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# Concomitant treatment with insulin and sodium–glucose cotransporter 2 inhibitors was associated with the renal composite outcome in Japanese patients with type 2 diabetes and chronic kidney disease: A propensity score-matched analysis

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**Keywords**
Insulin treatment, Renal composite outcome, Sodium–glucose cotransporter 2 inhibitors

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## ABSTRACT

**Aims/Introduction:** We previously reported that sodium–glucose cotransporter 2 inhibitor (SGLT2i) treatment was associated with an improvement of the albumin-to-creatinine ratio in Japanese patients with type 2 diabetes mellitus and chronic kidney disease. The present study clarified how concomitant insulin treatment (IT) with SGLT2i therapy influences the renal composite outcome (RCO).

**Materials and Methods:** We retrospectively evaluated 624 Japanese patients with type 2 diabetes mellitus and chronic kidney disease who underwent SGLT2i treatment. The renal composite outcome was set as progression of the stage of albuminuria or a ≥15% decrease in the estimated glomerular filtration rate per year. We developed a cohort model of patients managed with and without IT (Ins [+], Ins [−]) using propensity score matching methods. Furthermore, all patients in our study population were stratified into quintiles according to their propensity score.

**Results:** The incidence of the RCO was in Ins (+) patients significantly higher than that in Ins (−) ($P = 0.033$). The estimated hazard ratio for the RCO was 1.55 ($P = 0.035$) in Ins (+) patients. The change in the estimated glomerular filtration rate and albumin-to-creatinine ratio in the groups was not statistically significant. The analysis, which was based on the quintiles, showed a statistically significant difference between the Ins (+) and Ins (−) groups ($P = 0.01$); the odds ratio for the RCO in patients managed with IT was 2.20 ($P = 0.01$).

**Conclusions:** Concomitant administration of IT with SGLT2is influenced the RCO in Japanese patients with type 2 diabetes mellitus and chronic kidney disease. We might need to consider the influence of concomitant agents on the renoprotective effects of SGLT2i therapy.

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## INTRODUCTION

Sodium-glucose co-transporter 2 inhibitors (SGLT2is) are oral glucose-lowering agents that enhance the urinary excretion of glucose by inhibiting the SGLT2 in the renal proximal tubules. The Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) trial (empagliflozin)[1], the Canagliflozin Cardiovascular Assessment Study / the Canagliflozin Cardiovascular Assessment Study–Renal (CANVAS/CANVAS-R) program (canagliflozin)[2] and the Dapagliflozin Effect on Cardiovascular Events – Thrombolysis in Myocardial Infarction 58 (DECLARE-TIMI 58) trial (dapagliflozin)[3] were primarily cardiovascular outcome trials (CVOTs) that aimed to evaluate cardiovascular outcomes with the trial agent and establish its non-inferiority; however, they actually revealed the superiority of the cardiovascular outcome with the trial agent. In addition, the renoprotective effects of these SGLT2 is were demonstrated in the sub-analyses of these trials[2,4,5]. Furthermore the CREDENCE study (canagliflozin) showed the demonstrated a superior renal-composite outcome, as the primary end-point[6]. Thus, SGLT2is are considered to have a pronounced beneficial effect on the renal outcome.

By inducing substantial urinary glucose excretion, SGLT2is not only causes glucose-lowering effects, but also blood pressure (BP) reduction and body weight (BW) loss, as well as dyslipidemia and liver function improvements.

While the pleiotropic effects of SGLT2is have been discussed in detail related to the superiority of the cardiovascular or renal outcome, little is known about the mechanisms underlying these actions or the interaction with concomitant agents. Our group previously reported that SGLT2is improved the albumin-to-creatinine ratio (ACR) of Japanese patients with type 2 diabetes mellitus (T2DM) and chronic kidney disease (CKD)[7,8]. The same study also reported that insulin treatment was independently correlated with the renal composite outcome.

