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The effect of sodium glucose cotransporter 2 inhibition with empagliflozin on microalbuminuria and macroalbuminuria in patients with type 2 diabetes

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ABSTRACT

Aims/hypothesis Sodium glucose cotransporter 2 (SGLT2) inhibition lowers HbA_{1c}, systolic blood pressure and body weight in patients with type 2 diabetes and reduces renal hyperfiltration associated with type 1 diabetes, suggesting decreased intraglomerular hypertension. Since lowering HbA_{1c}, systolic blood pressure, weight and intraglomerular pressure is associated with anti-albuminuric effects in diabetes, we hypothesized that SGLT2 inhibition would reduce urine albumin to creatinine ratio (UACR) to a clinical meaningful extent.

Methods We examined the effect of the SGLT2 inhibitor empagliflozin on UACR by pooling data from patients with type 2 diabetes and prevalent microalbuminuria (UACR 30–300 mg/g; *n*=636) or macroalbuminuria (UACR >300 mg/g; *n*=215) who participated in 5 Phase III, randomised clinical trials. Primary assessment was defined as percentage change in geometric mean UACR from baseline to week 24.

Results After controlling for clinical confounders including baseline log-transformed UACR, HbA_{1c}, systolic blood pressure, and estimated glomerular filtration rate (according to Modification of Diet in Renal Disease formula), treatment with empagliflozin significantly reduced UACR in patients with microalbuminuria (-32% vs. placebo; *P*<0.001) or macroalbuminuria (-41% vs. placebo; *P*<0.001). Intriguingly, in regression models, the majority of UACR-lowering effect with empagliflozin was not explained by SGLT2 inhibition-related improvements in HbA_{1c}, systolic blood pressure or body weight.

Conclusions/interpretation In patients with type 2 diabetes and either micro- or macroalbuminuria, empagliflozin reduced UACR by clinically meaningful amounts. This

effect was largely independent of known metabolic or systemic haemodynamic effects of this drug class. Our results further support a direct renal effect of SGLT2 inhibitors. Prospective studies are needed to explore the potential of this intervention to alter the course of kidney disease in high-risk patients with diabetes.

Trial registration Clinicaltrials.gov NCT01177813 (Study 1);
NCT01159600 (Study 2); NCT01159600 (Study 3); NCT01210001 (Study 4);
NCT01164501 (Study 5).

Keywords: Empagliflozin, Macroalbuminuria, Microalbuminuria, Sodium glucose cotransporter 2, Type 2 diabetes, Urine albumin to creatinine ratio

Abbreviations

ACE	Angiotensin-converting enzyme
AE	Adverse event
ANCOVA	Analysis of covariance
CKD	Chronic kidney disease
DBP	Diastolic blood pressure
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
FAS	Full analysis set
IR	Immediate-release
LOCF	Last observation carried forward
MDRD	Modification of diet in renal disease formula

MTD	Maximum tolerated dose
RAAS	Renin angiotensin aldosterone system
SBP	Systolic blood pressure
SGLT2	Sodium glucose cotransporter 2
TS	Treated set
UACR	Urine albumin-to-creatinine ratio

Introduction

Diabetes is the leading cause of end-stage renal disease (ESRD) in the US, and accounts for >40% of patients requiring renal replacement therapy [1]. Current clinical indicators of underlying diabetic kidney disease and progression of nephropathy are the presence and worsening degree of albuminuria and/or progressive renal function decline. Although not an ideal biomarker of renal risk, the presence of microalbuminuria has been linked to an increased risk for both cardiovascular events and progression of kidney disease in patients with type 2 diabetes. Macroalbuminuria is more consistently associated with an elevated risk of both renal and cardiovascular complications [2, 3]. Therefore, regular assessment of albuminuria remains a clinical cornerstone to diagnose the onset of kidney disease in diabetes and to prospectively track progression of renal injury.

The current standard renal protective therapies in diabetes involve glycaemic and blood pressure control, and blockade of the renin angiotensin aldosterone system (RAAS) [4]. Therapeutic options for patients with persistent albuminuria in the setting of satisfactory A_{1c} and blood pressure control are limited to changes in lifestyle such as sodium restriction and weight loss, which have limited evidence and are often difficult to achieve [5, 6].

Empagliflozin is a highly-selective sodium glucose cotransporter 2 (SGLT2) inhibitor. In patients with type 2 diabetes, empagliflozin is consistently associated with declines in HbA_{1c}, systolic blood pressure (SBP) and body weight [7–10]. Empagliflozin has been shown to decrease renal hyperfiltration in patients with type 1 diabetes [11]. This renal physiological characteristic has been reported to be linked to the

development of nephropathy in type 1 and type 2 diabetes [12–14]. Furthermore, SGLT2 inhibition may improve this early glomerular haemodynamic abnormality through inhibition of sodium-glucose reabsorption at the proximal tubule [12–14]. Such a natriuretic effect increases sodium delivery to the distal tubule, thereby stimulating tubuloglomerular feedback, ultimately causing afferent renal arteriolar vasoconstriction and a reduction in intraglomerular pressure [11, 12]. Based on available data in healthy humans and in patients with type 1 diabetes, renal haemodynamic effects of SGLT2 inhibition appear to be RAAS-independent, as plasma and urinary levels of RAAS mediators increase modestly in response to the intervention, rather than decline [11, 13, 14]. The renal protective effect of empagliflozin, including effects on established clinical renal biomarkers such as albuminuria, remains to be systematically studied.

We therefore examined the effect of 24 weeks' treatment with empagliflozin on urine albumin-to-creatinine ratio (UACR) in patients with type 2 diabetes and either microalbuminuria (UACR 30–300 mg/g) or macroalbuminuria (UACR >300 mg/g) who participated in one of five, Phase III, randomised, placebo-controlled clinical trials. We hypothesized that the addition of empagliflozin to ongoing background of glucose- and blood pressure lowering therapy would reduce UACR compared with placebo.

Methods

Study population

This pooled analysis comprised patients with type 2 diabetes who participated in one of five Phase III clinical trials of empagliflozin, whose primary endpoints were related to glucose-lowering. Study designs and entry criteria for all five trials have been published

[7–10, 15] (see ESM Methods). In four studies, patients with eGFR We had were randomised to receive empagliflozin 10 mg, empagliflozin 25 mg or placebo once daily for 24 weeks as monotherapy or add-on therapy to background glucose-lowering medications. The fifth study included individuals with type 2 diabetes and chronic kidney disease (CKD) (EMPA-REG RENAL™) in which patients with CKD stage 2 received empagliflozin 10 mg, empagliflozin 25 mg or placebo, and patients with CKD stage 3 or stage 4 received empagliflozin 25 mg or placebo once daily for 52 weeks as add-on therapy [15]. UACR at 24 weeks was used in this analysis to remove the potential confounder of treatment length across trials.

Urine samples were taken at screening, the start of placebo run-in, baseline, week 12 and week 24. Urinary albumin and creatinine concentrations at respective time points were measured from a single, standardized spot urine sample and UACR was calculated at the central laboratory. UACR was therefore measured once per visit in a morning sample, in the fasted state and before intake of trial medication. In the total pooled population (*n*=3215), microalbuminuria (UACR 30–300 mg/g) was present in 636 (19.8%) individuals at baseline (248 patients who received placebo, 159 patients who received empagliflozin 10 mg and 229 patients who received empagliflozin 25 mg). In addition, macroalbuminuria (UACR >300 mg/g) was identified in 215 (6.7%) individuals at baseline (87 patients on placebo, 36 patients on empagliflozin 10 mg and 92 patients on empagliflozin 25 mg).

Endpoints

The primary assessment of this pooled analysis was defined as percentage change in geometric mean of the UACR from baseline to week 24 as described previously for other drug interventions targeting albuminuria [16, 17]. Empagliflozin 10 and 25 mg doses were pooled to define the overall magnitude of the drug effect compared to placebo.

Other efficacy endpoints were defined as changes in HbA_{1c}, body weight, and SBP from baseline to week 24. Selected safety endpoints were changes in eGFR (MDRD) over 24 weeks and overall adverse events (AEs), including the incidence of volume depletion related AEs and electrolyte imbalances, such as hyperkalaemia.

Statistical methods

The treated set (TS) comprised the pooled population of all randomised patients from the 5 studies who received at least one dose of study drug and were identified with prevalent albuminuria at baseline. All efficacy analyses were performed on the full analysis set (FAS) which included all patients from the TS who had a documented baseline HbA_{1c} and UACR value. Any efficacy data collected following initiation of hyperglycaemic rescue therapy were set to missing. Missing data were imputed using the last observation carried forward (LOCF) approach.

For all efficacy analyses, UACR data were initially log10-transformed due to their left-skewed distribution. Statistical procedures were conducted separately for patients with either micro- or macroalbuminuria at baseline. Changes from baseline to week 24 in UACR were analysed by analysis of covariance (ANCOVA) and adjusted for log10-transformed UACR at baseline, alongside baseline values for HbA_{1c}, eGFR (MDRD),

SBP, region, treatment and trial effect. The values were then back-transformed to obtain geometric means of the UACR ratios for depiction of 24-week treatment effects (with values expressed as percentage change in adjusted geometric mean of the UACR ratios). Further multivariable models were employed to explore the potential impact of concomitant treatment changes in HbA_{1c}, body weight, SBP, diastolic blood pressure (DBP) or pulse pressure (PP) and the combined effect of changes in HbA_{1c}, body weight, and SBP on UACR effects at week 24. We utilized individual ANCOVA models with baseline HbA_{1c}, SBP, log-transformed UACR, the baseline of the variable of interest, and the change from baseline in the variable or variables of interest as linear covariates in addition to the following fixed effects: treatment, baseline eGFR (MDRD), region and trial.

Changes in HbA_{1c}, body weight, and SBP at week 24 were evaluated on the FAS using an ANCOVA. Here, baseline HbA_{1c} and the respective baseline value of the endpoint in question were used as linear covariates. For all analyses baseline eGFR (MDRD), region, trial and treatment were included as fixed effects. Analyses for change in eGFR (MDRD) over 24 weeks and frequencies of AEs were descriptive and based on the TS.

