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# Growth differentiation factor-15 and the effect of empagliflozin in heart failure: Findings from the EMPEROR program

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## Aims

Growth differentiation factor-15 (GDF-15) is upregulated in part in response to cardiomyocyte stretch and stress, and it exerts a protective role that is mediated by its action to suppress signalling through insulin-like growth factor (IGF) and enhance signalling through adenosine monophosphate-activated protein kinase (AMPK). Sodium–glucose cotransporter 2 (SGLT2) inhibitors improve outcomes in heart failure, which has been experimentally linked to AMPK. This study aimed at evaluating the associations of GDF-15 with baseline characteristics, the prognostic significance of GDF-15, and the effect of empagliflozin on GDF-15 in patients with heart failure with a reduced and preserved ejection fraction.

## Methods and results

Growth differentiation factor-15 was determined in serum samples from the EMPEROR-Reduced and EMPEROR-Preserved trials. Cox regression and mixed models for repeated measures were used to study the association with outcomes and the effect of empagliflozin on GDF-15, respectively. We studied 1124 patients (560 placebo and 564 empagliflozin) with median GDF-15 levels at baseline of 2442 (interquartile range 1603–3780) pg/ml. Patients with higher GDF-15 levels were typically older men with more severe symptoms, higher N-terminal pro-B-type natriuretic peptide levels, worse kidney function and who were prescribed metformin. Baseline levels of GDF-15 were well correlated with levels of IGF-binding protein 7 ( $\rho = 0.64$ ). Higher levels of GDF-15 were independently associated with an increased risk of cardiovascular death, heart failure hospitalizations, and worse kidney outcomes. When considered as a continuous variable, for each doubling in GDF-15, the adjusted hazard ratio for cardiovascular death or heart failure hospitalization was 1.40 (95% confidence interval 1.15–1.71;  $p < 0.001$ ). The relative effect of empagliflozin on cardiovascular death and hospitalization for heart failure was most pronounced in patients with higher baseline levels of GDF-15 (interaction  $p$ -trend = 0.031). At week 52, when compared with placebo, empagliflozin increased GDF-15 by an additional 8% ( $p = 0.020$ ), an effect that was primarily seen in patients not receiving metformin, a known AMPK activator.

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## Conclusions

Growth differentiation factor-15 is a marker of worse heart failure severity, is an independent predictor of major heart failure outcomes and may be associated with more pronounced benefits of empagliflozin. GDF-15 is increased among metformin users, and empagliflozin was associated with an increase in GDF-15 levels, primarily in patients not receiving metformin.

## Keywords

Growth differentiation factor-15 • Heart failure • Empagliflozin • Metformin

## Introduction

Growth differentiation factor-15 (GDF-15) is a cell activation and stress response cytokine of the glial cell line-derived neurotrophic factor family within the transforming growth factor-beta superfamily of proteins. GDF-15 is systemically activated in pregnancy, cancer and multiple other stress conditions, including heart failure.<sup>1,2</sup> While the presence of the GDF-15 receptor has been found in the brainstem,<sup>3,4</sup> it is uncertain whether GDF-15 and its receptor are present in the human failing heart.<sup>5</sup> Elevated levels of GDF-15 have adverse prognostic significance in patients with heart failure.<sup>6,7</sup> GDF-15 exerts favourable actions on body weight, insulin sensitivity and systemic energy balance.<sup>8</sup>

Growth differentiation factor-15 prolongs cellular survival by two complementary actions. First, GDF-15 suppresses food intake and pro-growth pathways by an action to inhibit signalling through insulin-like growth factor (IGF).<sup>9</sup> Suppression of IGF-1 signalling can exert important cardioprotective effects, particularly in the aging heart.<sup>10</sup> Second, there is a strong interplay between GDF-15 and adenosine monophosphate-activated protein kinase (AMPK) – an important cardioprotective mediator of catabolic pathways required for the generation of ATP – with each acting to reinforce the other.<sup>11</sup> AMPK and IGF-1 exert opposing effects on cardiomyocyte programming and health, and upregulation of GDF-15 shifts the homeostatic balance in favour of AMPK.<sup>12,13</sup>

The interplay of GDF-15 and AMPK appears to underlie the action of metformin to lower body weight and exert its antidiabetic effects.<sup>14–16</sup> Additionally, metformin interacts directly with the promoter region for GDF-15 to stimulate its expression; the interplay between GDF-15 and metformin is such that levels of GDF-15 have been studied as a potential biomarker for the effect of metformin.<sup>17–19</sup> Since metformin is an established activator of AMPK, it is plausible that increased circulating GDF-15 levels might reflect intracellular activation of AMPK.