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## MATERIALS AND METHODS

### Study participants

The present study was a subanalysis of our previous survey. The participants and statistical methods have already been described [8]. Briefly, the study included 797 type 2 diabetes mellitus patients who were registered with and visited clinics of members of the Kanagawa Physicians Association between October 2018 and December 2018. The following inclusion criteria were applied: (i) type 2 diabetes mellitus patients treated with SGLT2is for the first time for >1 year before enrolment in the study; (ii) CKD (defined according to the K/DOQI clinical practice guidelines)[9]; and (iii) aged >20 years. The following exclusion criteria were applied: (i) type 1 diabetes mellitus; (ii) long-term dialysis patient; (iii) severe liver dysfunction (e.g., cirrhosis or severe infection); (iv) terminal stage malignancy; (v) pregnancy; (vi) poor adherence to SGLT2i therapy; and (vii) the intent to opt out of the study. A total of 34 patients were excluded based on these criteria.

To evaluate the renal outcome, a total of 624 patients in whom the ACR had been measured at two timepoints – baseline and at the end of the survey – were included in the present subanalysis. The median duration of SGLT2i therapy was 33.0 months (range 12–66 months). The following parameters were recorded at the start of SGLT2i therapy and at the time of the survey: age, sex, bodyweight, systolic and diastolic BP, serum creatinine level, glycated hemoglobin A1c (HbA1c) level, and ACR (mg/gCr). The estimated glomerular filtration rate (eGFR) was calculated as follows: eGFR (mL/min/1.73 $m^2$) = 194 × $age^{-0.287}$ × serum $creatinine^{-1.094}$ × (0.739 for women)[10]. The mean arterial pressure was calculated as follows: (systolic BP – diastolic BP) / 3 + diastolic BP.

### Statistical analysis

1. In the present study, the renal composite outcome was defined as worsening of the ACR and/or a >15% reduction in eGFR per year.
2. The participants were divided into two groups based on concomitant insulin treatment: patients with insulin treatment (Ins [+] group) and those without insulin treatment (Ins [−] group). Then, propensity scores were calculated for the Ins (+) group using a logistic regression model to estimate the likelihood of the disease according to the following parameters: baseline variables (age, sex, bodyweight, HbA1c, mean arterial pressure, eGFR and logarithmic value of ACR [LNACR] at baseline), type of SGLT2i, and concomitant administration of antihypertensive drugs and statins. As insulin use was related to the other concomitant hypoglycemic medications, these were not included in the logistic regression model that was used to determine the propensity score.
3. To address the discrepant effects of the clinical findings and baseline renal function on the outcomes of interest, and to fairly compare renal composite outcomes between patients with and without insulin treatment, we established cohort models of the Ins (+) and Ins (−) groups using propensity score matching. The following algorithm was applied for propensity score matching: 1:1 nearest neighbor match with a caliper value of 0.04, the width of which is equal to 0.2 of the standard deviation of the propensity score, without replacement. Although higher caliper widths are generally associated with an increased number of matches, they can

also reduce the balance between groups and add more bias in the estimation of treatment effects. In the present study, the caliper width was relatively low.The Ins (+) and Ins (−) groups were analyzed using an unpaired *t*-test or the Mann–Whitney rank sum test to analyze continuous variables in the unmatched cohort. In the propensity score-matched cohort, continuous variables were analyzed using a paired *t*-test or Wilcoxon's signed-rank test. We therefore used a caliper of width 0.04 to maximize the correct matches and reduce bias.

For the comparison of categorical data, the clinical, laboratory and pathological data were compared using the $\chi^2$-test in the unpaired cohort and McNemar's test in the propensity score-matched cohort.

1. Finally, we developed another cohort model with the use of propensity score stratification. The whole population was stratified into quintiles according to their corresponding propensity scores. First, the Breslow-Day test was carried out to examine the hypothesis that the odds ratio (OR) for the renal composite outcome is homogeneous in all stratified layers of propensity score. Next, the Mantel–Haenszel method was used for the analysis of these five categorical variables, and common ORs and 95% confidence intervals (CIs) were calculated.

The results are expressed as the mean with the standard deviation or percentage for categorical data. Two-tailed *P*-values of <0.05 were considered to show statistical significance. IBM SPSS Statistics 25.0 (IBM Inc., Armonk, NY, USA) was used to carry out all of the statistical analyses.

The special ethics committee of Kanagawa Medical Association of Japan (Krec304401.6 March 2018) approved the present study.