Results

Study population and baseline characteristics

Baseline characteristics are shown in Table 1. As expected, subjects with macroalbuminuria tended to have longer diabetes duration, higher baseline SBP, and lower eGFR (MDRD) compared to those with microalbuminuria. In microalbuminuric

patients, between 50-60% of patients were taking RAAS inhibitors at baseline. Macroalbuminuric patients tended to use more anti-hypertensive agents including RAAS blockers, and to take lipid-lowering and anti-platelet agents more frequently. Within the microalbuminuric and macroalbuminuric groups, patients allocated to placebo or empagliflozin were overall well balanced in terms of demographic factors, background anti-hypertensive therapies, metabolic parameters, blood pressure and level of albuminuria. Baseline eGFR was not significantly different between empagliflozin and placebo groups in patients with microalbuminuria or macroalbuminuria at baseline (p=0.475 and p=0.450, respectively). In the total study cohort, the proportion of patients with a medical history of diabetic retinopathy was 27.4% in the trial involving patients with CKD, and ranged from 1.4% to 10.2% in the other 4 studies.

Effects of empagliflozin on HbA_{1c}, body weight and blood pressure and eGFR

As expected, empagliflozin was associated with significant placebo-corrected declines in HbA_{1c} (electronic supplementary material [ESM] Figure 1a–b), body weight (ESM Figure 1c–d), SBP (ESM Figure 1e–f) and DBP diastolic blood pressure (ESM Figure 1g–h) in both groups of patients with either micro- or macroalbuminuria.

UACR effects of SGLT2 inhibition with empagliflozin

After 24 weeks of treatment with empagliflozin, UACR values significantly decreased in the microalbuminuria group (-32% [-41 to -22%] as compared to placebo; *P*<0.001, Figure 1a) and the macroalbuminuria group (-41% [-57 to -19%] as compared to placebo; p<0.001, Figure 1b). A pronounced effect of empagliflozin on UACR in both

albuminuria groups was observed with the first 12 weeks of treatment, and effects were maintained for the remainder of the 24-week treatment period (ESM Figure 2). The estimated effects were almost identical in a sensitivity analysis including baseline DBP, weight, gender, BMI, ethnicity, weight, and baseline RAAS use (data not shown). Replacing DBP by pulse pressure (PP) as a surrogate marker of arterial stiffness in this enriched model also resulted in the same effect (data not shown).

To also explore potential differential effects on UACR between the two empagliflozin doses, additional sensitivity analyses of the primary endpoint were conducted for the pool of studies 1 to 4. In patients with microalbuminuria, similar effects of empagliflozin 10 mg (*n*=141) and empagliflozin 25 mg (*n*=150) were observed after 24 weeks (adjusted mean difference: −0.04, [−0.13, 0.05]; *P*=0.431). The observation of no significant dose related difference on albuminuria was further confirmed in patients with macroalbuminuria (adjusted mean difference in UACR between empagliflozin 10 mg (*n*=27) and 25 mg (*n*=19): (adjusted mean difference: −0.36 [−0.78, 0.05]; *P*=0.084).

Contribution of changes in HbA_{1c}, body weight and blood pressure on UACR effects with empagliflozin

To further decipher the potential mechanisms underlying the observed decline in micro- and macroalbuminuria with empagliflozin at week 24, we employed a comprehensive ANCOVA model. This model accounted for changes in either HbA_{1c}, body weight, SBP and DBP from baseline to week 24 and thus adjusted for those variables that are known to be altered by SGLT2 inhibition. This is important because

the selected variables are generally also known to be associated with albuminuria in patients with type 2 diabetes.[1] This model showed that overall improvements in microalbuminuria with empagliflozin (-32%) were not significantly influenced by concomitant changes in HbA_{1c} (-2%) (Figure 2a and ESM Figure 3a). In contrast, in separate models the influence of body weight (-6%), as well as SBP (-9%) and DBP (-7%) had statistically significant, albeit modest, contributions to the overall UACR decline with empagliflozin (Figure 2b–d and ESM Figure 3b–d). In a model that included changes in HbA_{1c}, body weight and SBP simultaneously, these factors contributed a total of 15% to the overall UACR decline of 33% with empagliflozin vs. placebo (Figure 2e and ESM Figure 3e). That is, their individual effect on UACR appeared to be somewhat additive and, in total, account for about half of the UACR-lowering effect of empagliflozin. Finally, in patients with microalbuminuria, changes in UACR were only weakly correlated, albeit statistically significant in some cases, with changes in HbA1c (placebo r=0.129, p=0.042; empagliflozin r=0.021, p=0.673) or weight (placebo r=0.109, p=0.088; empagliflozin r=0.103, p=0.043). The association between UACR change vs. PP change was also weak, albeit statistically significant, and similar in the two groups (placebo r=0.143, p=0.024; empagliflozin r=0.141, p=0.006). These relationships indicate that the effect on UACR was not driven by changes in HbA1c, PP or weight, thus supporting the findings in the contribution analysis.

In patients with macroalbuminuria, changes in UACR with empagliflozin (-41%) were not significantly influenced by either changes in HbA_{1c} (+5%) nor by changes in body weight (-2%) (Figure 3a–b and ESM Figure 4a–b). As in patients with microalbuminuria, UACR lowering with empagliflozin was significantly, but again

modestly, related to changes in SBP (-9%) and DBP (-7%) (Figure 3c–d and ESM Figure 4c–d). In the model including changes in HbA_{1c}, body weight and SBP simultaneously, these factors contributed a total of 7% to the overall UACR decline of 40% with empagliflozin vs. placebo (Figure 3e and ESM Figure 4e). Thus, in both patients with microalbuminuria or macroalbuminuria, concomitant changes in glucose control, body weight, or blood pressure individually or together, only accounted for approximately half (at most) of the effect of empagliflozin to reduce albuminuria vs. placebo (approximately 5–15 percentage points out of a total reduction of approximately 30–40 percentage points). In macroalbuminuric patients, changes in UACR were not correlated with changes in HbA1c (placebo r=0.133, p=0.218; empagliflozin r=-0.046, p=0.607), weight (placebo r=-0.009, p=0.934; empagliflozin r=0.081, p=0.366) or PP (placebo r=0.190, p=0.078; empagliflozin r=0.061, p=0.492). These relationships indicate that the effect on UACR was not driven by changes in HbA1c, PP or weight, thus supporting the findings in the contribution analysis.

Sensitivity analyses for RAAS inhibitor use, completers vs. non-completers and for patients with and without changes in anti-hypertensive agents are included in the ESM Sensitivity Analyses.

Safety and adverse events

At weeks 12 and 24, eGFR showed a mild numerical decrease with empagliflozin and the reductions were similar among the micro- and macroalbuminuria groups (ESM Figure 5a–b).

Reported AEs are depicted in Table 2. The percentage of patients with urinary tract infections and genital infections was greater with empagliflozin than placebo. The percentage of patients with events consistent with volume depletion (including hypotension) was numerically greater with empagliflozin than placebo in patients with microalbuminuria (1.0% vs. 0.0%) or macroalbuminuria (2.3% vs. 1.1%) (Table 2). Of note, in this susceptible population of patients with underlying diabetic kidney disease, the percentage of patients with hyperkalemia was similar between the empagliflozin and placebo groups (Table 2). The data suggested comparable rates of AEs in empagliflozin treated patients taking a combination of a RAAS inhibitor and loop diuretic, with the caveat that the numbers were too small to draw reliable conclusions (data not shown).

Discussion

The main finding from this large analysis of a global clinical trial program supports our hypothesis that SGLT2 inhibition with empagliflozin reduces UACR in both the microalbuminuric and macroalbuminuric ranges in patients with type 2 diabetes. The quantity of the observed albuminuria-lowering effect was clinically meaningful and was only partially explained by expected empagliflozin-related improvements in variables otherwise associated with albuminuria reduction, namely HbA_{1c}, body weight or blood pressure. In this cohort, empagliflozin was generally well tolerated, aside from increased frequencies of urinary tract and genital infections and a numerical increase in cases of volume depletion in the micro- and macroalbuminuria groups vs. the placebo group.

Previous experimental work utilizing different animal models of kidney disease have demonstrated that SGLT2 inhibition alleviates renal damage. In Akita and

streptozotocin induced animal models of insulin deficient diabetes, SGLT2 inhibition reduced albuminuria [18–20]. SGLT2 inhibition has similar anti-albuminuric effects in animal models of type 2 diabetes [21], including recent evidence that empagliflozin reduced albuminuria, independent of effects on blood pressure or hyperglycaemia in BTBR ob/ob type 2 diabetic mice [22]. Previous clinical studies in type 2 diabetes patients with and without CKD have shown that SGLT2 inhibition is associated with an acute but modest decline in eGFR within 3-6 weeks of treatment initiation, followed by a period of stable renal function for up to 52-104 weeks, and this change is reversible after drug cessation for 2 weeks [17, 23, 24]. Notably, within this same treatment period renal safety assessments of SGLT2 inhibitors have also reported a reduction in UACR or urinary albumin excretion in patients with type 2 diabetes and CKD [15, 24]. Moreover, in a dedicated study of patients with CKD stage 3 the SGLT2 inhibitor dapagliflozin failed to show a significant effect on HbA_{1c} at 24 weeks, yet still reduced albuminuria, blood pressure and body weight [23]. Furthermore, dapagliflozin reduced eGFR acutely, followed by the maintenance of stable renal function over the subsequent 104 weeks of treatment [23]. Dapagliflozin treatment for 12 weeks was also shown to reduce UACR (combined microalbuminuria and macroalbuminuria) in a *post-hoc* analysis involving patients taking baseline RAAS blockade [25]. Similar to our observations, dapagliflozin was associated with changes in UACR after adjustment for potential confounding factors. Hence, existing experimental and clinical data suggest SGLT2 inhibitors as a drug class that may lead to reductions in urinary albumin excretion independent of known drug effects on blood pressure, HbA_{1c} or body weight.