Sodium–glucose cotransporter 2 (SGLT2) inhibitors reduce the risk of cardiovascular death and hospitalization for heart failure in large-scale trials of patients with type 2 diabetes, chronic kidney disease and heart failure.<sup>20</sup> The cardioprotective effects of these drugs are not mediated through an action on SGLT2 protein, since they are apparent in isolated cardiomyocytes that do not express SGLT2 and in whole animals in whom SGLT2 has been knocked out.<sup>21,22</sup> Instead, SGLT2 inhibitors appear to induce a state of starvation mimicry, which is characterized by upregulation of nutrient deprivation signalling pathways, in particular AMPK, and silencing of AMPK abrogates the favourable effects of SGLT2 inhibitors in cardiomyocytes.<sup>23</sup> Evidence that SGLT2 inhibitors may

activate AMPK in the clinical setting is provided by studies showing that SGLT2 inhibitors increase circulating levels of GDF-15 in patients with type 2 diabetes or with heart failure, especially in those who are not receiving treatment with metformin.<sup>24</sup> However, the number of patients evaluated in these earlier studies was small.

To provide further insights into these relationships, we investigated the associations of GDF-15 before and after treatment with placebo and empagliflozin in patients with chronic heart failure with a reduced or preserved ejection fraction, enrolled in the EMPEROR-Reduced and EMPEROR-Preserved trials, with a particular focus on potential interactions with metformin.

## Methods

### EMPEROR studies population

The EMPEROR-Reduced ( $n = 3730$ ) and EMPEROR-Preserved ( $n = 5988$ ) trials included a total of 9718 patients. The trials enrolled patients with New York Heart Association class II–IV heart failure with elevated circulating levels of natriuretic peptides across a broad range of ejection fractions. The design and results of the EMPEROR trials have been published previously.<sup>25,26</sup> The ethics committees of each of the participating institutions approved the protocol, and all patients gave written informed consent.

Eligible patients were randomized double-blind in a 1:1 fashion to receive placebo or empagliflozin 10 mg daily in addition to their usual therapy for heart failure, and patients were assessed at study visits for major heart failure outcomes. The primary endpoint for both trials was the combined risk of cardiovascular death or hospitalization for heart failure. The principal kidney outcome was the combined risk of a sustained decline in estimated glomerular filtration rate (eGFR) of at least 40%, a sustained decrease in eGFR to  $<10–15$  ml/min/1.73 m<sup>2</sup> or renal replacement therapy.

### Biosampling strategy

Participation in sampling for biobanking of plasma, serum, DNA, and urine was voluntary and not a prerequisite for participation in the trials. Biosamples were collected only after a separate informed consent had been signed, in accordance with local ethical and regulatory requirements. The list of countries that participated in biobanking for biomarker substudy as well as the principles and methodology of sample collection for biobanking in EMPEROR trials are described in the online supplementary *Methods*. Comparison of patient characteristics between those with and without biomarker measurements are displayed in online supplementary *Table S1*.

The measurements of GDF-15 as well as high-sensitivity cardiac troponin T (hs-cTnT), IGF-binding protein 7 (IGFBP-7), cancer antigen

125 (CA-125), angiotensin 2 (ANGPT-2), fatty acid-binding protein-3 (FABP-3) and bone morphogenetic protein 10 (BMP-10) were performed within the Biomarker Research Agreement of Boehringer Ingelheim, the sponsor of the EMPEROR trials and Roche Diagnostics International Ltd. The analysis of the markers listed above was performed using electrochemiluminescence immunoassay (Elecys<sup>®</sup>, Roche Diagnostics) in serum samples from the same patients that had been selected for the plasma proteomics analysis with Olink<sup>®</sup> Explore 1536 platform reported previously.<sup>27</sup> The measurements were performed at baseline ( $n = 1124$ ), week 12 ( $n = 1110$ ) and week 52 ( $n = 709$ ).

## Statistical analyses

The difference in GDF-15 values at baseline between patients from EMPEROR-Preserved and EMPEROR-Reduced was assessed via a *t*-test applied to the log-transformed biomarker values. Patients were grouped into tertiles according to baseline levels of GDF-15, and trends in demographic, clinical and physiological characteristics across these groups were analysed by ordinal regression. The correlation between GDF-15 and the proteins measured by Elecys<sup>®</sup> (IGFBP-7, CA-125, BMP-10, ANGPT-2, and FABP-3) and those in the Olink<sup>®</sup> Explore 1536 platform was assessed by Spearman correlation coefficients. Associations of baseline GDF-15 and major heart failure outcomes were analysed by fitting Cox regression models, adjusted for baseline age, sex, geographical region, diabetes status, left ventricular ejection fraction, eGFR, and trial (EMPEROR-Reduced or EMPEROR-Preserved), and in addition, adjusted for baseline hs-cTnT and N-terminal pro-B type natriuretic peptide (NT-proBNP), the most important predictors of the primary outcome in the EMPEROR studies<sup>28,29</sup> as well as treatment group (empagliflozin or placebo). Total hospitalizations were evaluated by fitting a joint frailty model together with cardiovascular death adjusting for the same covariates. In these models, GDF-15 was used either as a continuous variable, or as a categorical one reflecting the groups defined by its tertiles. Changes in GDF-15 levels over time were analysed using mixed models for repeated measurements (MMRM) comparing the change in plasma concentrations from baseline to week 12 and week 52 between treatment groups (empagliflozin vs. placebo), while adjusting for the prespecified baseline covariates above described as well as baseline GDF-15 levels. Heterogeneity of treatment effect on heart failure outcomes across the groups defined by the tertiles of GDF-15 was investigated by fitting Cox regression models adjusted by the prespecified covariates and including a GDF group by treatment interaction. An MMRM model was used to evaluate the treatment effect on the change of Kansas City Cardiomyopathy Questionnaire clinical summary score (KCCQ-CSS) from baseline, adjusting for the covariates previously described and with the addition of a GDF-15 group by treatment by visit interaction.

