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EDITORIAL

SGLT2 inhibitors in heart failure: insights from plasma proteomics

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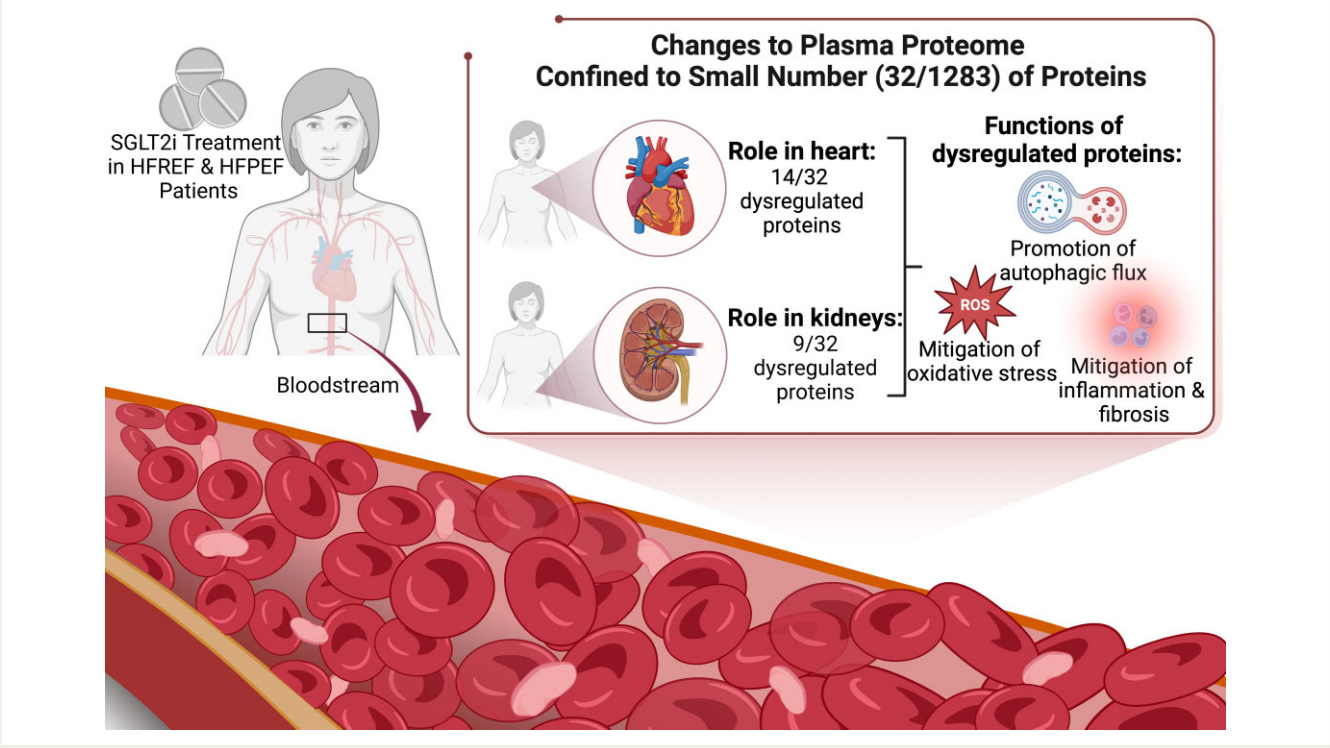
This editorial refers to 'Effect of empaglifozin on circulating proteomics in heart failure: mechanistic insights from the EMPEROR program' by F. Zannad *et al.*, https://doi.org/10.1093/eurheartj/ehac495.

Graphical Abstract



SGLT2 inhibitor treatment increased plasma proteins involved in the promotion of autophagic flux.