The albuminuria-lowering effect of SGLT2 inhibition may be due to several mechanisms. First, SGLT2 inhibition achieved through either pharmacological blockade or genetic knock-out models reduces renal hyperfiltration, which is considered to be a surrogate marker for intraglomerular pressure in humans [11, 12, 19, 26]. Such a mechanism would be expected to reduce albuminuria independent of changes in systemic blood pressure, similar to the effects of RAAS blockade - but through afferent vasoconstrictive effects rather than efferent vasodilatory effects. Since effects of SGLT2 inhibition on UACR occur in conjunction with small declines in eGFR over the initial 3-4 weeks in patients with type 2 diabetes, it is possible that UACR-lowering effects of these agents are due to reduced intraglomerular pressure. Consistent with this hypothesis is the preliminary observation that SGLT2 inhibition with empagliflozin reduces calculated afferent arteriolar tone and reduces calculated glomerular capillary pressure in patients with type 1 diabetes [12]. Furthermore, in the present pooled analysis, the effect of empagliflozin on UACR remained significant and of a clinically relevant magnitude (approximately 20-40% reduction) after controlling for on-treatment changes in blood pressure, body weight and HbA_{1c}. In fact, at most, only about half of the overall albuminuria-lowering effect of empagliflozin vs. placebo could be explained by concomitant changes in glucose, body weight or SBP (individually or together), with blood pressure changes apparently contributing most. This supports the hypothesis that reductions in UACR were predominantly mediated via mechanisms other than those expected to result in improvements in UACR such as reductions in glucose, blood pressure or body weight. The SGLT2 inhibition related mechanisms that are independent of these metabolic changes may be explained by intrarenal haemodynamic

effects characterized by alleviation of glomerular hypertension, as suggested from the reduction in renal hyperfiltration [11].

A second major mechanism that may contribute to albuminuria-lowering with SGLT2 inhibition relates to systemic vascular effects of these drugs. SGLT2 inhibition reduces blood pressure and arterial stiffness, effects that have been associated with renal protection [27]. Our current analysis suggests that changes in SBP only modestly account for the reduction in UACR, since effects on UACR remained significant even after controlling for changes in this clinical parameter. Our observations in patients with type 1 diabetes further support the concept that renal haemodynamic effects are disproportionately larger than blood pressure lowering, since hyperfiltration is corrected by ≈20% with SGLT2 inhibition even though SBP was reduced only modestly by 3 mmHg or <3%, respectively [11]. Therefore these clinical observations to date suggest that direct intrarenal effects of SGLT2 inhibition contribute importantly to intraglomerular pressure, leading to decreased UACR [11].

A third relevant mechanism that may contribute to albuminuria-lowering effects of SGLT2 inhibition relates to influences on pro-inflammatory pathways, a recognized hallmark of diabetic nephropathy that can contribute to albuminuria [28]. *In vitro and in vivo* work has suggested that sodium glucose cotransport inhibition reduces markers of inflammation and fibrosis [18, 19, 29–31]. Although we did not measure inflammatory markers in the present trials, future studies should assess the effect of SGLT2 inhibition on pro-inflammatory and pro-fibrotic mechanisms in diabetes and associated kidney disease. Similarly, SGLT2 inhibition lowers plasma uric acid by approximately 15% [32]. In light of the putative role of uric acid as a mediator of renal and cardiovascular disease

through activation of neurohormones and pro-inflammatory pathways, it is conceivable that reducing uric acid with empagliflozin may lead to salutary effects on UACR [33].

Finally, changes in effective circulating fluid volume via natriuresis are known to alter urinary albumin excretion, as demonstrated by the decline in UACR that is achieved through the use of either dietary sodium restriction, or intervention with thiazide diuretics, both of which potentiate the albuminuria-lowering effects of RAAS inhibitors [5, 34]. Since SGLT2 inhibition leads to a sustained and modest contraction of effective circulating fluid volume, the contribution of the osmotic-diuretic effect of SGLT2 inhibition to its albuminuria-lowering potential may be clinically relevant [13]. Furthermore, SGLT2 inhibition may lead to a reduction in natriuretic hormones such as atrial natriuretic peptide, which is elevated in the plasma of diabetic animals [35] and may play a role in hyperfiltration related to experimental diabetes [36].

It is important to highlight that while SGLT2 inhibition exerts renal protective effects in animals, including preservation of renal function, decreased glomerulosclerosis and tubulointerstitial fibrosis, and decreased albuminuria, these effects could be enhanced when combined with traditional ACE inhibition [37]. Whether this additive effect is achieved via haemodynamic or non-haemodynamic (i.e. anti-mitogenic or anti-inflammatory) effects is not known. However, it is tempting to speculate that similar potential benefits are possible in humans, and studies examining combination SGLT2 inhibition with RAAS inhibition in type 1 and type 2 diabetes are warranted as both primary and secondary renal complications prevention strategies. Our work has limitations that need to be considered. First, these trials were designed to investigate the glucose-lowering effects of empagliflozin in patients with type 2 diabetes

and, therefore, this pooled analysis of UACR should be interpreted as *hypothesis-generating*. Similarly, the majority of patients in this relatively healthy cohort had microalbuminuria rather than macroalbuminuria. Second, capturing of urinary albumin levels was merely based on predefined spot urine sample collections as part of the continuous and comprehensive safety assessments during all 5 individual trials. However, we consider it a strength that safety assessments were standardized across trials, as described for other classes of glucose-lowering drugs [38], and that laboratory measurements of albuminuria and kidney function were conducted by a central laboratory. UACR is subject to significant variability, which we attempted to mitigate by including a large sample size in this analysis. We were still able to detect a significant effect of SGLT2 inhibition on UACR – suggesting a genuine and robust effect. Moreover, randomisation and blinding in Phase III clinical trials makes it unlikely that variability played an important role in either the placebo or active treatment groups. Although our sample size was adequate to assess significant effects on albuminuria across different ranges of UACR, the total number of individuals with macroalbuminuria was modest. Finally, though we observe a substantial effect of empagliflozin on albuminuria reduction over 24 weeks, the effect on longer-term reduction and on glomerular function rate requires further study. However, the effect of SGLT2 inhibition on UACR with empagliflozin, dapagliflozin and canagliflozin is present by approximately 4 weeks and tends not to dissipate over time [15, 23, 24], highlighting that the uniform use of UACR endpoints at 24 weeks is acceptable for this analysis.

In conclusion, empagliflozin reduced albuminuria by a clinically meaningful amount in patients with either micro- or macroalbuminuria. Interestingly, changes in this

renal biomarker with empagliflozin were only modestly influenced by the previously established class effects of SGLT2 inhibitors, such as reduction in HbA_{1c}, body weight and blood pressure – effects otherwise expected to profoundly reduce UACR. Other mechanisms to reduce albuminuria, such as non-systemic improvements in intraglomerular hypertension may therefore be present during treatment with empagliflozin.

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Duality of interest DZIC has received speaker honoraria and acts as a consultant for Boehringer Ingelheim and BAP received operational funding with DZIC from Boehringer Ingelheim. PHG has received speaker honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Genzyme, Merck Sharp and Dohme, Novartis and Novo Nordisk, research grants from Eli Lilly and Roche, is an advisory board member for Abbott, AbbVie, Boehringer Ingelheim, Cebix, Eli Lilly Janssen, Medscape and Novartis, and is a board member for Medix Laboratories. MvE, SSL, SK, EP and HJW are employees of Boehringer Ingelheim. SSL owns shares in Novo Nordisk A/S and shares in dynamically traded investment funds which may own stocks from pharmaceutical companies. MEC has received speaker honoraria and is a member of advisory boards for Boehringer Ingelheim, Eli Lilly and AstraZeneca.

Contribution statement DZIC contributed to the analysis planning and interpretation of data and drafted the manuscript. SSL, EP, and MvE contributed to the analysis planning and interpretation of data, and reviewed and edited the manuscript. BAP, PHG, MC, SK and HJW contributed to the interpretation of data, and reviewed and edited the manuscript. All authors were fully responsible for all content and editorial decisions, and approved the final version. SSL is the guarantor of this work.

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Title and Legends

Figure 1 Adjusted geometric mean (gMean) of percentage change from baseline in UACR at week 24 in patients with microalbuminuria (a) or macroalbuminuria (b) at baseline. Abbreviations: CI, confidence interval; UACR, urine albumin-to-creatinine ratio.

Figure 2 Contribution of changes in HbA_{1c} (a), body weight (b), systolic blood pressure (SBP) (c), diastolic blood pressure (d) and HbA_{1c}, body weight and SBP (e) to changes in UACR with empagliflozin: Patients with microalbuminuria at baseline. Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; LOCF, last observation carried forward; SBP, systolic blood pressure; SE, standard error; UACR, urine albumin-to-creatinine ratio.

Figure 3 Contribution of changes in HbA_{1c} (a), body weight (b), systolic blood pressure (c), diastolic blood pressure (d) and HbA_{1c}, body weight and SBP (e) to changes in UACR with empagliflozin: Patients with macroalbuminuria at baseline. Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; LOCF, last observation carried forward; SBP, systolic blood pressure; SE, standard error; UACR, urine albumin-to-creatinine ratio.

Table 1 Baseline characteristics

	Patients with microalbuminuria at baseline		Patients with macroalbuminuria at baseline	
	Placebo (*n*=248)	**Empagliflozin (*n*=388)**	**Placebo (*n*=87)**	**Empagliflozin (*n*=128)**
Male	141 (56.9)	229 (59.0)	58 (66.7)	86 (67.2)
HbA_{1c}, %	8.15 (0.85)	8.17 (0.87)	8.13 (0.86)	8.20 (0.92)
HbA_{1c}, mmol/mol	66 (9)	66 (10)	65 (9)	66 (10)
Weight, kg	79.1 (19.2)	80.3 (19.5)	80.3 (20.2)	82.6 (18.9)
Body mass index, kg/m^2	29.2 (5.6)	29.4 (5.6)	29.8 (5.6)	29.7 (5.1)
Systolic blood pressure, mmHg	134.2 (17.0)	134.9 (16.9)	145.1 (19.2)	143.4 (18.9)
Diastolic blood pressure, mmHg	77.3 (9.6)	79.4 (9.5)	78.0 (11.2)	79.0 (10.0)
eGFR (MDRD), ml min^{-1} 1.73 m^{-2}	72.1 (30.4)	79.4 (26.0)	52.5 (29.0)	56.6 (29.0)
eGFR (MDRD), *n* (%)				
≥90 ml min^{-1} 1.73 m^{-2}	69 (27.8)	129 (33.2)	12 (13.8)	17 (13.3)
≥60 to <90 ml min^{-1} 1.73 m^{-2}	83 (33.5)	180 (46.4)	15 (17.2)	31 (24.2)
≥30 to <60 ml min^{-1} 1.73 m^{-2}	83 (33.5)	71 (18.3)	39 (44.8)	56 (43.8)
<30 ml min^{-1} 1.73 m^{-2}	13 (5.2)	8 (2.1)	21 (24.1)	24 (18.8)
UACR[a], mg/mmol	7.6 (4.6–13.3)	7.5 (4.7–13.4)	108.7 (58.2–231.3)	98.9 (57.4–197.3)
Race				
Asian	132 (53.2)	223 (57.5)	49 (56.3)	63 (49.2)
White	112 (45.2)	153 (39.4)	38 (43.7)	60 (46.9)
Other	4 (1.6)	12 (3.1)	0	5 (3.9)
Age, years	60.4 (10.2)	58.4 (10.5)	60.5 (10.3)	59.3 (9.9)
Time since diagnosis of T2D				
≤1 year	20 (8.1)	37 (9.5)	1 (1.1)	5 (3.9)