## Results

### Baseline characteristics by trial and GDF-15 tertiles

A total of 1124 patients had baseline GDF-15 measurements available, with an overall median (percentile<sub>25–75</sub>) of 2442 (1603–3780) pg/ml. Median GDF-15 levels at baseline were similar in patients from EMPEROR-Reduced and EMPEROR-Preserved (2449 [1574–3951] vs. 2396 [1630–3638] pg/ml,  $p = 0.36$ ). The median

(percentile<sub>25–75</sub>) GDF-15 levels in each of the groups defined by its tertiles were: 1374 (1110–1602) pg/ml (<T1 = 1831.6 pg/ml,  $n = 374$ ), 2442 (2106–2784) pg/ml ( $\geq$ T1 and <T2 = 3202.2 pg/ml,  $n = 375$ ) and 4719 (3777–6449) pg/ml ( $\geq$ T2,  $n = 375$ ).

Compared to patients with the lowest baseline GDF-15 levels (<T1), those with higher GDF-15 levels (>T2) were older, more likely to have worse heart failure symptoms and have been recently hospitalized for heart failure, had higher levels of NT-proBNP and hs-cTnT, and worse kidney function. Patients with higher GDF-15 levels were more likely to be treated with loop diuretics. As GDF-15 levels increased, there was a stepwise increase in the prevalence of type 2 diabetes, the use of metformin and other antidiabetic medications (Table 1).

### Correlation between GDF-15 and proteins assessed on Elecys<sup>®</sup> and Olink<sup>®</sup> platforms

At baseline, the strongest correlations of GDF-15 were seen with IGFBP7, FABP3, tumour necrosis factor receptor superfamily member 10B (TNFR10B), and tumour necrosis factor receptor superfamily member 27 (TNFR27) (Table 2). A correlation analysis between GDF-15 and other circulating proteins, including those assessed with the Olink<sup>®</sup> proteomics, are reported in online supplementary Table S2 and Figure S1. The full list of correlations is provided in the online supplementary Data S1.

### Association of baseline GDF-15 levels and subsequent outcomes

As GDF-15 levels increased across the groups defined by the tertiles, there was a stepwise increase in the combined risk of cardiovascular death or heart failure hospitalization. When considered as a continuous variable, the hazard ratio (HR) was 1.40 (95% confidence interval [CI] 1.15–1.71,  $p < 0.001$ ) for each two-fold increase in GDF-15, adjusted for all prespecified covariates as well as NT-pro BNP and hs-cTnT. The results were similar for each component of the primary endpoint, and the composite kidney outcome (Table 3 and Figure 1).

### Effect of empagliflozin on GDF-15 levels at 12 and 52 weeks

Relative changes of GDF-15 levels from baseline to week 12 were similar in the empagliflozin and placebo groups (ratio of relative change = 1.02, 95% CI 0.98–1.07,  $p = 0.35$ ); however, compared to placebo, empagliflozin showed an 8% higher relative increase in GDF-15 levels from baseline to week 52 (ratio of relative change = 1.08, 95% CI 1.01–1.15,  $p = 0.02$ ) (Figure 2). At 52 weeks, the increase in GDF-15 levels with empagliflozin (vs. placebo) was more pronounced among metformin non-users than among metformin users at week 52 (interaction  $p = 0.06$ ) (online supplementary Table S3). Adjusting for metformin use at baseline did not influence the associations of GDF15 with the primary outcome (online supplementary Table S4).