## RESULTS

Among the 624 participants, 71 (11.4%) were identified as having achieved the renal composite outcome. As we reported previously, the use of canagliflozin, the use of insulin, the mean arterial pressure after SGLT2i treatment and age showed significant associations with the renal composite outcome (OR 2.42, 95% CI 1.26–4.68, $P < 0.01$; OR 2.15, 95% CI 1.27–3.65, $P < 0.01$; 1.05, 95% CI 1.03–1.08, $P < 0.01$; and OR 1.03, 95% CI 1.00–1.05, $P = 0.04$, respectively)[8].

### Propensity score-matched cohort model

Table 1 shows the baseline characteristics before and after propensity score matching. The two groups showed significant differences in the HbA1c, eGFR, LNACR, and use of glucagon-like peptide-1 receptor agonist, sulphonyl urea and β-blockers in the unmatched cohort model (*P*-values were <0.001, 0.03, <0.001, <0.001, <0.001 and <0.001, respectively). An absolute standardized difference $<1.96\text{x}\sqrt{2/n}$ for measured covariates suggested that the balance between the groups was appropriate[11]. In this matched cohort model, this borderline ($n = 149$ in each group, then $1.96\text{x}\sqrt{2/n} = 0.23$) and all of the standardized differences of the clinical characteristics used as the covariates in the logistic analysis to calculate the propensity score were <0.14. Regarding the hypoglycemic medications that were not used as covariates in the logistic analysis that was used to calculate the propensity score in this model, we observed a significant difference in the use of sulfonylurea between the two groups in the propensity score-matched model ($P < 0.01$). The histogram of the propensity scores before and after propensity score matching is shown in Figure S1.

### Comparison of the renal composite outcome among the 149 propensity score-matched patients in the Ins(+) and Ins(−) groups

Table 2 shows the significantly higher incidence of the renal composite outcome in the Ins(+) group in comparison with the Ins(−) group, with 10 (6.7%) and 23 (15.4%) events reported, respectively ($P = 0.02$). The incidence of the renal composite outcome and changes in clinical parameters after SGLT2is treatment are also shown in Table 2. The change in the eGFR and LNACR did not differ between the two groups to a statistically significant extent; however, the HbA1c value in patients in the Ins(+) group was greater than that in the Ins (−) group ($P = 0.01$).

### The cohort model using propensity score stratification

The mean prevalence of the incidence of the renal composite outcome in each quintile (divided based on propensity score) was as follows [Figure 1]): Q1, propensity score ≤0.12; Q2, propensity score >0.12 to ≤0.18; Q3, propensity score propensity score >0.18 to ≤0.27; Q4, propensity score >0.27 to ≤0.40; and Q5, propensity score >0.40. The *P*-value by the Breslow-Day test was 0.22; thus the hypothesis that the OR for the renal composite outcome is homogeneous in all stratified layers of propensity score was not rejected. Based on this, the analysis by the Mantel–Haenszel method showed a significant difference between the groups ($P = 0.01$). The common OR for the renal composite outcome in the Ins(+) group was 2.20 (95% CI 1.22–3.95, $P = 0.01$).

## DISCUSSION

The present study showed that, in patients using SGLT2is, for which there is abundant evidence of renoprotective effects, a different effect than previously reported was observed in the group receiving concomitant insulin treatment. Despite the lack of significant differences between the Ins(+) and Ins(−) groups with respect to changes in the eGFR or LNACR, it was found that the composite renal outcome in the Ins (+) group was significantly less favorable in comparison with the non-treated group in the propensity score-matched model. The combination of SGLT2is and insulin treatment is widely used in the clinical setting. Therefore, it would be useful to clarify whether

