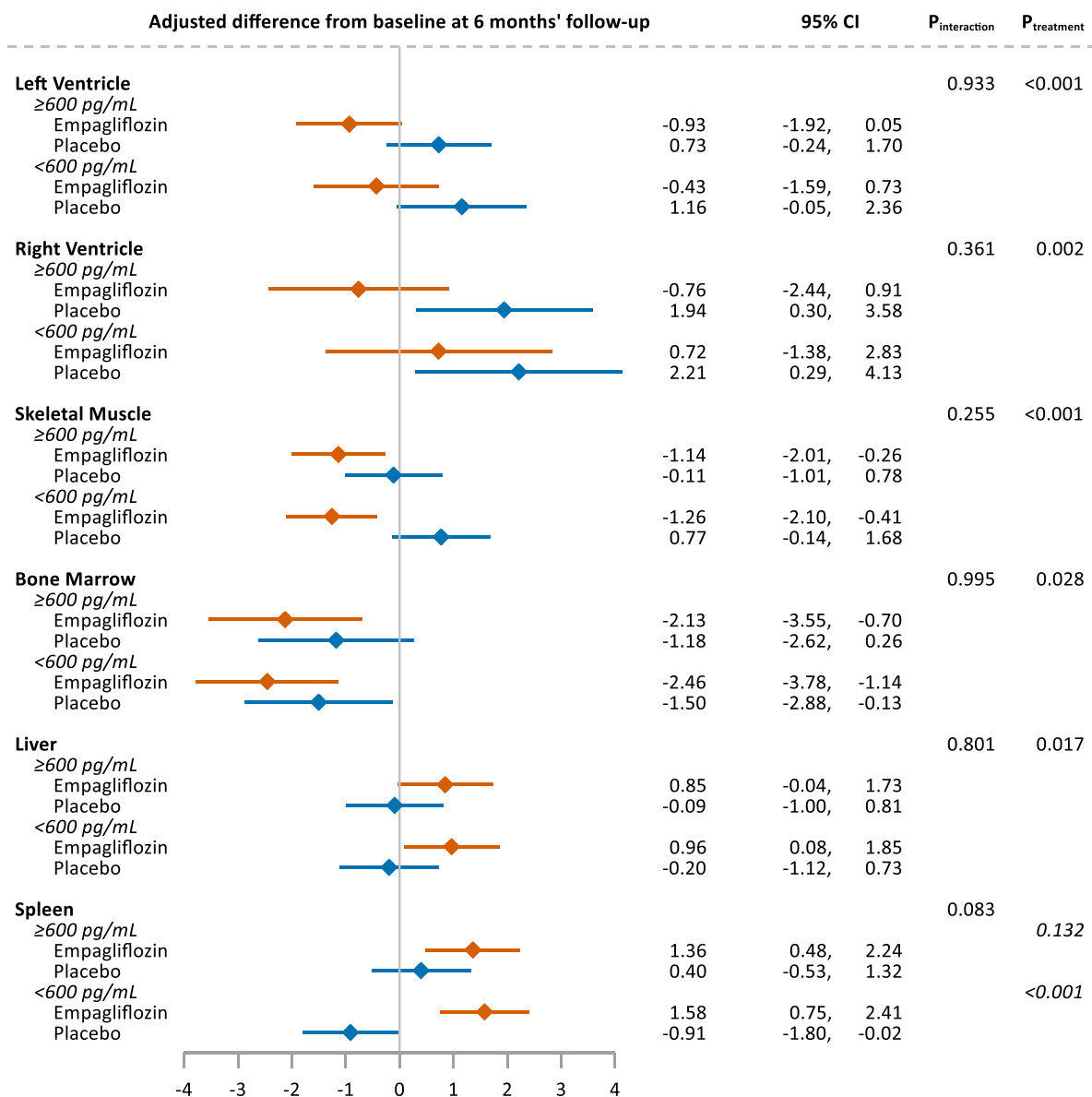
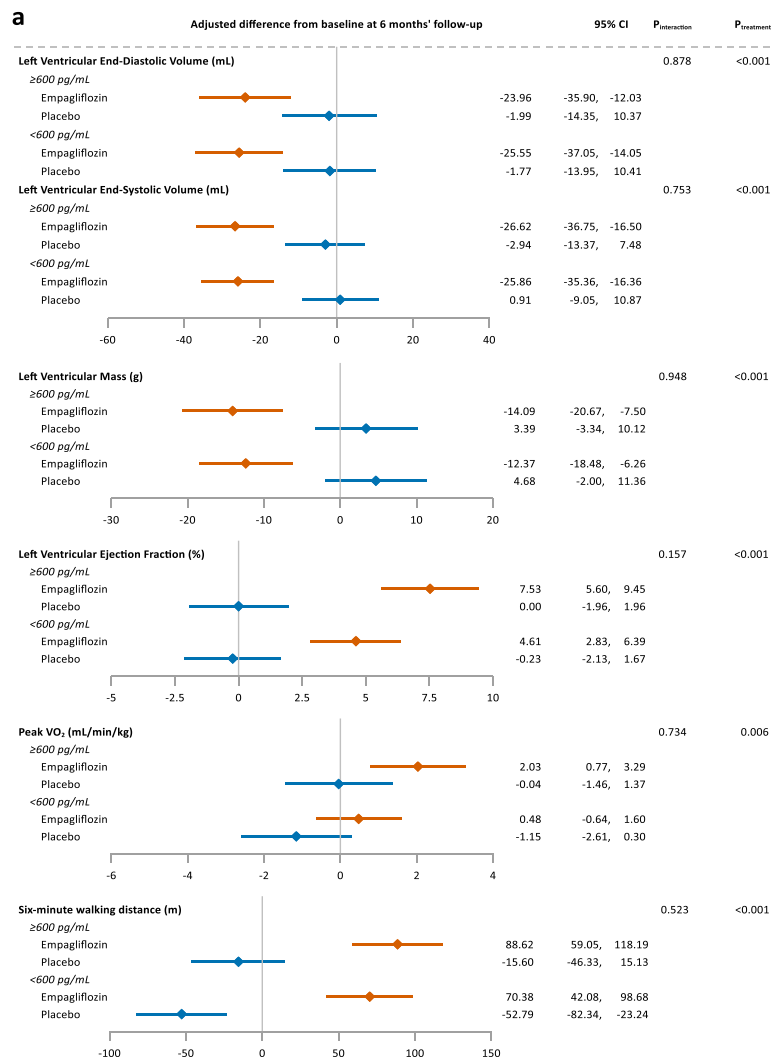
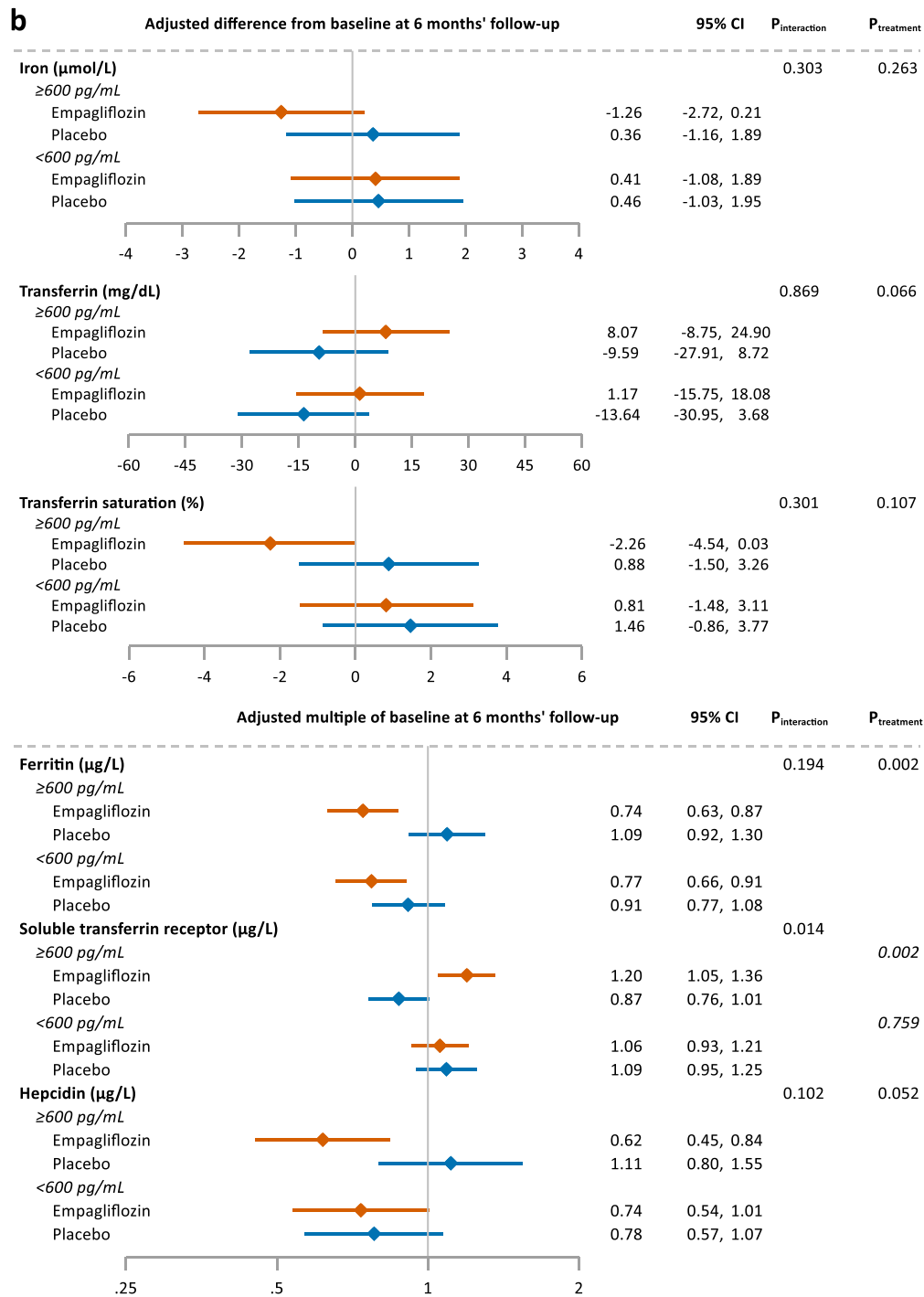


Figure 4. Changes in indices of left ventricular structure and function and exercise capacity (a), laboratory iron markers and hepcidin (b), and erythropoietin, erythroferrone and red blood cell indices (c) after 6 months' treatment with empagliflozin or placebo by norepinephrine level subgroup (≥ 600 vs. < 600 pg/mL) in the EMPATROPISM-FE trial.

Results are reported as subgroup- and treatment-specific adjusted change from baseline values with corresponding 95% confidence interval (CI). P-values for the interaction term ($P_{\text{interaction}}$); $P_{\text{interaction}} > 0.1$: $P_{\text{treatment}}$ -values refer to the effects of empagliflozin vs. placebo in the entire cohort (regular font), $P_{\text{interaction}} \leq 0.1$: $P_{\text{treatment}}$ -values refer to the effects of empagliflozin vs. placebo within subgroup by norepinephrine ($P_{\text{treatment}}$, *italic font*).