**Table 1** Patient characteristics across growth differentiation factor-15 tertiles

Characteristics	GDF-15 level at baseline			p-value
	<T1 (n = 374)	≥ T1 and <T2 (n = 375)	≥T2 (n = 375)	
GDF-15 <sup>a</sup> , pg/ml	1374 (1110–1602)	2442 (2106–2784)	4719 (3777–6449)	NA
Age, years	66.5 ± 10.6	70.9 ± 9.4	72.6 ± 8.9	<0.001
Women, n (%)	133 (35.6)	117 (31.2)	110 (29.3)	0.067
Race, n (%)				0.002
White	304 (81.3)	313 (83.5)	330 (88.0)	
Black/African American	11 (2.9)	10 (2.7)	4 (1.1)	
Asian	31 (8.3)	36 (9.6)	29 (7.7)	
Other (including mixed race)	25 (6.7)	13 (3.5)	7 (1.9)	
Region, n (%)				0.18
North America	45 (12.0)	43 (11.5)	67 (17.9)	
Latin America	75 (20.1)	62 (16.5)	79 (21.1)	
Europe	215 (57.5)	226 (60.3)	192 (51.2)	
Asia	27 (7.2)	34 (9.1)	25 (6.7)	
Other	12 (3.2)	10 (2.7)	12 (3.2)	
Ischaemic HF, n (%)	152 (40.6)	160 (42.7)	186 (49.6)	0.013
PCI/CABG, n (%)	126 (33.7)	130 (34.7)	175 (46.7)	<0.001
NYHA class, n (%)				0.001
II	318 (85.0)	304 (81.1)	278 (74.1)	
III	55 (14.7)	69 (18.4)	97 (25.9)	
IV	1 (0.3)	2 (0.5)	0	
Duration of HF, years	4.8 ± 5.5	4.6 ± 4.7	5.7 ± 6.0	0.02
Prior HFH 12 months, n (%)	98 (26.2)	99 (26.4)	113 (30.1)	0.23
Diabetes, n (%)	125 (33.4)	173 (46.1)	245 (65.3)	<0.001
Hypertension, n (%)	293 (78.3)	320 (85.3)	320 (85.3)	0.010
AFib/flutter, n (%)	156 (41.7)	183 (48.8)	208 (55.5)	<0.001
SBP, mmHg	128 ± 16	130 ± 16	126 ± 15	0.015
Heart rate, bpm	70 ± 12	71 ± 12	71 ± 12	0.38
Weight, kg	84.0 ± 18.1	83.5 ± 19.9	82.2 ± 18.3	0.21
BMI, kg/m <sup>2</sup>	29.6 ± 5.4	29.2 ± 5.8	29.2 ± 5.7	0.32
LVEF, %	40 ± 15	41 ± 15	39 ± 15	0.53
LVEF <50%, n (%)	255 (68.2)	252 (67.2)	265 (70.7)	0.46
eGFR, ml/min	72 ± 18	61 ± 19	49 ± 18	<0.001
eGFR <60 ml/min	108 (28.9)	196 (52.3)	272 (72.5)	<0.001
UACR <sup>a</sup> , mg/g	10 (4–33)	18 (7–62)	24 (9–109)	<0.001
Haemoglobin, g/dl	14.0 ± 1.3	13.7 ± 1.5	13.2 ± 1.7	<0.001
NT-proBNP <sup>a</sup> , pg/ml	1050 (612–1705)	1360 (694–2301)	1994 (995–3561)	<0.001
hs-cTnT <sup>a</sup> , ng/L	14 (10–21)	18 (14–27)	28 (19–39)	<0.001
ACEi/ARB/ARNi, n (%)	340 (90.9)	323 (86.1)	314 (83.7)	0.004
Beta-blockers, n (%)	346 (92.5)	346 (92.3)	343 (91.5)	0.59
MRAs, n (%)	217 (58.0)	193 (51.5)	193 (51.5)	0.072
Loop diuretics, n (%)	251 (67.1)	286 (76.3)	314 (83.7)	<0.001
Metformin, n (%)	56 (15.0)	90 (24.0)	130 (34.7)	<0.001
Insulin, n (%)	29 (7.8)	52 (13.9)	77 (20.5)	<0.001
Sulfonylureas, n (%)	15 (4.0)	47 (12.5)	60 (16.0)	<0.001
DPP4 inhibitors, n (%)	9 (2.4)	16 (4.3)	31 (8.3)	<0.001

Median baseline GDF-15 levels were similar between EMPEROR-Reduced and EMPEROR-Preserved: 2449 (1573–3951) vs. 2395 (1630–3638) pg/ml ( $p = 0.36$ ).

ACEi, angiotensin-converting enzyme inhibitor; AFib, atrial fibrillation; ARB, angiotensin II receptor blocker; ARNi, angiotensin receptor–neprilysin inhibitor; BMI, body mass index; CABG, coronary artery bypass grafting; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; HF, heart failure; HFH, heart failure hospitalization; hs-cTnT, high-sensitivity cardiac troponin T; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SBP, systolic blood pressure; T1, first tertile; T2, second tertile; UACR, urinary albumin-to-creatinine ratio.

<sup>a</sup>Median (percentile<sub>25–75</sub>).