**Table 1** | Baseline characteristics before and after propensity score matching

| | Unmatched cohort ($n$ = 624) | | | Matched cohort ($n$ = 298) | | |
|---|---|---|---|---|---|---|
| | Ins (+) ($n$ = 169) | Ins (−) ($n$ = 455) | $P$-value | Ins (+) ($n$ = 149) | Ins (−) ($n$ = 149) | Standardized difference |
| Age (years) | 60.1 ± 11.6 | 60.6 ± 11.4 | NS (0.66) | 60.0 ± 11.3 | 60.1 ± 11.3 | 0.01 |
| Gender (male) | 107 (63.3%) | 303 (66.6%) | NS (0.44) | 96 (64.4%) | 98 (65.8%) | 0.03 |
| BMI (kg/m$^2$) | 29.5 ± 4.8 | 28.9 ± 5.0 | NS (0.19) | 28.2 ± 4.5 | 28.3 ± 5.2 | 0.02 |
| BW (kg) | 80.2 ± 16.3 | 79.1 ± 16.5 | NS (0.47) | 79.8 ± 16.9 | 80.8 ± 17.1 | 0.06 |
| MAP (mmHg) | 96.1 ± 13.1 | 97.3 ± 11.8 | NS (0.29) | 95.5 ± 12.7 | 95.1 ± 11.7 | 0.03 |
| SBP (mmHg) | 134.8 ± 18.4 | 134.8 ± 16.0 | NS (0.98) | 133.6 ± 17.9 | 133.2 ± 16.3 | 0.02 |
| DBP (mmHg) | 76.8 ± 12.7 | 78.5 ± 12.0 | NS (0.11) | 76.5 ± 12.1 | 76.1 ± 12.0 | 0.03 |
| HbA1c (mmol/mol [%]) | 71.2 ± 14.8 (8.67 ± 1.35) | 61.4 ± 14.4 (7.77 ± 1.32) | <0.001 | 69.4 ± 13.8 (8.50 ± 1.26) | 68.8 ± 17.8 (8.45 ± 1.63) | 0.06 |
| eGFR (mL/min/1.73 m$^2$) | 76.0 ± 21.8 | 80.4 ± 21.8 | 0.03 | 77.5 ± 21.5 | 75.6 ± 21.3 | 0.09 |
| LNACR | 1.75 ± 0.66 | 1.54 ± 0.63 | <0.001 | 1.72 ± 0.64 | 1.72 ± 0.63 | 0.003 |
| Administration periods (months) | 32.9 ± 10.9 | 32.3 ± 10.5 | NS (0.54) | 33.2 ± 11.1 | 33.3 ± 10.0 | 0.02 |
| Types of SGLT2is | | | | | | |
| Ipragliflozin | 44 (26.0%) | 92 (20.2%) | NS (0.12)* | 40 (26.8%) | 35 (23.5%) | 0.09 |
| Dapagliflozin | 28 (16.6%) | 74 (16.3%) | NS (0.93)* | 24 (16.1%) | 30 (20.1%) | 0.14 |
| Tofogliflozin | 18 (10.7%) | 58 (12.7%) | NS (0.48)* | 16 (10.7%) | 18 (12.1%) | 0.07 |
| Luseogliflozin | 13 (7.7%) | 41 (9.0%) | NS (0.60)* | 12 (8.1%) | 12 (8.1%) | 0.0 |
| Canagliflozin | 20 (11.8%) | 58 (12.7%) | NS (0.76)* | 18 (12.1%) | 16 (10.7%) | 0.07 |
| Empagliflozin | 23 (13.6%) | 67 (14.7%) | NS (0.72)* | 18 (12.1%) | 17 (11.4%) | 0.03 |
| SGLT2is were changed during the treatment periods | 23 (13.6%) | 65 (14.3%) | NS (0.83)* | 21 (14.1%) | 21 (14.1%) | 0.0 |
| Concomitant treatments (at the survey) | | | | | | |
| RAS inhibitors | 89 (52.7%) | 236 (51.9%) | NS (0.86)* | 78 (52.3%) | 76 (51.0%) | 0.03 |
| Ca channel blocker | 85 (50.3%) | 192 (42.2%) | NS (0.07)* | 72 (48.3%) | 64 (43.0%) | 0.11 |
| β-Blocker | 33 (19.5%) | 43 (9.5%) | <0.001* | 23 (15.4%) | 25 (16.8%) | 0.05 |
| Statins | 210 (61.0%) | 172 (61.4%) | NS (0.65) * | 85 (57.1%) | 89 (59.7%) | 0.05 |
| Concomitant hypoglycemic treatments (at the survey) | | | | | | $P$-value |
| DPP4 inhibitor | 86 (50.9%) | 256 (56.3%) | NS (0.23)* | 76 (51.0%) | 91 (61.1%) | NS (0.12)† |
| GLP1Ra | 44 (26.0%) | 58 (12.7%) | <0.001* | 37 (24.8%) | 24 (16.1%) | NS (0.09)† |
| Metformin | 98 (58.0%) | 287 (63.1%) | NS (0.25)* | 88 (59.1%) | 99 (66.4%) | NS (0.19)† |
| SU | 30 (17.8%) | 160 (35.2%) | <0.001* | 26 (17.5%) | 66 (44.3%) | <0.01† |
| Pioglitazone | 27 (16.0%) | 89 (19.6%) | NS (0.31)* | 20 (13.4%) | 31 (20.8%) | NS (0.14)† |