>1 to 5 years	55 (22.2)	108 (27.8)	15 (17.2)	20 (15.6)
>5 years	173 (69.8)	243 (62.6)	71 (81.6)	103 (80.5)
Background antihypertensive medication[b]	168 (67.7)	259 (66.8)	73 (83.9)	113 (88.3)
Agents acting on renin-angiotensin system	142 (57.3)	203 (52.3)	57 (65.5)	96 (75.0)
ACE-inhibitor, plain	59 (23.8)	83 (21.4)	29 (33.3)	47 (36.7)
ACE-inhibitor, combination[c]	6 (2.4)	18 (4.6)	2 (2.3)	6 (4.7)
Angiotensin II antagonist, plain	60 (24.2)	75 (19.3)	26 (29.9)	41 (32.0)
Angiotensin II antagonist, combination[c]	20 (8.1)	32 (8.2)	6 (6.9)	10 (7.8)
Other	3 (1.2)	2 (0.5)	4 (4.6)	5 (3.9)
Diuretics	45 (18.1)	55 (14.2)	30 (34.5)	39 (30.5)
Loop diuretics	22 (8.9)	16 (4.1)	22 (25.3)	25 (19.5)
Thiazides	16 (6.5)	22 (5.7)	5 (5.7)	13 (10.2)
Low−ceiling diuretics, excl. thiazides	9 (3.6)	13 (3.4)	2 (2.3)	6 (4.7)
Potassium−sparing agents	2 (0.8)	4 (1.0)	1 (1.1)	2 (1.6)
Diuretics and potassium−sparing agents in combination	1 (0.4)	2 (0.5)	1 (1.1)	1 (0.8)
Other diuretics	0	2 (0.5)	0	0
Calcium channel blockers	74 (29.8)	110 (28.4)	41 (47.1)	60 (46.9)
β-blockers	49 (19.8)	73 (18.8)	32 (36.8)	50 (39.1)
Other	14 (5.6)	18 (4.6)	18 (20.7)	13 (10.2)
Background lipid-lowering	121 (48.8)	169 (43.6)	56 (64.4)	79 (61.7)

medication				
Background ASA	91 (36.7)	105 (27.1)	40 (46.0)	62 (48.4)

Abbreviations: ACE, angiotensin-converting enzyme; ASA, acetylsalicylic acid; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; SD, standard deviation; T2D, type 2 diabetes; UACR, urine albumin-to-creatinine ratio.

Data are n (%) or mean (SD) in the full analysis set (randomised patients who received ≥1 dose of study drug and had a baseline HbA_{1c} measurement) unless otherwise stated. [a]Median (1st quartile–3rd quartile). [b]Patients could be receiving ≥1 type of antihypertensive medication. [c]Combination with diuretics or calcium channel blockers or other combinations.

Table 2 Adverse events (AEs) in patients with microalbuminuria or macroalbuminuria at baseline

	Patients with microalbuminuria		Patients with macroalbuminuria	
	Placebo (*n*=248)	**Empagliflozin (*n*=388)**	**Placebo (*n*=87)**	**Empagliflozin (*n*=128)**
One or more AE(s)	180 (72.6)	259 (66.8)	68 (78.2)	91 (71.1)
One or more drug-related[a] AE(s)	48 (19.4)	74 (19.1)	24 (27.6)	26 (20.3)
AE(s) leading to treatment discontinuation	7 (2.8)	4 (1.0)	4 (4.6)	3 (2.3)
One or more severe AE(s)	10 (4.0)	16 (4.1)	8 (9.2)	9 (7.0)
One or more serious AE(s)	9 (3.6)	19 (4.9)	7 (8.0)	10 (7.8)
Deaths	0	0	2 (2.3)	0
Confirmed hypoglycaemic AEs[b]	29 (11.7)	35 (9.0)	21 (24.1)	24 (18.8)
Events consistent with urinary tract infection[c]	24 (9.7)	31 (8.0)	8 (9.2)	15 (11.7)
Events consistent with genital infection[d]	2 (0.8)	11 (2.8)	0	4 (3.1)
Events consistent with volume depletion[e]	0	4 (1.0)	1 (1.1)	3 (2.3)
Decreased renal function[f]	0	1 (0.3)	3 (3.4)	6 (4.7)
Bone fractures	1 (0.4)	4 (1.0)	1 (1.1)	0
Hyperkalaemia[g]	3 (1.2)	2 (0.5)	1 (1.1)	2 (1.6)

Abbreviations: AE, adverse event; MedDRA, Medical Dictionary for Drug Regulatory Activities.

Data are n (%) in the treated set (randomised patients who received ≥1 dose of study drug). [a]As defined by the investigator; [b]Plasma glucose ≤3.9 mmol/l and/or requiring assistance; [c]Based on 77 preferred terms; [d]Based on 89 preferred terms; [e]Based on 8 preferred terms. [f]Based on the narrow standardized MedDRA query “decreased renal function”; [g]Based on 1 preferred term.

Figure 1: Adjusted geometric mean (gMean) of percentage change from baseline in UACR at week 24 in patients with microalbuminuria (a) or macroalbuminuria (b) at baseline. ANCOVA in FAS (LOCF). **p*=0.001, ***p*<0.001

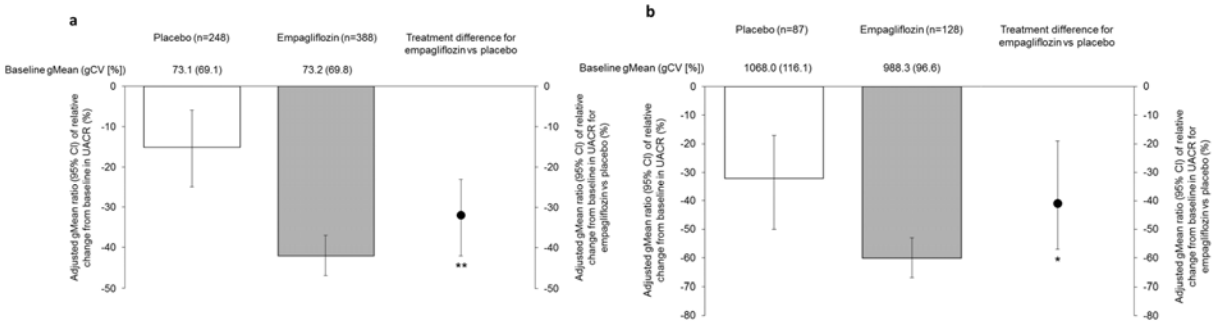


Figure 2: Contribution of changes in HbA_{1c} (a), body weight (b), systolic blood pressure (SBP) (c), diastolic blood pressure (d) and HbA_{1c}, body weight and SBP (e) to changes in UACR with empagliflozin: Patients with microalbuminuria at baseline. Treated set (LOCF). *p<0.05