**Table 2** Top baseline correlations of serum growth differentiation factor-15 with Olink® plasma proteins and other serum Roche® proteins

Top correlated proteins	Spearman correlation with GDF-15 (Roche®)
Insulin-like growth factor binding protein-7 (log) <sup>a</sup>	0.64
Fatty acid binding protein-3 (log) <sup>a</sup>	0.63
Tumour necrosis factor receptor superfamily member 10B	0.61
Tumour necrosis factor receptor superfamily member 27	0.61

GDF-15, growth differentiation factor-15.

Rho correlation &gt;0.6 presented.

<sup>a</sup>Roche® proteins.

## Effect of empagliflozin on study outcomes according to baseline GDF-15 levels

The effect of empagliflozin (vs. placebo) to reduce the incidence of the primary composite outcome of heart failure hospitalization or cardiovascular death was more pronounced in patients with higher baseline GDF-15 levels (>T1) than in those with lower GDF-15 levels (<T1) (HR 1.30 [95% CI 0.66–2.57] [<T1], HR 0.77 [95% CI 0.45–1.33] [≥T1 and <T2], HR 0.55 [95% CI 0.36–0.84] [≥T2], interaction *p*-trend = 0.031). A directionally concordant but not statistically significant tendency was observed in both the individual components of the primary outcome, and all-cause death (interaction *p*-trend = 0.059 for heart failure hospitalization, 0.52 for cardiovascular death and 0.30 for all-cause death). The effect of empagliflozin to reduce the time to intensification of diuretics and to improve KCCQ-CSS at week 52 was consistent irrespective of baseline concentration of GDF-15 (interaction *p*-trends 0.72 and 0.38, respectively) (Table 4). Baseline use of metformin did not influence the effect of empagliflozin on the primary endpoint in patients with type 2 diabetes in EMPEROR-Reduced (interaction *p* = 0.28), and EMPEROR-Preserved (interaction *p* = 0.96).

## Discussion

In the EMPEROR trials, circulating levels of GDF-15 were particularly increased in patients with the highest prevalence of risk factors at baseline, especially those who were older, had a longer duration of heart failure with more severe symptoms, higher levels of natriuretic peptides and worse kidney function. Therefore, it was not surprising that circulating levels of GDF-15 had important prognostic significance. For example, when GDF-15 was considered as a continuous variable (per doubling in concentration), the risk of cardiovascular death or hospitalization for heart failure increased by 1.4-fold, the risk of sustained kidney function deterioration increased by 2-fold, and the risk of death increased by

**Table 3** Outcome associations of growth differentiation factor-15

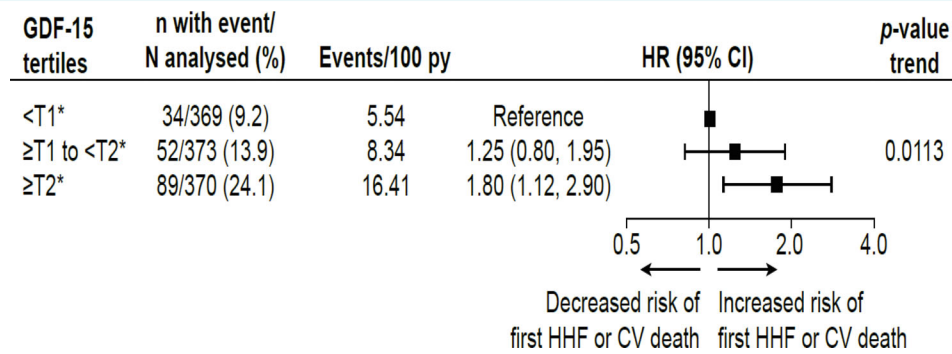
Outcome by GDF-15 level at baseline	<T1 (n = 374), n (%) events IR per 100py	≥T1 and <T2 (n = 375), n (%) events IR per 100py	≥T2 (n = 375), n (%) events IR per 100py	HR (95% CI) <i>p</i> -value T2 vs. T1	HR (95% CI) <i>p</i> -value for a two-fold increase
CV death or HFH	34 (9.2) IR = 5.5	52 (13.9) IR = 8.3	89 (24.1) IR = 16.4	1.25 (0.80–1.95) <i>p</i> = 0.32	1.40 (1.15–1.71) <i>p</i> < 0.001
HFH	21 (5.7) IR = 3.4	38 (10.2) IR = 6.1	68 (18.4) IR = 12.5	1.38 (0.80–2.39) <i>p</i> = 0.25	1.31 (1.04–1.66) <i>p</i> = 0.022
Total HFH <sup>a</sup>	33 IR = 5.3	60 IR = 9.1	120 IR = 19.9	1.13 (0.62–2.04) <i>p</i> = 0.70	1.36 (1.05–1.76) <i>p</i> = 0.019
CV death	18 (4.9) IR = 2.9	21 (5.6) IR = 3.2	42 (11.4) IR = 7.0	1.00 (0.52–1.92) <i>p</i> = 0.99	1.56 (1.16–2.11) 0.004
All-cause death	24 (6.5) IR = 3.8	33 (8.8) IR = 5.0	61 (16.5) IR = 10.1	1.11 (0.64–1.91) <i>p</i> = 0.72	1.69 (1.32–2.17) <i>p</i> < 0.001
Kidney endpoint <sup>b</sup>	2 (0.5) IR = 0.4	13 (3.5) IR = 2.4	24 (6.5) IR = 5.0	6.5 (1.43–29.2) <i>p</i> = 0.015	2.04 (1.35–3.09) <i>p</i> < 0.001