Values are expressed as the mean ± standard deviation or $n$/total $n$ (%), and analyses were carried out using an unpaired $t$-test or the $\chi^2$-test* on the unmatched cohort model. McNemar's test† was carried out for the comparison of the use of concomitant hypoglycemic treatments on the matched cohort model. BMI, body mass index; BW, bodyweight; DBP, diastolic blood pressure; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP1Ra, glucagon-like peptide-1 receptor agonist; HbA1c, glycated hemoglobin A1c; Ins, insulin; LNACR, logarithmic value of albumin-to-creatinine ratio; MAP, mean atrial pressure; NS, not significant; RAS, renin–angiotensin system inhibitor; SBP, systolic blood pressure; SGLT2i, sodium–glucose cotransporter 2 inhibitor; SU, sulfonylurea.

or not the combination of insulin treatment and SGLT2is attenuates the renoprotective effect of SGLT2is.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial previously reported that insulin intensive therapy not only failed to significantly reduce cardiovascular events compared with conventional therapy, but also significantly increased mortality by 22%[12]. However, no statistically direct causal relationship was found, and why strict glycemic control increased mortality remains unclear. One possible reason is that it was too late to carry out strict glycemic control in patients with a nearly 10-year history of diabetes; alternatively, the goal HbA1c level in the intensified therapy group was set at <6.0%, and the value reached approximately 6.5% relatively rapidly (within ~6 months), so such rapid glycemic control itself might have been harmful. The present results are similar to these previous findings in that the study design does not allow us to statistically show causality for whether or not concomitant insulin treatment had a direct influence on the increase in renal composite events.

To our knowledge, no large clinical studies have used improvement in renal composite events with insulin as a primary end-point. In the Kumamoto Study, which examined the

**Table 2** | Incidence of the renal composite outcome and changes in the logarithmic value of albumin-to-creatinine ratio and estimated glomerular filtration rate

| (a) The renal outcomes | Ins (+) ($n$ = 149) | Ins (−) ($n$ = 149) | $P$-value | |
|---|---|---|---|---|
| (1) Incidence of a renal composite outcome | 23 (15.4%) | 10 (6.7%) | 0.02* | |
| (2) Incidence of a progression of albuminuria | 19 (12.8%) | 10 (6.7%) | 0.11* | |
| (3) Incidence of an annual decrease in eGFR by ≥15%. | 4 (2.7%) | 0 (0%) | 0.13* | |
| (b) Clinical findings after SGLT2i treatment | | | | The difference and 95% CI (lower, upper) compared with patients in Ins (−) group |
| eGFR at the survey (mL/min/1.73 m$^2$) | 71.2 ± 19.0 | 71.8 ± 21.3 | 0.81† | −0.6 [−5.7, 4.4] |
| the change in eGFR (mL/min/1.73 m$^2$) | −6.3 ± 10.7 | −3.8 ± 11.8 | 0.06† | −2.5 [−5.1, 0.1] |
| the annual eGFR change | −2.3 ± 8.1 | −1.4 ± 6.9 | 0.32† | −0.9 [−2.6, 0.9] |
| LNACR at the survey | 1.64 ± 0.68 | 1.58 ± 0.64 | 0.46† | 0.05 [−0.09, 0.20] |
| the change in LNACR | −0.08 ± 0.47 | −0.14 ± 0.46 | 0.68 | 0.05 [−0.05, 0.16] |
| BMI (kg/m$^2$) | 28.2 ± 4.5 | 28.3 ± 5.2 | 0.89† | −0.1 [−1.2, 1.1] |
| BW (kg) | 76.8 ± 15.5 | 77.1 ± 16.7 | 0.72† | −0.3 [−4.2, 3.6] |
| MAP (mmHg) | 91.7 ± 9.9 | 94.0 ± 91.7 | 0.05† | −2.3 [−4.6, 3.6] |
| SBP (mmHg) | 127.7 ± 14.1 | 130.3 ± 16.0 | 0.15† | −2.5 [−6.0, 0.9] |
| DBP (mmHg) | 73.7 ± 10.8 | 75.9 ± 11.3 | 0.08† | 0.3 [−4.5, 0.0] |
| HbA1c (mmol/mol [%]) | 61.4 ± 14.4 (7.8 ± 1.3) | 57.7 ± 11.9 (7.44 ± 1.1) | 0.01† | 3.8 [0.9, 6.7] (0.3 [0.1, 0.6]) |