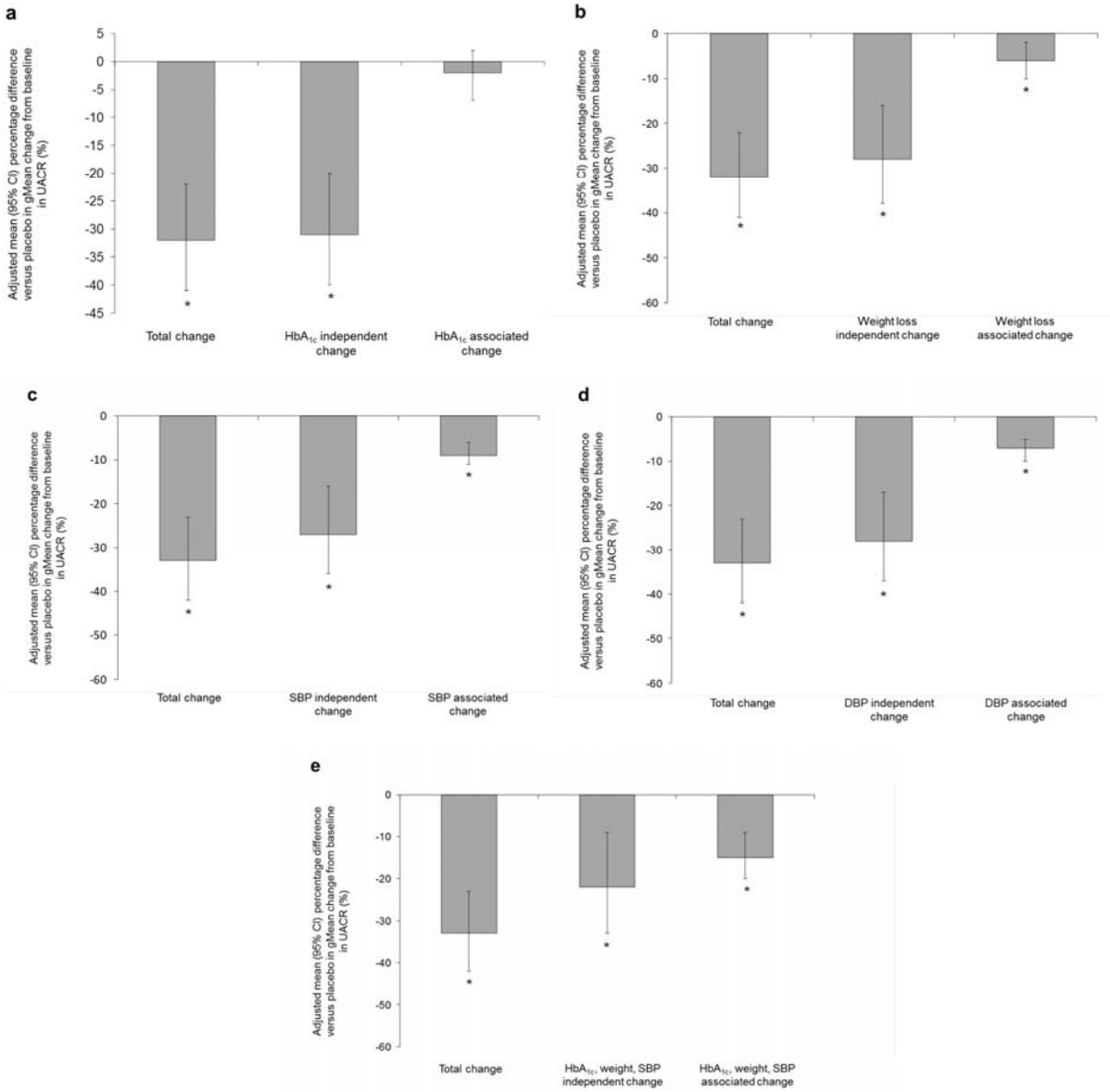
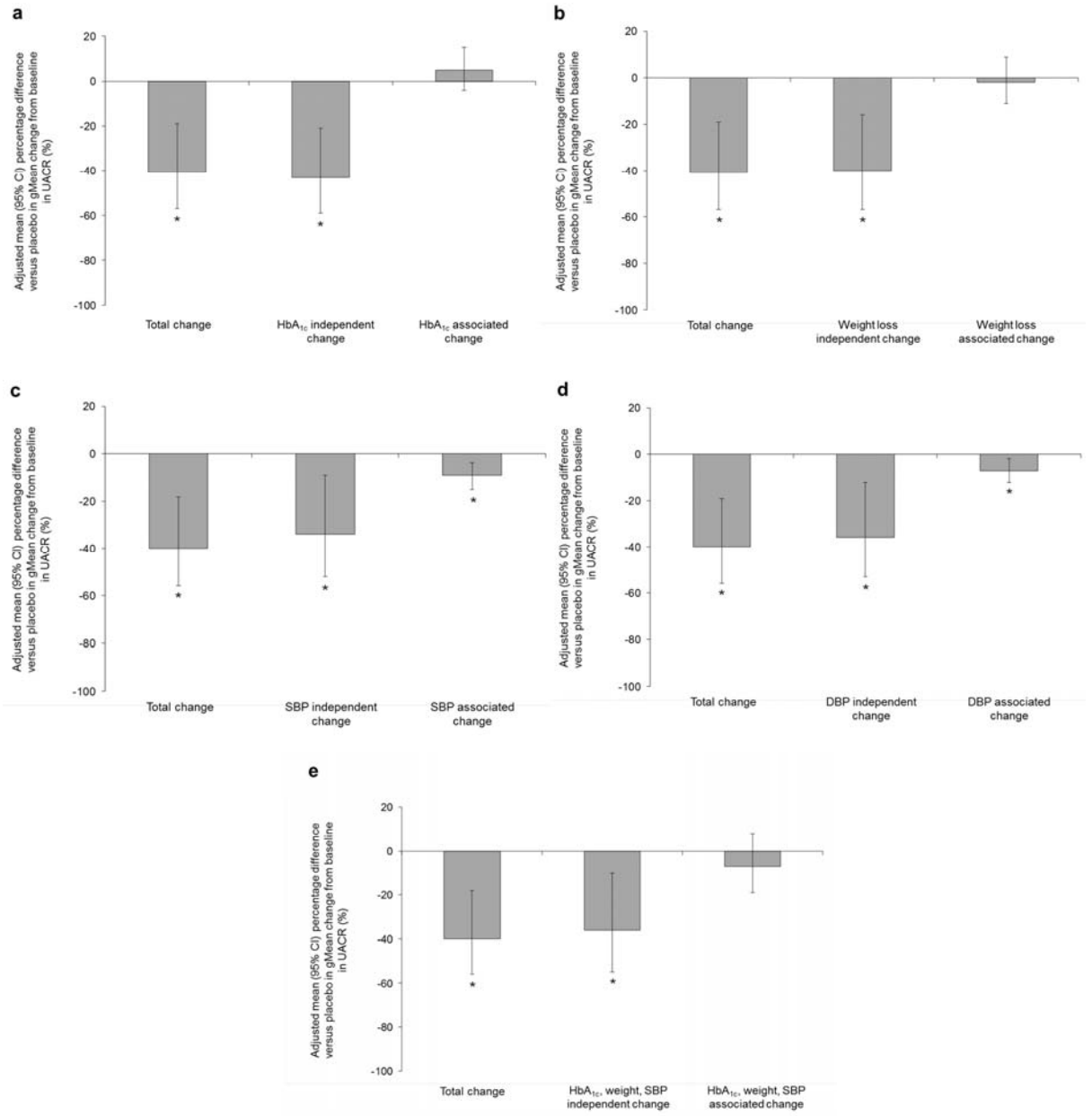


Figure 3: Contribution of changes in HbA_{1c} (a), body weight (b), systolic blood pressure (c), diastolic blood pressure (d) and HbA_{1c}, body weight and SBP (e) to changes in UACR with empagliflozin: Patients with macroalbuminuria at baseline. Treated set (LOCF). *p<0.05



Electronic supplementary material (ESM)

ESM Figure S1 Changes from baseline in HbA_{1c}, weight, systolic blood pressure and diastolic blood pressure at week 24 in patients with microalbuminuria or macroalbuminuria at baseline.

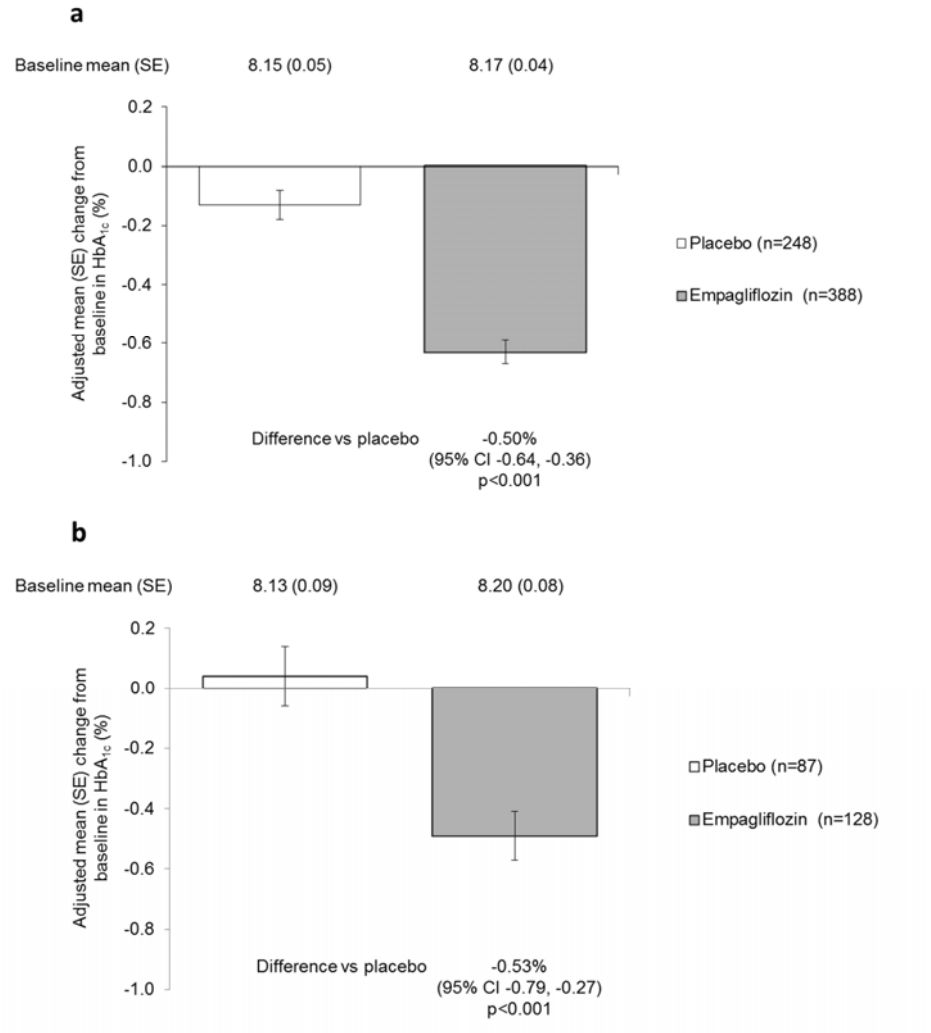
ESM Figure S2 Ratio of relative change from baseline in urine albumin-to-creatinine ratio over 24 weeks in patients with microalbuminuria or macroalbuminuria at baseline.

