Insulin–glucagon-like peptide-1 receptor agonist relay and glucagon-like peptide-1 receptor agonist first regimens in individuals with type 2 diabetes: A randomized, open-label trial study

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Keywords
Glucagon-like peptide-1 receptor agonists, Glucose toxicity, Insulin–glucagon-like peptide-1 receptor agonist relay regimen

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J Diabetes Investig 2022; 13: 965–974
doi: 10.1111/jdi.13749

Clinical Trial Registry
Efficacy and Safety of GLP-1 first therapy compared with insulin GLP-1 relay therapy in type 2 diabetes with inadequate glucose control: a randomized, open-label, multicenter parallel-group study (UMIN000014140).

ABSTRACT
Aims/Introduction: Glucagon-like peptide-1 receptor agonists (GLP-1 RA) might be less effective in patients with severe hyperglycemia, because hyperglycemia downregulated the GLP-1 receptor in an animal study. To examine this hypothesis clinically, we compared the glucose-lowering effects of GLP-1 receptor agonist liraglutide with and without prior glycemic control.

Materials and Methods: In an open-label, parallel trial, participants with poorly controlled type 2 diabetes were recruited and randomized to receive once-daily insulin therapy, degludec (Insulin–GLP-1 RA relay group, mean 16.8 ± 11.4 IU/day), for 12 weeks and then liraglutide for 12 weeks or subcutaneous injections of GLP-1 RA, liraglutide (GLP-1 RA first group, 0.9 mg), for 24 weeks. The primary efficacy end-points consisted of changes in the levels of fasting plasma glucose and glycated hemoglobin (HbA1c).

Results: The median fasting plasma glucose and HbA1c before the study were 210.0 mg/dL and 9.8%, respectively. The levels of fasting plasma glucose and HbA1c significantly decreased in the Insulin–GLP-1 RA relay group (P < 0.001) and GLP-1 RA first group (P < 0.001) by week 24, although no intergroup differences were observed. The reduction of HbA1c in the Insulin–GLP-1 RA relay group tended to be larger than that in the GLP-1 RA first group in the lowest CPR (C-peptide immunoactivity) quartile (P = 0.072). The adverse events consisted of gastrointestinal problems, followed by hypoglycemia.

Conclusions: The GLP-1 receptor agonist is overall effective without prior glycemic control with insulin in participants with poorly controlled type 2 diabetes. However, in participants with insulinopenic type 2 diabetes, prior glycemic control with insulin might overcome glucose toxicity-induced GLP-1 resistance.

INTRODUCTION
Emerging evidence suggests that treatment with glucagon-like peptide-1 (GLP-1) receptor agonists (RA) has beneficial effects on cardiovascular and mortality outcomes in patients with type 2 diabetes. Recent guidelines recommend starting GLP-1 RA among patients with type 2 diabetes who have established atherosclerotic cardiovascular diseases as part of glycemic management. However, the clinical guidelines consistently recommend that the early introduction of insulin for inadequately controlled type 2 diabetes patients, especially with ongoing...
catabolic features, such as weight loss and ketosis if symptoms of hyperglycemia are present, or when glycated hemoglobin (HbA1c) levels >10% (86 mmol/mol) or blood glucose levels ≥300 mg/dL (16.7 mmol/L)³.