Values are shown as the mean ± standard deviation or $n$/total $n$ (%), analyses were carried out using *McNemar's test or †paired $t$-test. BMI, body mass index; BW, bodyweight; CI, confidence interval; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; Ins, insulin; LNACR, logarithmic value of albumin-to-creatinine ratio; MAP, mean arterial pressure; SBP, systolic blood pressure; SGLT2i, sodium–glucose cotransporter 2 inhibitor.

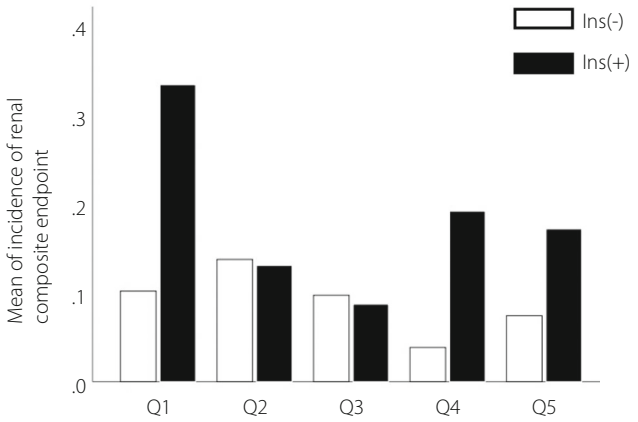


**Figure 1** | Mean incidence of the renal composite outcome according to propensity score quintiles. All patients were stratified into quintiles based on the corresponding propensity score, as follows: Q1, PS ≤0.12; Q2, PS >0.12 to ≤0.18; Q3, PS >0.18 to ≤0.27; Q4, PS >0.27 to ≤0.40; and Q5, PS >0.40. PS, propensity score.

effect of enhanced glycemic control by multiple insulin injection (MIT) on development and progression of microvascular complications in 110 Japanese type 2 diabetes patients receiving insulin treatment, the cumulative incidence of nephropathy (proteinuria) after 8 years in the primary prevention group was 11.5% for the MIT group and 43.5% for the conventional insulin therapy group ($P$ = 0.029), and in the secondary intervention group, the value was 16.0% for the MIT group and 40.0% for the conventional insulin therapy group ($P$ = 0.043). The rates in the conventional insulin therapy group were significantly lower in comparison to those in the CIT group[13].

These studies suggest that although the improvement of glycemic control by insulin might be expected to have a renoprotective effect, a rapid decrease in blood glucose level, which causes hypoglycemia, might also eliminate the renoprotective effect and further fail to inhibit cardiovascular events. Regarding the mechanism by which hypoglycemia itself increases cardiovascular events, various pathways have been postulated, including the induction of inflammatory responses through vascular endothelial growth factor and interleukin-6, vascular endothelial dysfunction, and adverse effects on the blood coagulation system[14]. The fact that many of these mechanisms overlap in the pathogenesis and progression of renal injury[15,16] is considered an important point when assessing the mechanisms underlying increased renal events in patients receiving insulin therapy. Regarding the possible involvement of hypoglycemia, the study protocol should originally have been designed to examine the

presence of insulin-induced hypoglycemia, and the type and dosage of insulin in the present study. The type and dose of insulin were investigated in our study, but the relationship with renal events was not statistically significant (data not shown). In addition, hypoglycemia was not investigated in this study, which was considered to be a limitation and a future issue to address.