CI, confidence interval; CV, cardiovascular; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; HFH, heart failure hospitalization; HR, hazard ratio; hs-cTnT, high-sensitivity cardiac troponin T; IR, incidence rate; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PY, patient-years.

Models adjusted for age, sex, eGFR, LVEF, region, diabetes, treatment (empagliflozin or placebo), NT-proBNP, hs-cTnT, and study (EMPEROR-Preserved or EMPEROR-Reduced).

<sup>a</sup>Joint frailty model.

<sup>b</sup>eGFR reduction ≥40% or end-stage renal disease.



**Figure 1** Time to heart failure HF hospitalization or cardiovascular death by growth differentiation factor-15 (GDF-15) levels at baseline. Summary of estimates from a Cox regression model for time to hospitalization for heart failure (HHF) or cardiovascular (CV) death by tertiles of GDF-15 at baseline.

1.7-fold, after correction for other prognostic variables, including the level of natriuretic peptides and cardiac troponin. Our findings are remarkably similar to those in previous studies.<sup>6,30–32</sup> Prior reports in patients with heart failure have also proposed potential relationships of GDF-15 with characteristics reflecting disease severity (e.g. congestion, inflammation, or iron deficiency), without influence of ejection fraction on GDF-15 levels.<sup>33,34</sup> Therefore, it seems likely that GDF-15 is a general marker of disease severity rather than an indicator of any specific clinical feature, heart failure phenotype, or underlying disease mechanism.

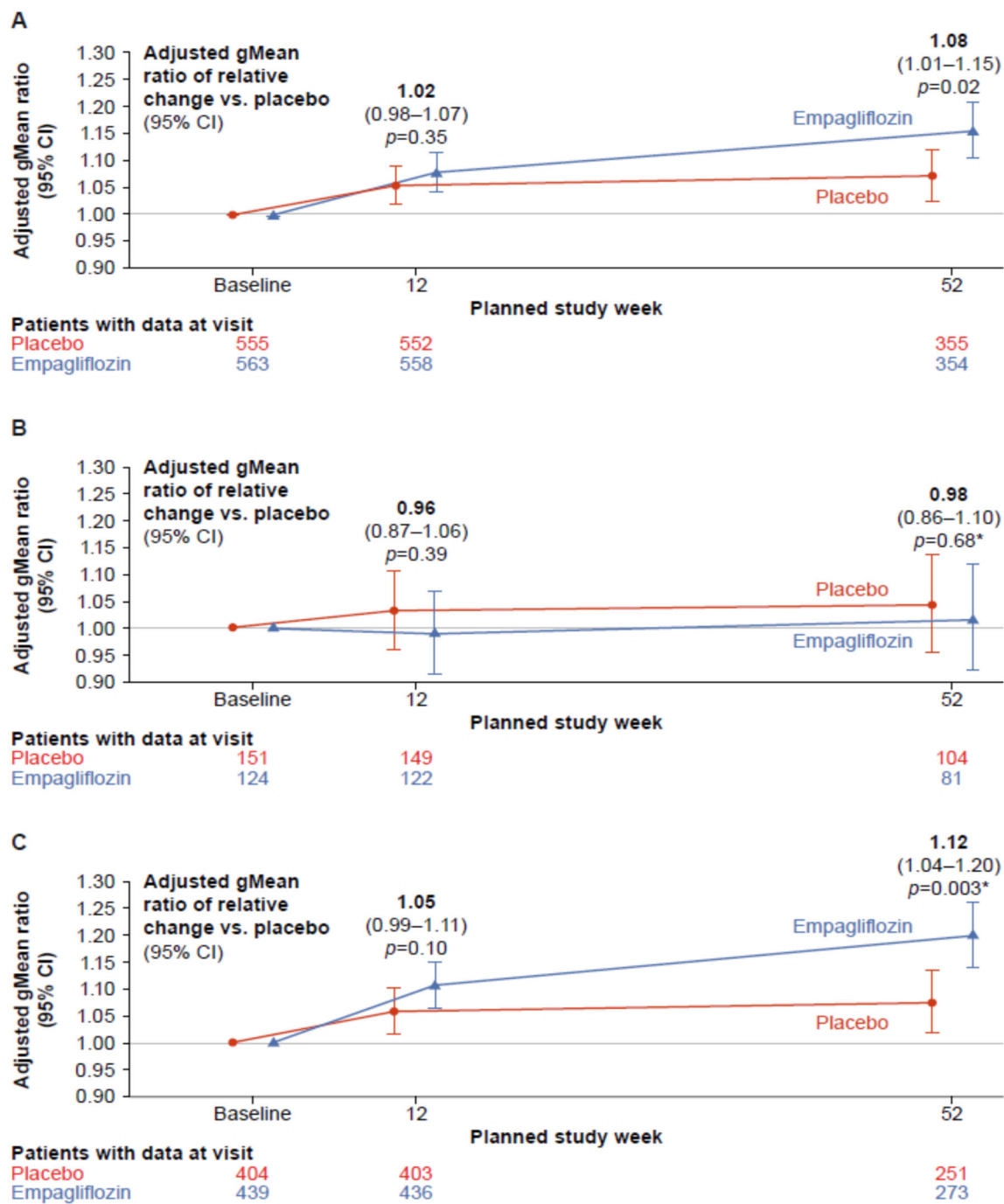
Growth differentiation factor-15 acts primarily through a brain-stem receptor, where it regulates appetite and body weight, as well as its metabolic effects (e.g. lipolysis and fatty acid oxidation).<sup>35,36</sup> When these actions are considered collectively, GDF-15 may be conceptualized as a means of reducing the energy requirements of the organism. It is therefore noteworthy that we observed a linear relationship between GDF-15 and IGF-binding proteins, which function to inhibit the interaction of IGF with its receptors.<sup>10</sup> We also found a correlation between circulating GDF-15 levels and FABP3, a fatty acid transport protein that is upregulated during the stimulation of fatty acid oxidation.<sup>37</sup> Finally, we found significant associations between circulating GDF-15 levels and circulating levels of tumor necrosis factor superfamily members. The failing heart produces marked quantities of tumor necrosis factor, which (like GDF-15) has been implicated in the pathogenesis of the weight loss and muscle wasting seen in severe heart failure.<sup>38</sup>