In the meta-analysis⁵ of the Liraglutide Effect and Action in Diabetes 5 (LEAD-5) study⁶ and Exenatide once weekly versus insulin glargine for type 2 diabetes (DURATION-3)⁷ by baseline HbA1c quartile between 7.0 and 11.0%, HbA1c values decreased approximately in parallel, with numerically greater reductions in GLP-1RAs than insulin in all quartiles. In contrast, GLP-1 RAs reduce fasting plasma glucose levels to a lesser extent in the highest HbA1c quartiles than basal insulin⁵,⁷, suggesting that GLP-1RA is less effective in reducing fasting plasma glucose in patients with severe hyperglycemia. Indeed, insulin responses to GLP-1RA are substantially reduced in participants with type 2 diabetes compared with non-diabetic control participants⁸. The near-normalization of blood glucose for 4 weeks by intensive insulin therapy improves b-cell responsivenes to GLP-1⁹. In a single-arm study, GLP-1 RA liraglutide maintained glycemic control subsequent to intensive insulin therapy¹⁰. The incretin effect in participants with type 2 diabetes is reported to be reduced because of a defect in b-cell sensitivity to GLP-1 in humans¹¹. This phenomenon might be partly explained by the finding that hyperglycemia downregulates the GLP-1 receptor, which is restored by glucose normalization with phlorizin, in the b-cells in partially pancreatectomized rats and db/db mice¹². However, no past studies have ever directly tested the involvement of glucose toxicity-mediated GLP-1 resistance. Therefore, we hypothesized that eliminating the glucose toxicity with insulin therapy might be a solution to the hyperglycemia-associated GLP-1 resistance. To test this hypothesis, we evaluated the effect of prior insulin therapy on GLP-1 resistance to achieve adequate glycemic goals. The present study questions which of ‘Insulin–GLP-1 RA relay regimen’ or ‘GLP-1 RA first regimen’ is a better treatment option for type 2 diabetes under glucose toxicity states.

MATERIALS AND METHODS

Study design
This was a 24-week, randomized, open-label, parallel-group trial carried out at seven hospitals in Japan between 16 April 2014 and 30 May 2015. This study was designed in accordance with the principles stated in the Declaration of Helsinki. The protocol was reviewed and approved by the institutional review board of each study site (Innovative Clinical Research Center, Kanazawa University, approval number; 2013-081 [1538], 16 April 2014).

Participants
The inclusion criteria consisted of the following: (i) being aged ≥20 years; (ii) having type 2 diabetes mellitus that was poorly controlled (HbA1c levels ≥8.0% 12 weeks before screening); and (iii) having received diet therapy and/or treatment with oral antihyperglycemic drugs for ≥12 weeks. The exclusion criteria consisted of the following: (i) having hypersensitivity or a contraindication to liraglutide or insulin treatment, degludec; (ii) having a medical history and/or complications from diabetic ketoacidosis or severe hypoglycemia; (iii) experiencing severe infection or trauma pre-/post-surgery; (iv) receiving insulin or GLP-1 RA therapy 4 weeks before screening; (v) receiving glucocorticoid therapy; (vi) having poorly controlled hypertension (systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg); (vii) having severe retinopathy; (viii) having a medical history and/or complications from chronic conditions not suitable for the study (e.g., cancer, ischemic heart disease and heart failure); (ix) being pregnant or breastfeeding; and (x) having psychiatric or psychosocial conditions that preclude participation, as assessed by the investigators. Before participation, all eligible individuals provided written informed consent.

Randomization and masking
Participants were randomized in a 1:1 ratio using a computer-generated randomization sequence to the Insulin–GLP-1 RA relay group or GLP-1 RA first group. As this was an open-label trial, no one (participants, investigators and site staff) was masked to the treatment allocation.

Procedures
This was a therapeutic intervention wherein participants received subcutaneous injections of either 0.9 mg liraglutide (Victoza® Subcutaneous Injection; Novo Nordisk, Tokyo, Japan; GLP-1 RA first group) for 24 weeks or insulin degludec (Tresiba® Novo Nordisk; Insulin–GLP-1 RA relay group) once per day for 12 weeks, followed by injections of liraglutide once at bedtime for 12 weeks (Figure S1). Liraglutide injected subcutaneously with a pen device was started at 0.3 mg/day, and escalated by 0.3 mg/3 days to 0.9 mg/day (maximum allowable dosage in Japan). The initiation and titration of degludec were modified according to past studies¹³,¹⁴ and a diabetes treatment guide published by the Japan Diabetes Society¹⁵. The starting doses for insulin degludec were 4 IU at bedtime. Using the self-report data on glucose monitoring at several times including FPG and pre-meal glucose values, target glycemic levels were pre-meal glucose values of 80–110 mg/dL, the investigators and participants carried out daily insulin dose titrations. If participants were currently taking oral hypoglycemic agents, they continued with their baseline doses throughout the study, except for dipeptidyl peptidase-4 inhibitors. Dipeptidyl peptidase-4 inhibitors were stopped after intervention in both groups. The ingestion of any other antihyperglycemic medications was prohibited. During the study period, all participants underwent nutritional and exercise counseling provided by experienced practitioners. In brief, each individual was prescribed a diet to maintain or achieve a body mass index (BMI) of 22.30 kcal/kg/day, 50–60% kcal from carbohydrates, 20–30% kcal from fat and 15–20% kcal from protein. All participants underwent exercise counseling (5–6 metabolic equivalent estimations for 30 min daily) during the study.