As shown in Table 1, the baseline HbA1c value was higher in the Ins (+) group in the unmatched cohort, and even with propensity score matching, glycemic control was poor in the Ins (+) group, even with SGLT2is. Furthermore, the mean body mass index of Ins (+) group was 28.2 kg/m$^2$, as shown in Table 2. In the present study, we did not evaluate homeostasis model assessment-insulin resistance. However, it is widely known that insulin treatment causes lipogenesis and weight gain, resulting in the induction of insulin resistance[17]. This suggests that a large number of patients in the Ins (+) group had some extent of insulin resistance, as their blood glucose levels did not decrease despite insulin treatment. There are many reports that insulin resistance or hyperinsulinism caused by insulin resistance has pathological significance in the kidney. Excessive insulin reportedly increases the expression of nicotinamide adenine dinucleotide phosphate-oxidase in the podocyte, enhances oxidative stress and increases albumin permeability in the podocyte[18]. In addition, clinical studies have reported an association between insulin resistance, and the development and progression of albuminuria in patients with CKD[19], suggesting a possible association of insulin resistance or hyperinsulinemia conditions and increased renal events in the Ins (+) group in this study. Similarly, the proximal tubular $Na^+$-$H^+$ exchanger 3 contributes to 70% of Na reabsorption, and hyperinsulinemia conditions, such as insulin-treated patients and metabolic syndrome, lead to increased Na reabsorption through $Na^+$-$H^+$ exchanger 3 and renal injury through an angiotensin II-mediated mechanism[20]. Furthermore, hyperinsulinemia acts on the distal tubular epithelial Na channel to promote Na reabsorption[21], and might cause renal damage due to hypertension, independent of the renin–angiotensin system; that is, Na-dependent. The impact of hyperinsulinemia on renal function has been reported from clinical studies in Japanese individuals[22]. This might confirm the mechanism of insulin's impact on the kidneys, discussed above, in real clinical practice. The present results also suggest that hyperinsulinemia might have a negative effect on renal protection.

However, the items regarding insulin resistance and blood insulin concentrations were not investigated in the present study, which is also a limitation and an issue to be addressed in the future.

Table 3 summarizes the impact of baseline insulin use on organ protection in four previous large clinical studies using SGLT2is. Some studies did not examine the effect of baseline insulin use on outcomes due to differences in the backgrounds of the patients recruited for each study and the end-points established. However, no evidence has yet been reported of adverse effects of insulin use on organ protection. In the EMPA-REG OUTCOME trial, there were no significant differences in three-point major adverse cardiovascular events (MACE), cardiovascular death, hospitalization for heart failure or the renal outcome between patients with and without insulin use at baseline[23]. The CANVAS program examined only three-point MACE in the presence/absence of insulin, and the results showed no significant effects[2], whereas the DECLARE-TIMI58 trial showed no significant differences in three-point MACE, cardiovascular death, hospitalization for heart failure or the renal outcome between groups. In the DECLARE-TIMI58 trial, insulin use was not found to have any significant effect on three-point MACE, cardiovascular death, hospitalization for heart failure or the renal outcome, nor was any significant effect observed in the data analysis after adjustment for baseline body mass index and eGFR[24]. The CREDENCE trial did not examine insulin use at baseline for any of the outcomes[25]. In this respect, the present results are noteworthy, because they differ from these previous findings. As a possible reason for the

**Table 3** | Outcomes with SGLT2is by baseline insulin therapy

| Outcome | EMPA-REG OUTCOME[23] | CANVAS[2] | DECLARE-TIMI 58[24] | CREDENCE[25] |
|---|---|---|---|---|
| SGLT2i | Empagliflozin | Canagliflozin | Dapagliflozin | Canagliflozin |
| 3-point MACE | $P = 0.28$ | $P = 0.96$ | $P = 0.0586$ ($P = 0.0786$)* | – |
| CV death | $P = 0.92$ | – | ($P = 0.8199$)* | – |
| HHF | $P = 0.72$ | – | $P = 0.6594$ ($P = 0.5231$)* | – |
| Renal | $P = 0.05$ | – | $P = 0.4844$ ($P = 0.4273$)* | – |