Our findings confirm those of previous reports that identified elevated GDF-15 levels among metformin users.<sup>18,19,24</sup> Metformin interacts directly with the promoter that regulates GDF-15 expression, and thus, GDF-15 has been proposed as a biomarker of metformin use.<sup>17</sup> This relationship is noteworthy, since metformin acts (in part) as an activator of AMPK.<sup>25</sup> Like GDF-15, AMPK exerts cardioprotective effects and acts to promote catabolism (including lipolysis and fatty acid oxidation).<sup>26–28</sup> AMPK and GDF-15 act to reinforce each other as part of a positive feedback loop,<sup>11,15</sup> and GDF-15 appears to mediate the effects of metformin on body weight and energy balance.<sup>14</sup>

It has been shown that the cardioprotective effects of SGLT2 inhibitors in isolated cardiomyocytes are dependent on the activation of AMPK, and the favourable actions of SGLT2 inhibition on the heart are abrogated when AMPK is silenced.<sup>22</sup> These observations may explain why the effects of empagliflozin appeared to be most marked in patients with the higher levels of GDF-15, in whom AMPK signalling is preserved and upregulated. The action of SGLT2 inhibitors to stimulate AMPK can also explain the increase in circulating levels of GDF-15 in patients receiving empagliflozin, which was not observed in patients who were also receiving metformin. The 8% increase that we observed in the EMPEROR programme at 52 weeks was similar to the 9% increase that was observed in the Empire-HF trial at 12 weeks<sup>24</sup>; an effect that was observed primarily among patients not receiving metformin who experienced a relative increase in GDF-15 of 12% in both studies, although at different time points. These observations are paralleled by findings from the EMPA-REG OUTCOME trial that the effect of empagliflozin to reduce serious adverse kidney outcomes appeared to be attenuated in patients receiving metformin.<sup>29</sup> Although the CANVAS investigators reported no effect of canagliflozin on GDF-15 in patients with type 2 diabetes at 1 year and lower levels of GDF-15 at years 3 and 6, most patients in that report were receiving metformin.<sup>39</sup> Other studies in patients with type 2 diabetes noted an increase in GDF-15 with empagliflozin, albeit with no metformin interaction.<sup>26</sup> As the increase in GDF-15 with SGLT2 inhibition might reflect upregulation of AMPK signalling, it is noteworthy that increases in GDF-15 have not been seen with sacubitril/valsartan, which signals through natriuretic peptides.<sup>40</sup>

## Limitations

The findings of this paper represent a post-hoc exploratory analysis and should be interpreted with caution. Attenuation of the effect of empagliflozin on GDF-15 levels in metformin users was based on relatively small numbers of patients in the current study as in an earlier report,<sup>38</sup> and due to our limited sampling, our findings cannot define the time course of the effect of empagliflozin on GDF-15. Correlation analysis between conventional ELISA