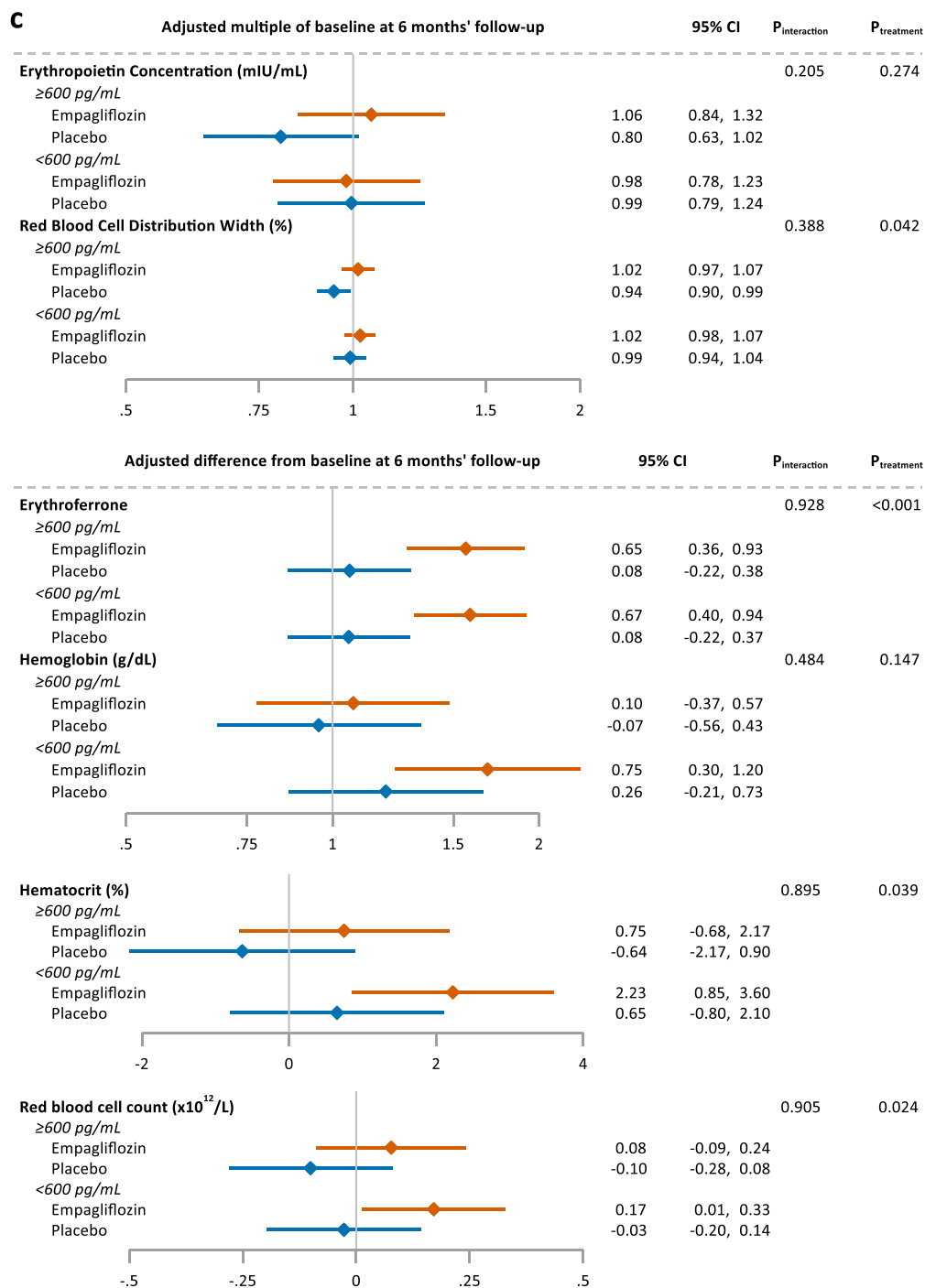
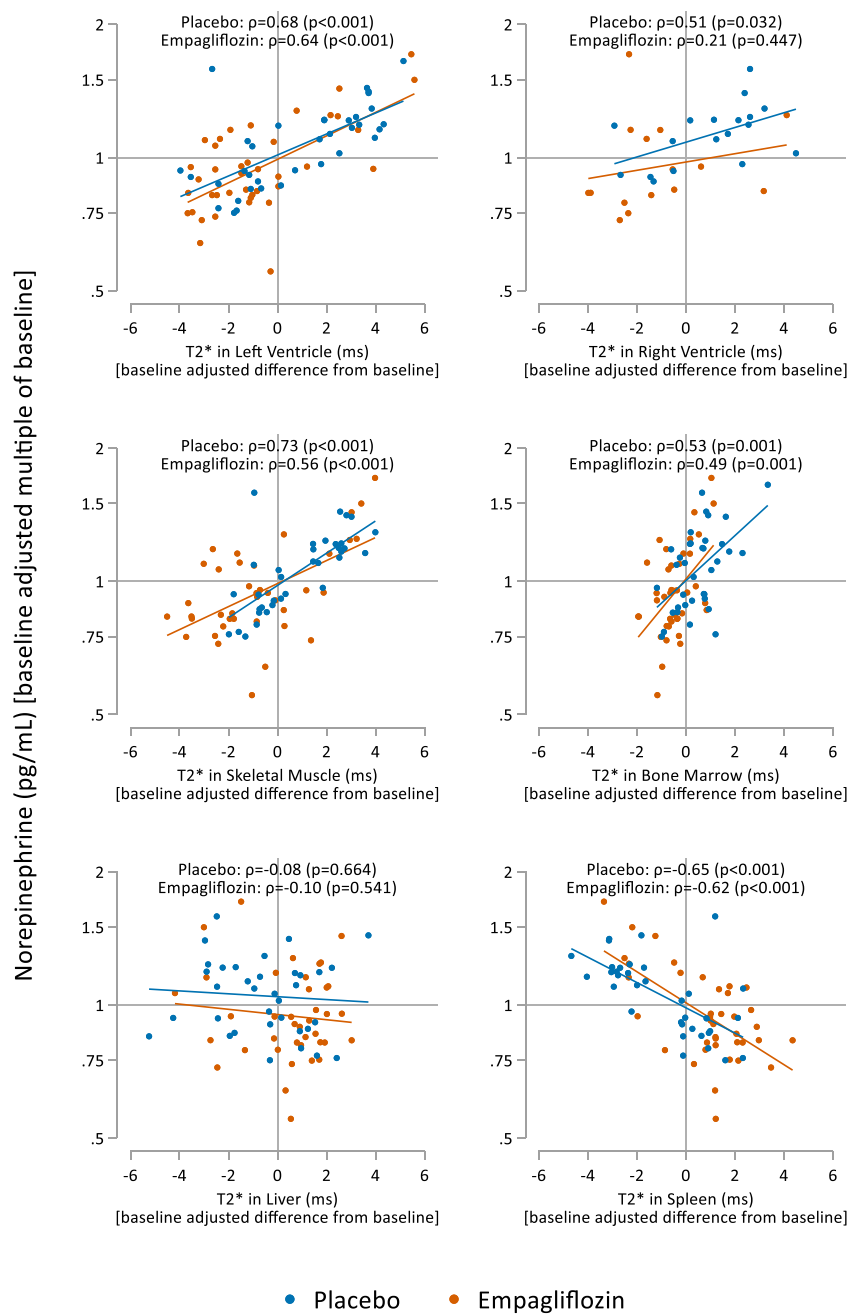


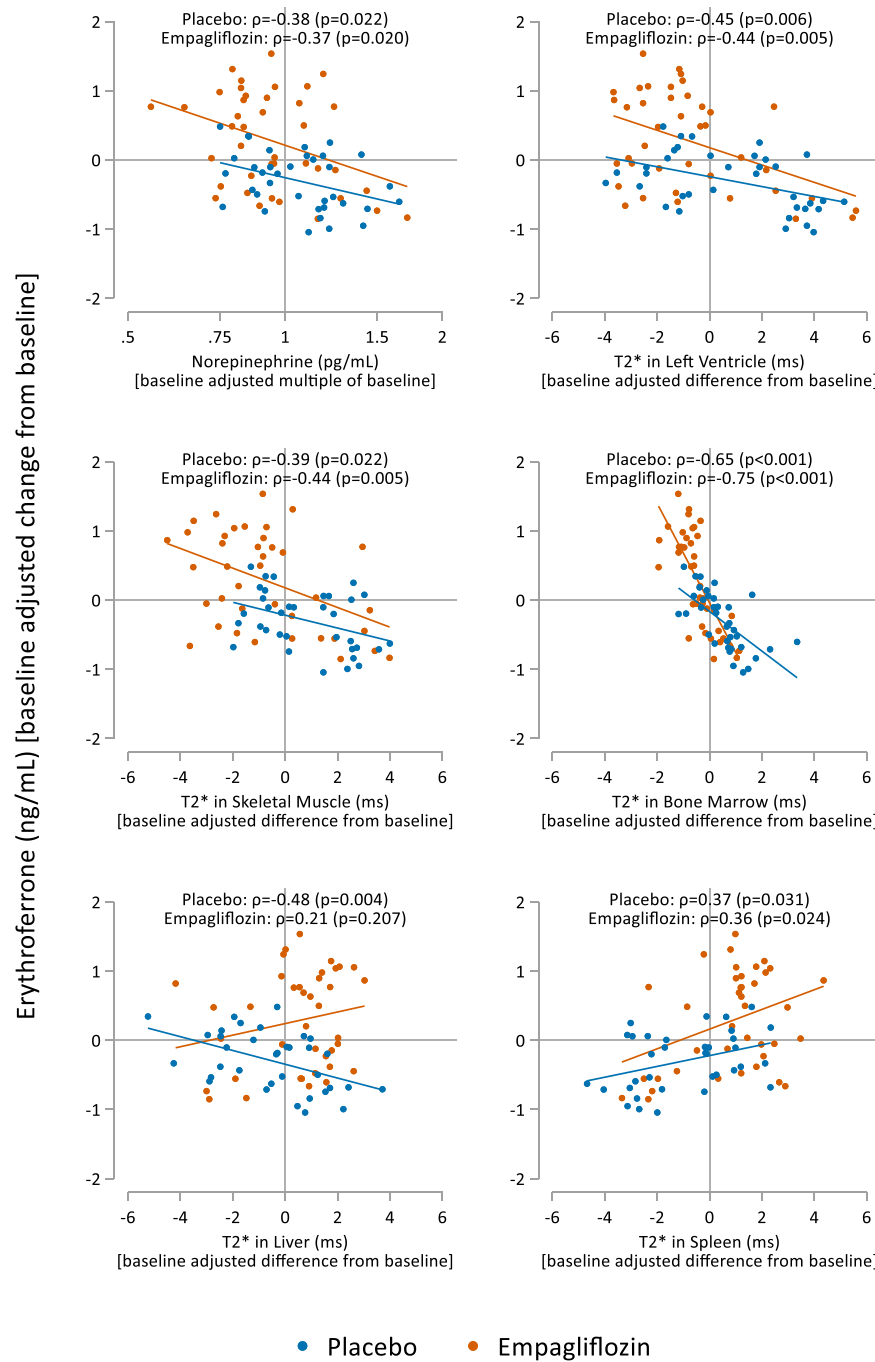
Figure 5. Correlations between baseline-adjusted changes from baseline in norepinephrine levels and T2* (a) and between baseline-adjusted changes in erythroferrone and changes in norepinephrine levels and T2* (b) at the 6-month follow-up visit in the EMPATROPISM-FE trial.

Individual data points are color-coded by treatment (empagliflozin or placebo). Pearson correlations were calculated for each treatment group.

a



b



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