Sodium–glucose co-transporter-2 (SGLT2) inhibitors were originally developed to improve glycaemic control in patients with type 2 diabetes. Triggered by concerns of increased risk of ischaemic heart disease conferred by some antidiabetic medications,[1] regulatory authorities mandated large cardiovascular outcome trials for the approval of new diabetes drugs. SGLT2 inhibitors were the first therapeutic class that reduced not only the risk of death in diabetic patients but, unexpectedly, also the risk of hospitalization for heart failure (HF). A similar protective effect was also observed in patients with chronic kidney disease and in HF patients without diabetes. By now, clinical benefits have been demonstrated in trials on HF with reduced ejection fraction (DAPA-HF, EMPEROR-Reduced)[2–4] as well as on HF with preserved ejection fraction (EMPEROR-Preserved, DELIVER).[5,6] Thus, SGLT2 inhibitors seem to emerge as a central pillar

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of HF management across a wider spectrum of left ventricular ejection fraction.[7,8]

SGLT2 inhibitors reduce renal glucose reabsorption by inhibiting SGLT2 in the luminal epithelial membrane of the proximal convoluted tubule, lowering blood glucose in an insulin-independent manner.[9] However, improved glycaemic control is unlikely to be responsible for the cardiovascular benefits conferred by SGLT2 inhibitors. The onset of the protective effects of SGLT2 inhibitors is rapid and detectable within weeks. Protective effects mediated by improved glycaemic control would be expected to occur within years.[9] Notably, the reduction in body weight with SGLT2 inhibitors is sustained. In contrast, loop diuretics do not cause further weight loss beyond the first few days of treatment. Thus, SGLT2 inhibitors may induce a more favourable diuretic profile with a new steady state.[9] Other pleiotropic effects of SGLT2 inhibitors have been reported, including a reduction in inflammation, oxidative stress, fibrosis, and sympathetic nervous system activation, and improved mitochondrial function and myocardial efficiency. Yet, it remains elusive how SGLT2 inhibitors mediate protection in HF.[9] The advent of -omics technologies may contribute towards a more holistic understanding of molecular processes, enabling the identification of pathways that are altered in response to therapy.

In this issue of *the European Heart Journal*, Zannad *et al.*[10] explore the circulating proteome in HF patients with reduced ejection fraction and preserved ejection fraction before and after treatment with empaglifozin (the EMPEROR-Reduced and EMPEROR-Preserved trials, respectively). As participation in biobanking was not mandatory for the trials, not all EMPEROR patients were included. The patients in the proteomics substudy had a lower ejection fraction (40% vs. 45%), and HF patients with reduced ejection fraction and preserved ejection fraction were combined. The present proteomics study comprises a substantial number of patients [600 (16%) of 3730 EMPEROR-Reduced participants and 539 (9%) of 5988 EMPEROR-Preserved participants]. These are >10-fold more patients than the largest previous proteomics study on SGLT2 inhibitors[11]

The authors use a commercial solution for targeted proteomics measurements: the Olink® Explore 1536 platform allowed the quantification of 1283 proteins in this study. Measurements were performed at baseline, and after 12 weeks and 52 weeks of empaglifozin initiation. Differentially regulated proteins compared with placebo were identified. Using a strict false discovery threshold of 1%, only 32 of 1283 (2.5%) proteins were differentially regulated between the placebo group and the empaglifozin group at week 12. Changes from baseline to week 12 were concordant with changes from baseline to week 52. However, the detected plasma protein changes were small (~10–20%). A higher cut-off, i.e. a minimum of a 1.5- or 2-fold change, would not have returned differentially regulated plasma proteins. Thus, the circulating proteome remained remarkably stable after SGLT2 inhibitor treatment.