966 J Diabetes Invest Vol. 13 No. 6 June 2022 © 2022 The Authors. Journal of Diabetes Investigation published by AASD and John Wiley & Sons Australia, Ltd
Clinical end-points
The primary clinical end-points were evaluated at week 24, and consisted of changes in the levels of FPG, 1,5-anhydroglucitol and HbA1c from baseline.

The secondary clinical end-points were evaluated at week 24, and consisted of changes in blood pressure, heart rate, laboratory parameters, treatment satisfaction, safety and tolerability. Specifically, endothelial function was assessed as the reactive hyperemia index and measured using the EndoPAT 2000 (Itamar Medical, Caesarea, Israel)\(^\text{(16)}\). As the additional end-point, for HbA1c levels particularly, participants were stratified into quartiles (Q1, 7.6–8.7%; Q2, 8.8–9.6%; Q3, 9.7–10.9%; Q4, 11.0–16.5%) according to baseline HbA1c levels to determine their influence on the treatment outcomes. Treatment satisfaction was evaluated using the Diabetes Treatment Satisfaction Questionnaire (DTSQ)\(^\text{(17)}\). The overall treatment satisfaction score was calculated as the sum of DTSQ items 1 (Satisfaction), 4 (Convenience), 5 (Flexibility), 6 (Understanding), 7 (Recommend to others) and 8 (Wish to continue).

Finally, safety and tolerability were surveyed on all participants who received at least one dose of the study medication (the full analysis set [FAS]), and assessed by reviewing the number of general adverse, hypoglycemic and hyperglycemic events, frequency of treatment discontinuations, bodyweight, BMI, waist circumference, and urinary ketone levels.

Statistical analysis
The sample size required to detect a \(-0.9%\) and \(-1.24%\) change in HbA1c levels among the GLP-1 RA first and insulin–GLP-1 RA relay groups, respectively, with a power of 80% (\(\alpha = 0.05\), one-tailed; \(\beta = 0.20\)) and standardized effect size of 0.6 was 50 participants per group\(^\text{(18,19)}\). Considering a dropout rate of 10–15%, we aimed to recruit 120 participants.

The results are expressed as the median (interquartile range). The analyses were carried out on the FAS and a per protocol set up to week 24 using the Wilcoxon signed-rank test for intergroup comparisons, and Student’s \(t\)-test or the Mann–Whitney \(U\)-test for intragroup comparisons. The Statistical Package for the Social Sciences (version 25.0; SPSS, Chicago, IL, USA) was used to carry out the statistical tests; \(P\)-values <0.05 showed significance.

This study was registered at the University Hospital Medical Information Network (UMIN000014140). The funder of the study had no role in the study design, data collection, data analysis, data interpretation or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS
Participant characteristics
In total, 120 individuals participated in the study (\(n = 60\) in the Insulin–GLP-1 RA relay group, \(n = 60\) in the GLP-1 RA first group). Of these, 91 took the study medication up to week 24, whereas 13 discontinued it due to an adverse event or withdrew participation (\(n = 3\) in the Insulin–GLP-1 RA relay group, \(n = 1\) in the GLP-1 RA first group). During follow up, 12 participants were lost (\(n = 3\) in the Insulin–GLP-1 RA relay group, \(n = 9\) in the GLP-1 RA first group; Figure S2). The groups were well balanced with respect to baseline demographics and disease characteristics both in FAS and PSS (Table S1).