**Figure 2** Relative changes (empagliflozin vs. placebo) in the circulating levels of growth differentiation factor-15 at week 12 and week 52, overall and by metformin use. (A) All patients. (B) For patients using metformin at baseline. (C) For patients not using metformin at baseline. CI, confidence interval. \*Interaction  $p$ -value for treatment-by-metformin use at baseline, for week 12 = 0.12; for week 52 = 0.06.

assay (Elecys<sup>®</sup>, Roche Diagnostics) and antibody-based proximity extension assay (Olink<sup>®</sup> Explore 1536) involved different protein quantification and detection range between the two methods. In the analysis of the effect of empagliflozin across GDF-15 tertiles, no adjustments were performed for test multiplicity, and subgroup

findings were often based on few events and may be subject to the play of chance, requiring further replication. Finally, GDF-15 was measured in a subsample of 1124 patients of the EMPEROR studies, and, despite random selection, sampling errors might have occurred potentially limiting the generalizability of these results.



**Table 4** Effect of empagliflozin across growth differentiation factor-15 levels at baseline

Outcome by GDF-15 levels	Placebo <sup>a</sup>	Empagliflozin <sup>a</sup>	HR or difference (95% CI)	Interaction p-trend
Cardiovascular death or HFH				0.031
<T1	15/187 (8.0)	19/187 (10.2)	1.30 (0.66–2.57)	
≥T1 and <T2	30/192 (15.6)	23/183 (12.6)	0.77 (0.45–1.33)	
≥T2	53/181 (29.3)	38/194 (19.6)	0.55 (0.36–0.84)	
HFH				0.059
<T1	8/187 (4.3)	13/187 (7.0)	1.66 (0.69–4.02)	
≥T1 and <T2	22/192 (11.5)	17/183 (9.3)	0.76 (0.40–1.44)	
≥T2	38/181 (21.0)	31/194 (16.0)	0.60 (0.37–0.98)	
Total HFH				0.23
<T1	13/187	20/187	1.63 (0.68–3.92)	
≥T1 and <T2	33/192	29/183	0.98 (0.48–1.98)	
≥T2	70/181	52/194	0.64 (0.35–1.19)	
Intensification of diuretics				0.72
<T1	27/187 (14.4)	22/187 (11.8)	0.74 (0.42–1.30)	
≥T1 and <T2	36/192 (18.8)	24/183 (13.1)	0.64 (0.38–1.07)	
≥T2	45/181 (24.9)	37/194 (19.1)	0.64 (0.41–1.00)	
KCCQ-CSS change from baseline to week 52				0.38
<T1	1.79 (−0.54–4.13)	3.77 (1.45–6.10)	1.98 (−1.3–5.27)	
≥T1 and <T2	1.48 (−0.78–3.74)	4.72 (2.42–7.02)	3.24 (0.01–6.46)	
≥T2	2.86 (0.41–5.32)	6.94 (4.63–9.25)	4.07 (0.72–7.43)	
Cardiovascular death				0.52
<T1	9/187 (4.8)	9/187 (4.8)	1.03 (0.41–2.61)	
≥T1 and <T2	13/192 (6.8)	8/183 (4.4)	0.66 (0.27–1.60)	
≥T2	25/181 (13.8)	19/194 (9.8)	0.70 (0.38–1.28)	
All-cause death				0.30
<T1	12/187 (6.4)	12/187 (6.4)	1.02 (0.46–2.28)	
≥T1 and <T2	18/192 (9.4)	15/183 (8.2)	0.91 (0.46–1.81)	
≥T2	37/181 (20.4)	27/194 (13.9)	0.66 (0.40–1.09)	
Renal endpoint <sup>b</sup>				–
<T1	0	2/187 (1.1)	NC	
≥T1 and <T2	11/192 (5.7)	2/183 (1.1)	NC	
≥T2	8/181 (4.4)	16/194 (8.2)	NC	

CI, confidence interval; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; HFH, heart failure hospitalization; HR, hazard ratio; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire clinical summary score; NC, not comparable.

<sup>a</sup>Reported as n/N (%) for all endpoints except KCCQ-CSS change from baseline to week 52 where adjusted mean (95% CI) is reported.

<sup>b</sup>eGFR reduction ≥40% or end-stage renal disease. Two subgroups with <14 events, no model was produced.

## Conclusions

High circulating levels of GDF-15 are a marker of heart failure severity, are an independent predictor of major adverse heart failure and kidney outcomes and may be associated with more pronounced cardiovascular benefits of empagliflozin. SGLT2 inhibition was associated with an increase in GDF-15 levels, primarily in patients not receiving metformin. These findings should be considered hypothesis-generating and require evaluation in further studies.

## Supplementary Information

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

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