Based on previous literature, 14 of the 32 proteins (44%) are known to have effects in the heart, with an over-representation for proteins involved in the promotion of autophagic flux, i.e. autophagic degradation activity (five proteins). Autophagy is essential for maintenance of cellular homeostasis by transferring damaged or ageing cytoplasmic material to the lysosome for degradation. In pre-clinical models, an impairment of autophagy leads to cardiomyopathies.[12] Previous experimental studies suggested that the cardioprotective effects of SGLT2 inhibitors may be attributed to improved autophagy.[13] The present study provides further evidence that SGLT2 inhibitors may induce a beneficial state of starvation mimicry with increased nutrient deprivation signalling and autophagic flux. Of course, proteins identified in human plasma, including those with a role in autophagy, exert more than one function in different tissues. Proteins such as transferrin receptor protein 1 and erythropoietin have their main function in iron metabolism and erythropoiesis, respectively, whereas their role in autophagy is considered secondary. Some of the other differentially regulated proteins have been implicated in the reduction of oxidative stress, inhibition of apoptosis, inflammation, and fibrosis, and higher regenerative capacity of the heart. Notably, SGLT2 is not expressed in cardiomyocytes. Thus, the benefits of SGLT2 inhibitors on cardiomyocytes must be indirect. Another major cluster of differentially regulated proteins (9 of 32, i.e. 28%) are known to mediate effects in kidneys, with three of them being involved in tubular sodium transport and two of them inhibiting renal inflammatory and fibrotic processes. Promotion of autophagy was again apparent among the differentially regulated proteins related to the kidneys (three of nine).

The Olink® Explore 1536 platform enables plasma proteome analysis at scale. This targeted proteomics approach includes 1470 protein assays. Unlike aptamer-based proteomics platforms, which tend to rely on single binders, the proximity-extension assays by Olink® require the recognition of a protein by two antibodies. The read-out is based on next-generation sequencing and depends on the accessibility of epitopes that enable these two antibodies to get in close proximity. However, the human genome encodes ~20 000 proteins. Unlike targeted proteomics, untargeted proteomics by mass spectrometry (MS) does not rely on the availability of binders. MS quantifies peptides directly but identifies more abundant plasma proteins, which are underrepresented in the Olink® 1536 platform. Compared with MS, Olink® offers higher throughput and better sensitivity. Thus, the different plasma proteomics approaches can be considered complementary.

Most of the 32 differentially regulated plasma proteins after SGLT2 inhibitor treatment exert their function intracellularly. It is unclear to what extent their plasma levels reflect intracellular protein concentrations. Moreover, plasma proteomic analyses do not reveal the cellular origin of differentially regulated proteins. In fact, it is unlikely that the heart is the main source. An important confounder is the renal filtration of many circulating proteins. While results were also adjusted for estimated glomerular filtration rate (eGFR), the eGFR has notable limitations. Thus, even though adjustment by eGFR did not substantially alter the findings, there could still be residual confounding by improved kidney function upon SGLT2 inhibition. This would be supported by the observation that the overall changes in plasma protein levels were more modest and that nearly all significant proteins were increased at week 12 and week 52 in the empaglifozin group compared with placebo. Only kidney injury molecule-1 was reported to be significantly reduced.

In addition to proteomics, the application of other -omics technologies provides further molecular information. A recent metabolomics study[14] has suggested that SGLT2 inhibitors may improve cardiac energy metabolism by shifting fuel selection towards increased ketone body and free fatty acid consumption. Similar to autophagy, these findings are also indicative of a state of starvation mimicry. In addition to the proteome and the metabolome, the circulating transcriptome, in particular cardiometabolic microRNAs,[15] can contribute insights into biological processes. The quest for new biomarkers is important as the most established protein biomarker for HF, N-terminal probrain natriuretic peptide (NT-proBNP), is not profoundly altered by SLGT2 inhibitors.

In summary, subtle changes were found in the plasma proteome after SGLT2 inhibition. Among the few differentially regulated proteins, proteins related to autophagy appeared over-represented, in line with previous experimental studies implicating autophagy in the cardioprotective

effects of SGLT2 inhibitors. However, further experimental evidence is needed to support this mechanism of protection by SGLT2 inhibitors. Moreover, alternative explanations are plausible, i.e. effects on iron homeostasis and erythropoiesis based on changes in proteins such as transferrin receptor protein 1 and erythropoietin. Future proteomics studies would also benefit from investigating HF patients with reduced and preserved ejection fraction separately. Compared with EMPEROR-Reduced, the beneficial effects of SGLT2 inhibitors in EMPEROR-Preserved were more modest. Thus, the precise mechanisms by which SGLT2 inhibitors improve cardiovascular outcomes in HF remain to be defined.