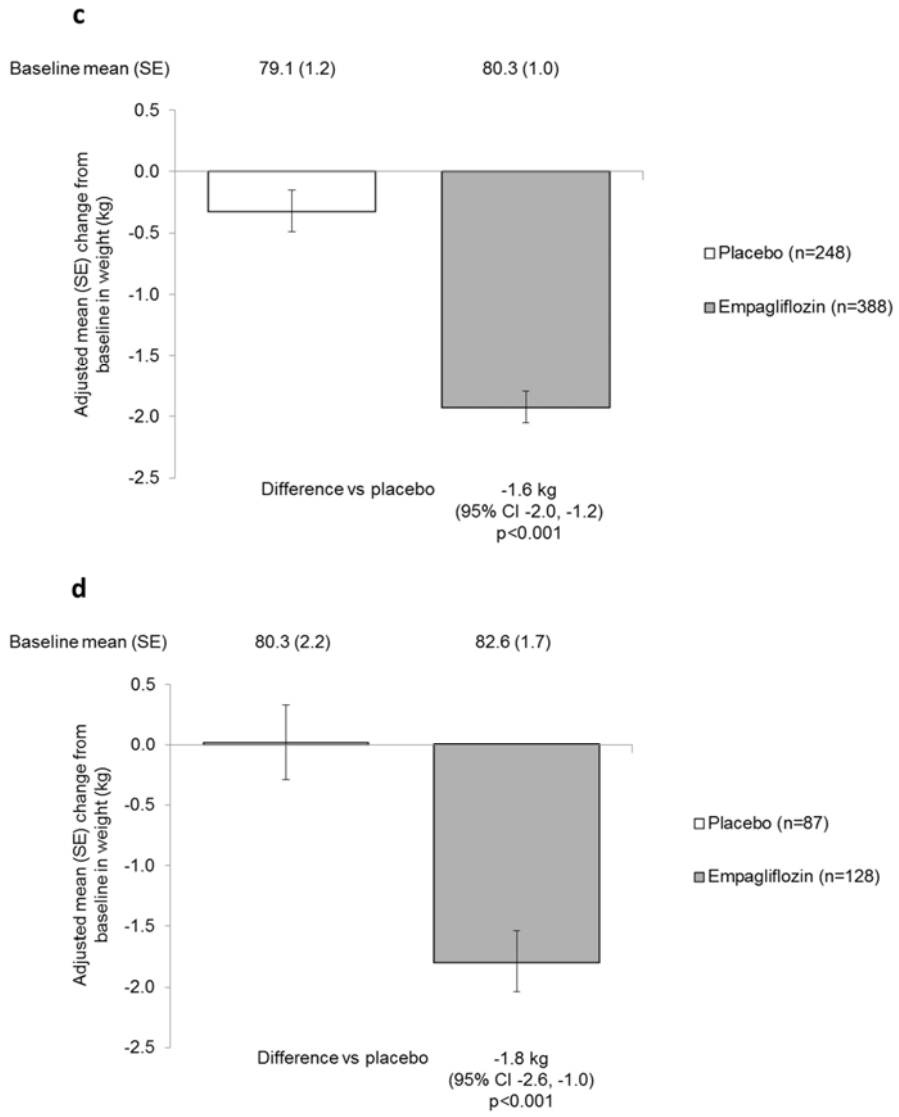
ESM Figure S3 Contribution of changes in HbA_{1c}, body weight, SBP, diastolic blood pressure, and HbA_{1c}, body weight and SBP to changes in UACR with empagliflozin in patients with microalbuminuria at baseline.

ESM Figure S4 Contribution of changes in HbA_{1c}, body weight, SBP, diastolic blood pressure, and HbA_{1c}, body weight and SBP to changes in UACR with empagliflozin in patients with macroalbuminuria at baseline.

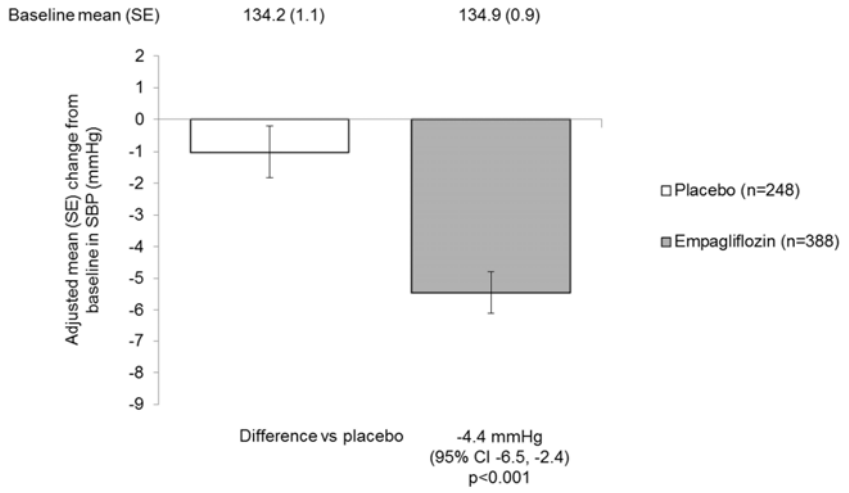
ESM Figure S5 eGFR over 24 weeks in patients with microalbuminuria or macroalbuminuria at baseline.

ESM Figure 1 Changes from baseline in HbA_{1c}, weight and SBP at week 24. Change in HbA_{1c} in patients with microalbuminuria at baseline (a), change in HbA_{1c} in patients with macroalbuminuria at baseline (b), change in weight in patients with microalbuminuria at baseline (c), change in weight in patients with macroalbuminuria at baseline (d), change in SBP in patients with microalbuminuria at baseline (e), change in SBP in patients with macroalbuminuria at baseline (f), change in DBP in patients with microalbuminuria at baseline (g), change in DBP in patients with macroalbuminuria at baseline (h). ANCOVA in FAS (LOCF)

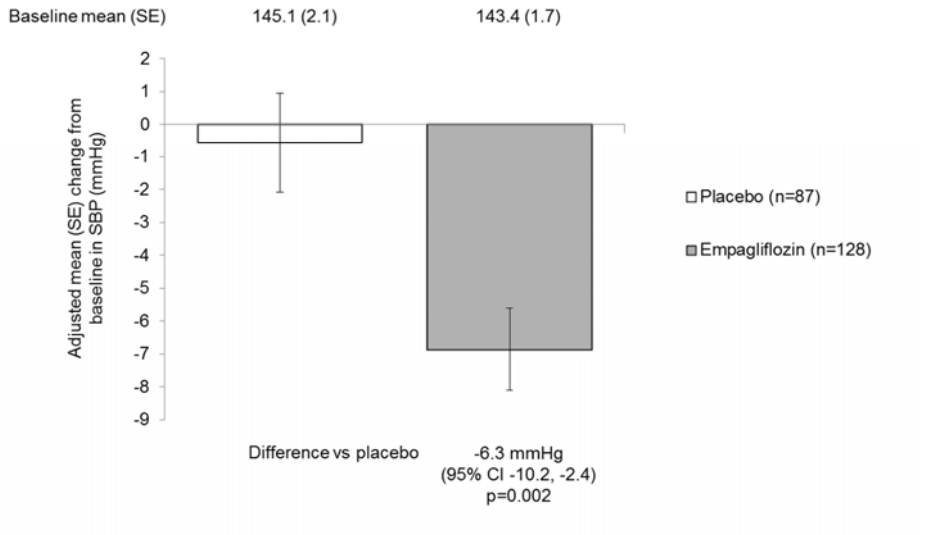



c
Baseline mean (SE)
79.1 (1.2)
80.3 (1.0)
Adjusted mean (SE) change from baseline in weight (kg)
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Placebo (n=248)
Empagliflozin (n=388)
Difference vs placebo
-1.6 kg
(95% CI -2.0, -1.2)
p<0.001
d
Baseline mean (SE)
80.3 (2.2)
82.6 (1.7)
Adjusted mean (SE) change from baseline in weight (kg)
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Placebo (n=87)
Empagliflozin (n=128)
Difference vs placebo
-1.8 kg
(95% CI -2.6, -1.0)
p<0.001

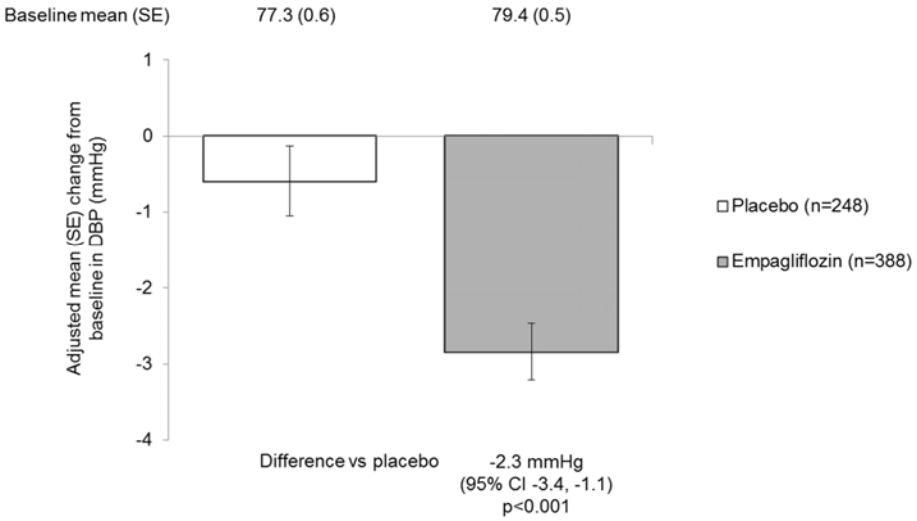
e


Baseline mean (SE)
134.2 (1.1)
134.9 (0.9)
Adjusted mean (SE) change from baseline in SBP (mmHg)
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
Placebo (n=248)
Empagliflozin (n=388)
Difference vs placebo
-4.4 mmHg
(95% CI -6.5, -2.4)
p<0.001

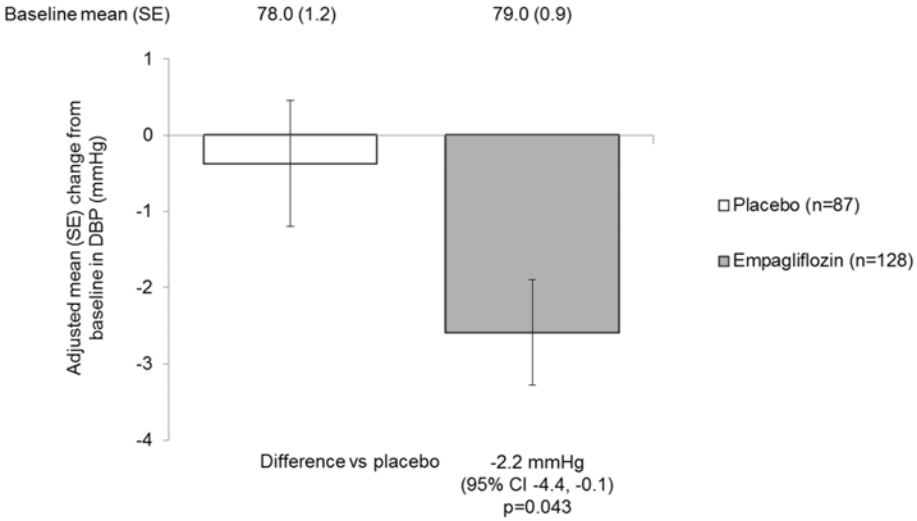
f


Baseline mean (SE)
145.1 (2.1)
143.4 (1.7)
Adjusted mean (SE) change from baseline in SBP (mmHg)
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
Placebo (n=87)
Empagliflozin (n=128)
Difference vs placebo
-6.3 mmHg
(95% CI -10.2, -2.4)
p=0.002