*The baseline insulin subgroup was adjusted for baseline BMI, eGFR (CKD-EPI), diabetes duration, HbA1c, ASCVD versus multiple risk factors, history of heart failure, history of MI and all other baseline hypoglycemic treatment. Abbreviations; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CV, cardiovascular; HbA1c, glycated hemoglobin A1c; HHF, hospitalization for heart failure; MACE, major adverse cardiovascular events; MI, myocardial infarction; SGLT2i, sodium-glucose co-transporter inhibitor; EMPA-REG OUTCOME, The Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients; CANVAS, The Canagliflozin Cardiovascular Assessment Study; DECLARE-TIMI 58, The Dapagliflozin Effect on Cardiovascular Events – Thrombolysis in Myocardial Infarction 58; CREDENCE, The Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation.

difference in the present results from previous reports, the effect of the duration of diabetes in Ins (+) group should not be underestimated. In other words, the DECLARE-TIMI58 trial adjusted for the duration of diabetes, but the present study did not. However, the EMPA-REG OUTCOME trial found no significant difference in renal outcomes in the Ins (+) group, despite the fact that they had a longer diabetes duration. Although it is not appropriate to describe the duration of diabetes in our study patients as exactly the same, we would like to discuss it a little here, citing our separate data, as it might be helpful. Our study group also carried out a retrospective survey for the patients who received glucagon-like peptide-1 receptor agonist treatment. The participants in the glucagon-like peptide-1 receptor agonist survey were patients who had visited the clinics of the members of the Kanagawa Physicians Association between July and October 2020. As many of the patients were enrolled from the same institution, it is assumed that a certain percentage overlapped with those included in this analysis. In this glucagon-like peptide-1 receptor agonist survey, data on the duration of type 2 diabetes mellitus were collected; however, it was difficult to collect accurate information on this point. More than two-thirds of patients reported the duration as 'unknown', and most had had type 2 diabetes mellitus for >10 years. From the perspective of concomitant treatment with an SGLT2i or insulin, the distribution of the duration of type 2 diabetes mellitus did not show a significant difference ($P = 0.17$ for a concomitant SGLT2i, and $P = 0.67$ for concomitant insulin; Table S1). Although the clinical background characteristics were balanced by propensity score matching methods, and most patients in the present study were suspected of having had type 2 diabetes mellitus for >10 years, as in our other survey, we cannot deny that the duration of type 2 diabetes mellitus might have influenced the renal outcome. Therefore, further prospective studies are warranted.

The present study was associated with some limitations. First, as described above, although the Ins (+) group would be expected to have a longer duration of diabetes than the Ins (−) group, the duration of diabetes was not investigated in the present study; thus, no adjustment was carried out for this factor. Furthermore, limitations, including racial differences, the small sample size, and the type and dose of insulin used, need to be addressed. In the future, prospective large-scale clinical studies are expected to address the question of whether or not the renoprotective effects of SGLT2is are attenuated by concomitant insulin therapy.

In conclusion, concomitant insulin treatment with an SGLT2i had an effect on the renal composite outcomes of Japanese patients with type 2 diabetes mellitus and CKD. We might need to consider the influence of concomitant agents on the renoprotective effects of SGLT2is.

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## DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: The special ethics committee of Kanagawa Medical Association of Japan approved the present research protocol (Krec304401.6 March 2018).
Informed consent: The opt out consent procedure was used in this study.
Registry and the registration no. of the study/trial: This study was carried out after receiving approval from the special ethics committee of Kanagawa Medical Association of Japan (approval no. Krec304401.6 March 2018).
Animal studies: N/A.

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## SUPPORTING INFORMATION

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

**Figure S1** | The histogram of the propensity score in the unmatched and matched cohorts.

**Table S1** | Duration of type 2 diabetes in patients included in the glucagon-like peptide-1 receptor agonist survey.