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Data availability

No new data were generated or analysed in support of this research.

References

1. Pouwels KB, van Grootheest K. The rosiglitazone decision process at FDA and EMA. What should we learn? *Int J Risk Saf Med* 2012;**24**:73–80. https://doi.org/10.3233/JRS-2012-0559
2. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, *et al.* Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med* 2019;**381**:1995–2008. https://doi.org/10.1056/NEJMoa1911303
3. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, *et al.* Cardiovascular and renal outcomes with empagliflozin in heart failure. *N Engl J Med* 2020;**383**:1413–1424. https://doi.org/10.1056/NEJMoa2022190
4. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, *et al.* SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019;**393**:31–39. https://doi.org/10.1016/S0140-6736(18)32590-X
5. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, *et al.* Empagliflozin in heart failure with a preserved ejection fraction. *N Engl J Med* 2021;**385**:1451–1461. https://doi.org/10.1056/NEJMoa2107038
6. Solomon SD, McMurray JJV, Claggett B, de Boer RA, DeMets D, Hernandez AF, *et al.* Dapagliflozin in heart failure with mildly reduced or preserved ejection fraction. *N Engl J Med* 2022;**27**:1089–1098. https://doi.org/10.1056/NEJMoa2206286
7. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, *et al.* 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J* 2021;**42**:3599–3726. https://doi.org/10.1093/eurheartj/ehab368
8. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, *et al.* 2022 AHA/ACC/HFSA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association joint committee on clinical practice guidelines. *Circulation* 2022;**145**:e895–e1032. https://doi.org/10.1161/CIR.0000000000001063
9. Zelniker TA, Braunwald E. Mechanisms of cardiorenal effects of sodium–glucose co-transporter 2 inhibitors: JACC state-of-the-art review. *J Am Coll Cardiol* 2020;**75**:422–434. https://doi.org/10.1016/J.JACC.2019.11.031
10. Zannad F, Ferreira JP, Butler J, Filippatos G, Januzzi JL, Sumin M, *et al.* Effect of empagliflozin on circulating proteomics in heart failure: mechanistic insights from the EMPEROR program. *Eur Heart J* 2022;**43**:ehac495. https://doi.org/10.1093/eurheartj/ehac495
11. Ferrannini E, Murthy AC, Lee YH, Muscelli E, Weiss S, Ostroff RM, *et al.* Mechanisms of sodium–glucose cotransporter 2 inhibition: insights from large-scale proteomics. *Diabetes Care* 2020;**43**:2183–2189. https://doi.org/10.2337/dc20-0456
12. Bravo-San Pedro JM, Kroemer G, Galluzzi L. Autophagy and mitophagy in cardiovascular disease. *Circ Res* 2017;**120**:1812–1824. https://doi.org/10.1161/CIRCRESAHA.117.311082
13. Moellmann J, Mann PA, Kappel BA, Kahles F, Klinkhammer BM, Boor P, *et al.* The sodium–glucose co-transporter-2 inhibitor ertugliflozin modifies the signature of cardiac substrate metabolism and reduces cardiac mTOR signalling, endoplasmic reticulum stress and apoptosis. *Diabetes Obes Metab* 2022;**24**:2263–2272. https://doi.org/10.1111/dom.14814
14. Selvaraj S, Fu Z, Jones P, Kwee LC, Windsor SL, Ilkayeva O, *et al.* Metabolomic profiling of the effects of dapagliflozin in heart failure with reduced ejection fraction: DEFINE-HF. *Circulation* 2022;**146**:808–818. https://doi.org/10.1161/CIRCULATIONAHA.122.060402
15. Gutmann C, Khamina K, Theofilatos K, Diendorfer AB, Burnap SA, Nabeebaccus A, *et al.* Association of cardiometabolic microRNAs with COVID-19 severity and mortality. *Cardiovasc Res* 2022;**118**:461–474. https://doi.org/10.1093/cvr/cvab338