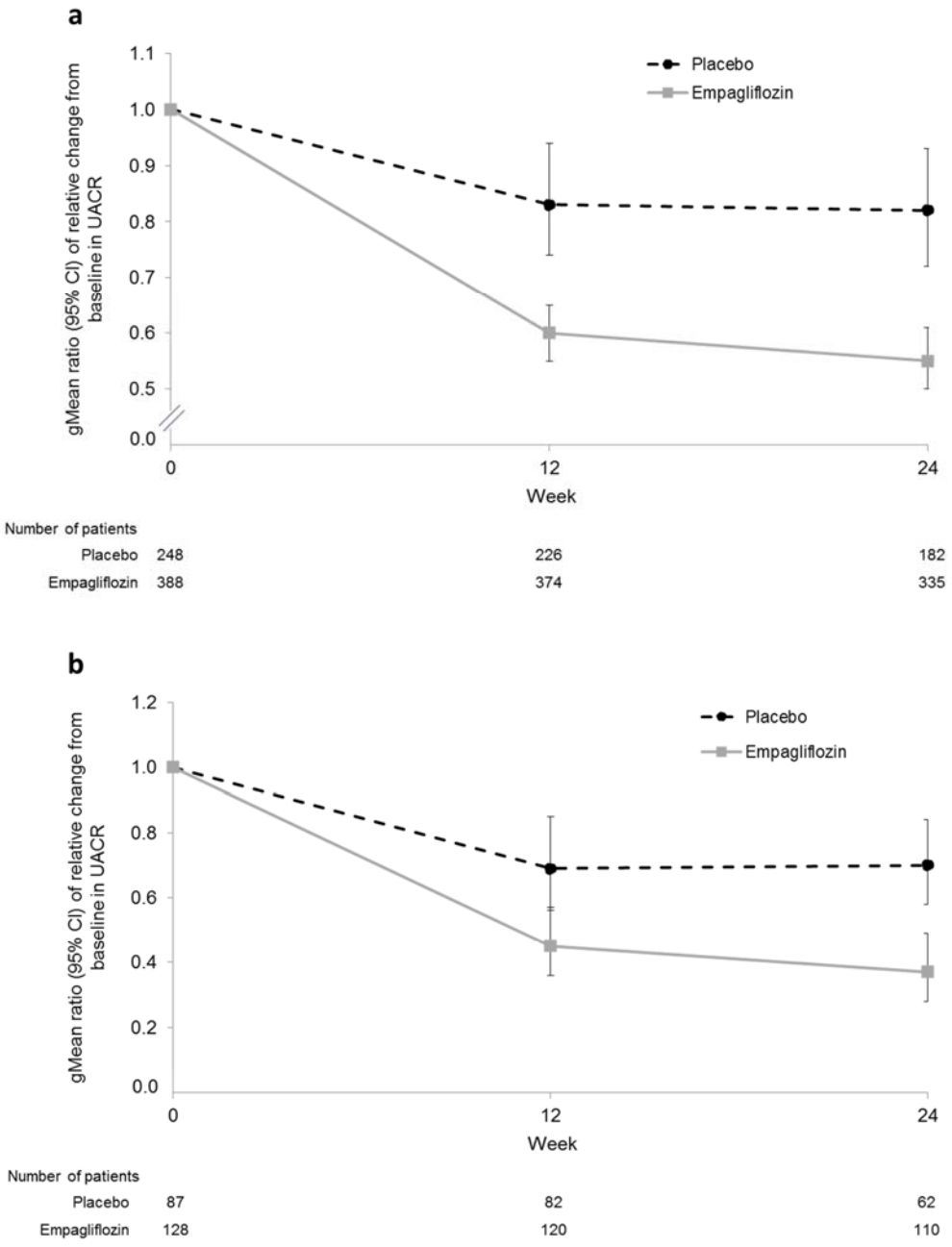
g


Baseline mean (SE)
77.3 (0.6)
79.4 (0.5)
Adjusted mean (SE) change from baseline in DBP (mmHg)
1
0
-1
-2
-3
-4
Placebo (n=248)
Empagliflozin (n=388)
Difference vs placebo
-2.3 mmHg
(95% CI -3.4, -1.1)
p<0.001

h


Baseline mean (SE)
78.0 (1.2)
79.0 (0.9)
Adjusted mean (SE) change from baseline in DBP (mmHg)
1
0
-1
-2
-3
-4
Placebo (n=87)
Empagliflozin (n=128)
Difference vs placebo
-2.2 mmHg
(95% CI -4.4, -0.1)
p=0.043

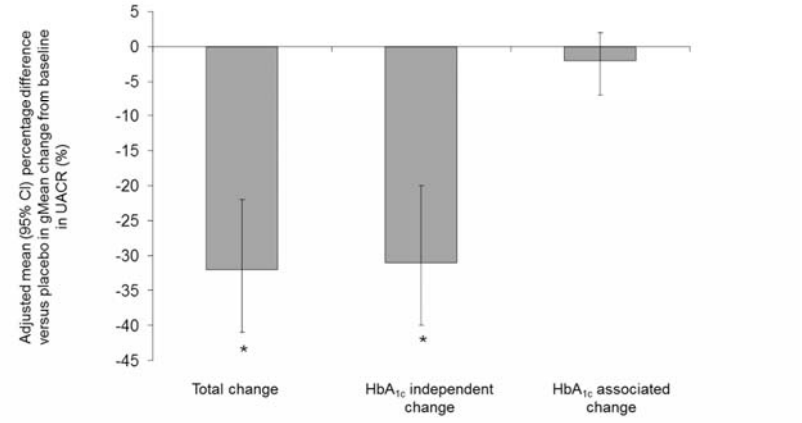
ESM Figure 2 Ratio of relative change from baseline in UACR over 24 weeks in patients with microalbuminuria (a) or macroalbuminuria (b) at baseline. Treated set. Descriptive statistics, observed cases.



ESM Figure S3 Contribution of changes in HbA_{1c} (a), body weight (b), systolic blood pressure (SBP) (c), diastolic blood pressure (d) and HbA_{1c}, body weight and SBP (e) to changes in UACR with empagliflozin: Patients with microalbuminuria at baseline. Treated set (LOCF). *p<0.05

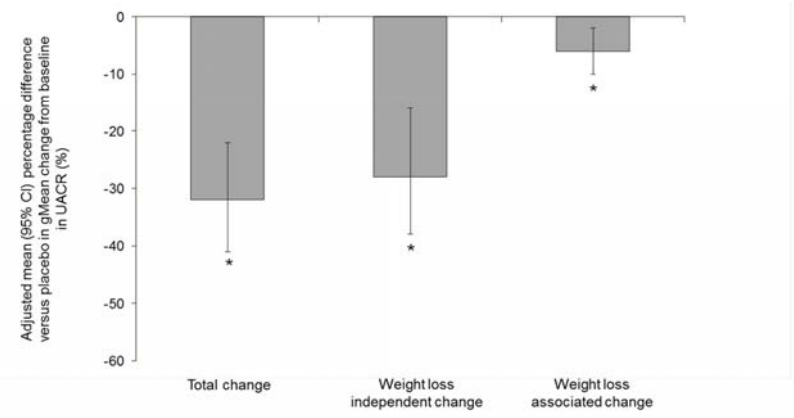
a

	Placebo	Empagliflozin
n	248	388
Baseline mean (SE) HbA_{1c} (%)	8.15 (0.05)	8.17 (0.04)
Baseline gMean (gCV [%]) UACR (mg/g)	73.1 (69.1)	73.2 (69.8)



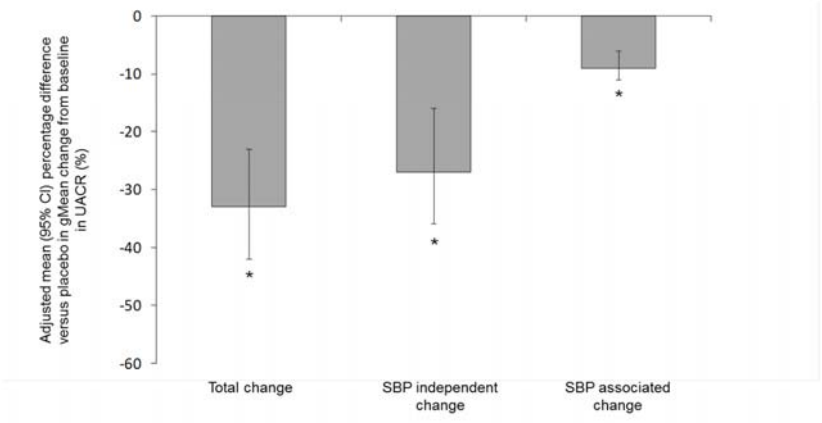
b

	Placebo	Empagliflozin
n	248	388
Baseline mean (SE) body weight (kg)	79.1 (1.2)	80.3 (1.0)
Baseline gMean (gCV [%]) UACR (mg/g)	73.1 (69.1)	73.2 (69.8)



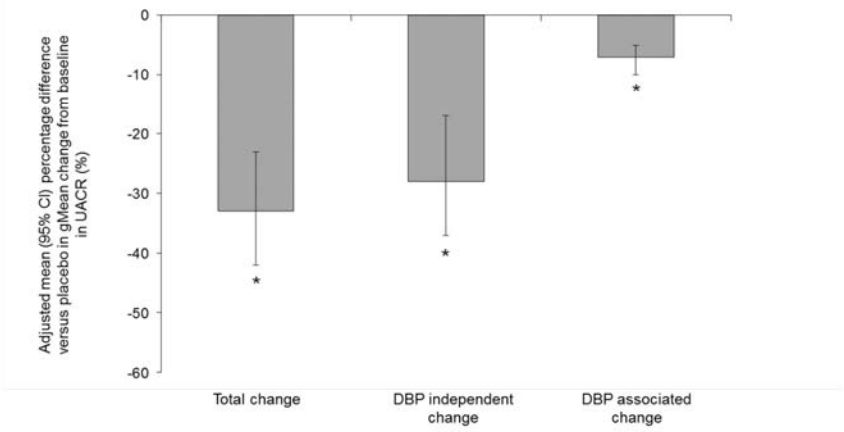
c

	Placebo	Empagliflozin
n	248	388
Baseline mean (SE) SBP (mmHg)	134.2 (1.1)	134.9 (0.9)
Baseline gMean (gCV [%]) UACR (mg/g)	73.1 (69.1)	73.2 (69.8)



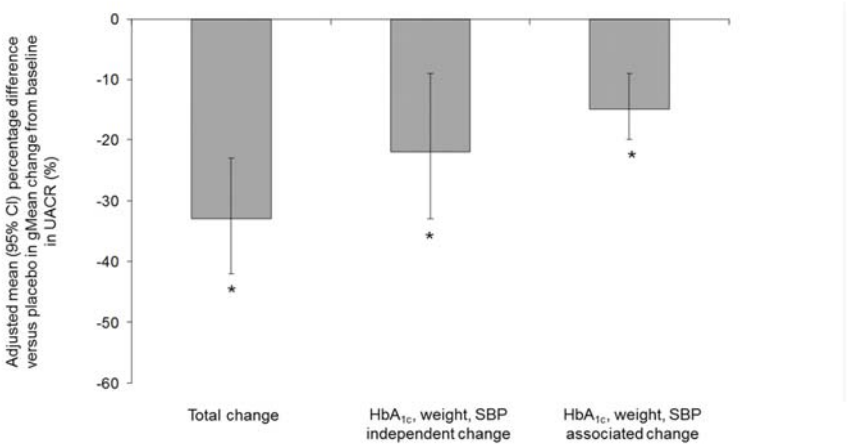
d

	Placebo	Empagliflozin
n	248	388
Baseline mean (SE) DBP (mmHg)	77.3 (0.6)	79.4 (0.5)
Baseline gMean (gCV [%]) UACR (mg/g)	73.1 (69.1)	73.2 (69.8)



e

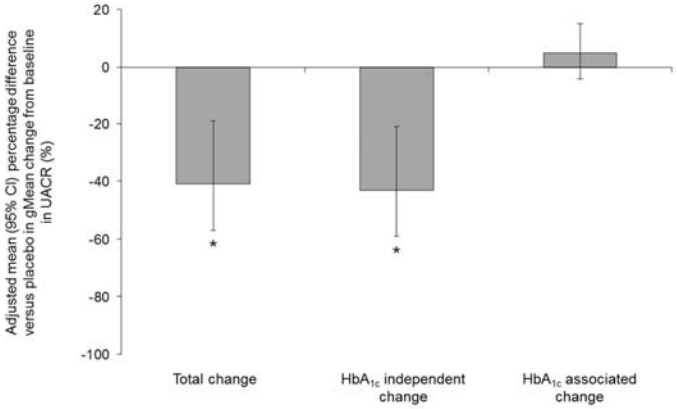
	Placebo	Empagliflozin
n	248	388
Baseline mean (SE) HbA1c (%)	8.15 (0.05)	8.17 (0.04)
Baseline mean (SE) body weight (kg)	79.1 (1.2)	80.3 (1.0)
Baseline mean (SE) SBP (mmHg)	134.2 (1.1)	134.9 (0.9)
Baseline gMean (gCV [%]) UACR (mg/g)	73.1 (69.1)	73.2 (69.8)



ESM Figure S4 Contribution of changes in HbA_{1c} (a), body weight (b), systolic blood pressure (SBP) (c), diastolic blood pressure (d) and HbA_{1c}, body weight and SBP (e) to changes in UACR with empagliflozin: Patients with macroalbuminuria at baseline. Treated set (LOCF). *p<0.05

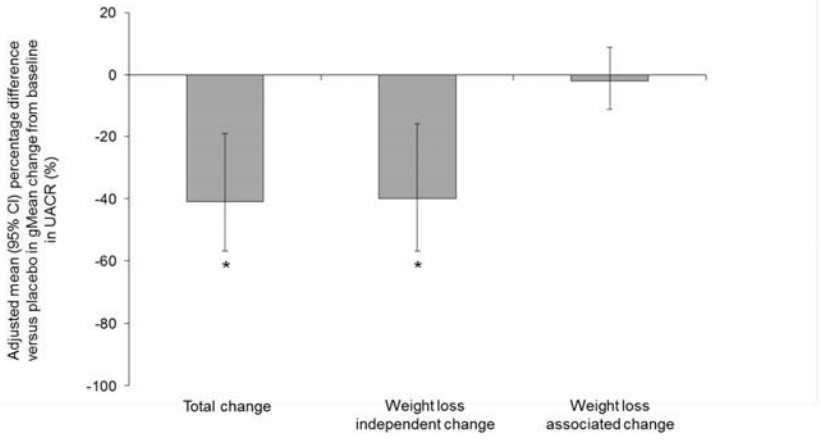
a

	Placebo	Empagliflozin
n	87	128
Baseline mean (SE) HbA_{1c} (%)	8.13 (0.09)	8.20 (0.08)
Baseline gMean (gCV [%]) UACR (mg/g)	1068.0 (116.1)	988.3 (96.6)



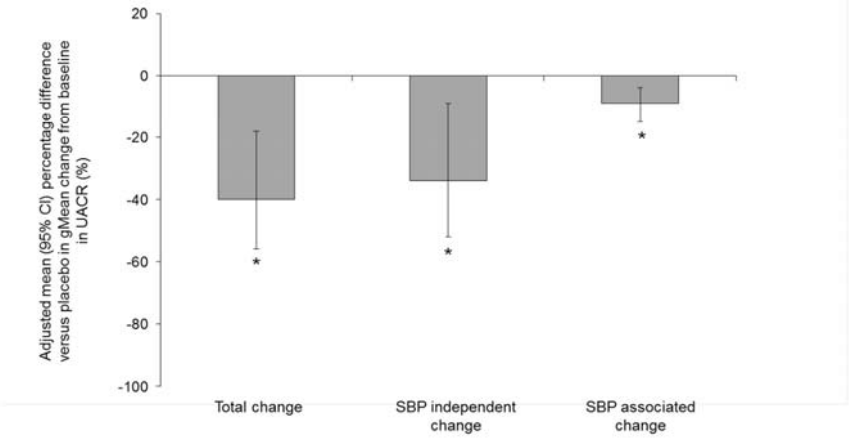
b

	Placebo	Empagliflozin
n	87	128
Baseline mean (SE) body weight (kg)	80.3 (2.2)	82.6 (1.7)
Baseline gMean (gCV [%]) UACR (mg/g)	1068.0 (116.1)	988.3 (96.6)



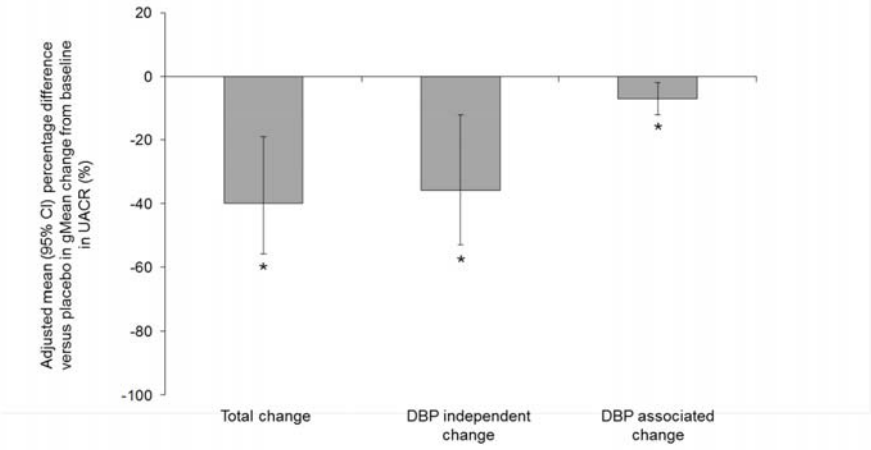
c

	Placebo	Empagliflozin
n	87	128
Baseline mean (SE) SBP (mmHg)	145.1 (2.1)	143.4 (1.7)
Baseline gMean (gCV [%]) UACR (mg/g)	1068.0 (116.1)	988.3 (96.6)



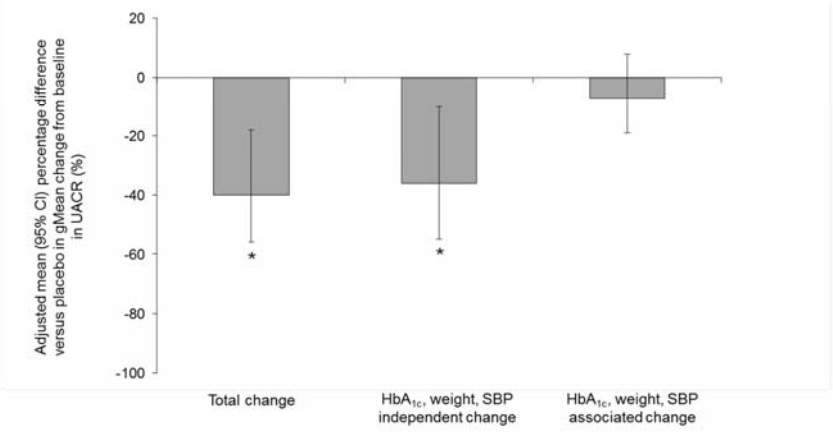
d

	Placebo	Empagliflozin
n	87	128
Baseline mean (SE) DBP (mmHg)	78.0 (1.2)	79.0 (0.9)
Baseline gMean (gCV [%]) UACR (mg/g)	1068.0 (116.1)	988.3 (96.6)



e

	Placebo	Empagliflozin
n	87	128
Baseline mean (SE) HbA1c (%)	8.13 (0.09)	8.20 (0.08)
Baseline mean (SE) body weight (kg)	80.3 (2.2)	82.6 (1.7)
Baseline mean (SE) SBP (mmHg)	145.1 (2.1)	143.4 (1.7)
Baseline gMean (gCV [%]) UACR (mg/g)	1068.0 (116.1)	988.3 (96.6)



ESM Figure 5 eGFR over 24 weeks in patients with microalbuminuria (a) or macroalbuminuria (b) at baseline. Treated set. Descriptive statistics. MDRD, Modification of Diet in Renal Disease.