The median age of the participants in FAS was 61.0 years; BMI, 25.8 kg/m\(^2\); diabetes duration, 4.0 years; and baseline levels for FPG, HbA1c and CPR, 210.0 mg/dL, 9.8% (8.8–11.1%) and 2.2 ng/mL, respectively. The percentage of newly diagnosed with diabetes was 33.6% (39 participants). Microalbuminuria was present in 17 out of 116 for this study. No study participant experienced macroalbuminuria. The participants have no or mild diabetic complications, such as diabetic nephropathy. The average insulin dose at 12 weeks in FAS given to the Insulin–GLP-1 RA relay group was 16.8 ± 11.4 IU/day (0.26 ± 0.17 kg/\(\text{IU/day}\)).

Glucose metabolism
At 24 weeks, the FPG levels significantly decreased from baseline for the Insulin–GLP-1 RA relay group (215.0–126.0 mg/dL, \(P < 0.001\)) and GLP-1 RA first group (204.0–131.5 mg/dL, \(P < 0.001\)), with no significant differences between groups (\(-68.0 [-145.0 to –120.0] \text{mg/dL in the Insulin–GLP-1 RA relay group,} [–43.0 [–116.0 to –8.0] \text{mg/dL in the GLP-1 RA first group,} \(P = 0.155\)). Similarly, the HbA1c levels significantly decreased from baseline for the Insulin–GLP-1 RA relay group (9.8–6.5%, \(P < 0.001\)) and the GLP-1 RA first group (9.7–6.8%, \(P < 0.001\)), with no significant differences between groups (\(–2.8% [–4.2 to –1.3] \text{in the Insulin–GLP-1 RA relay group,} [–2.1% [–4.0 to –0.7] \text{in the GLP-1 RA first group,} \(P = 0.286\)). The differences between the groups for these primary clinical outcomes were unremarkable (Table 1). As 55 out of 116 participants (47.4%) did not take any anti-diabetes medications, we examined the subanalysis between with and without anti-diabetes medications (Table S7). Both in the Insulin–GLP-1 RA relay group and GLP-1 RA first group, there were no significant differences in FPG and HbA1c changes between participants with and without prior anti-diabetes medications. In addition, the proportion of participants who attained the HbA1c level (HbA1c <7.0%) from FAS was 61.0% in the Insulin–GLP-1 RA relay group and 47.4% in the GLP-1 RA first group, with no significant differences between groups (\(P = 0.140\)).

At 12 weeks, the levels of FPG, HbA1c and 1,5-anhydroglucitol were significantly reduced in the Insulin–GLP-1 RA relay group (215.0–121.0 mg/dL, 9.8–7.1% and 2.4–7.4 µg/mL, \(P < 0.001\) for all) and the GLP-1 RA first group (204.0–126.0 mg/dL, 9.7–7.1% and 2.2–8.7 µg/mL, \(P < 0.001\) for all), although there were no significant differences between them (–60.0 [–123.0 to –90.0] mg/dL, –2.5% [–3.5 to –1.0], and 3.8 [0.0–9.8] µg/mL in the Insulin–GLP-1 RA relay group, –39.0 [–102.0 to –13.0] mg/dL, –1.7 [–3.9 to –0.4] % and 5.5 [0.0–13.1] µg/mL in the GLP-1 RA first group, \(P = 0.286, 0.284\), and 0.326; Table S3).
Table 1 | Changes in the characteristics of patients between baseline and 24 weeks

|                          | Insulin–GLP-1 RA relay | GLP-1 RA first |
|--------------------------|------------------------|----------------|
|                          | Before | 24 weeks | p* | Before | 24 weeks | p* | p** |
| Fasting plasma glucose (mg/dL) | 215.0 (152.5–299.0) | 126.0 (89.0–148.0) | 0.000 | 204.0 (157.8–252.5) | 131.5 (108.0–153.3) | 0.000 | 0.155 |
| Hemoglobin A1c (%)        | 9.8 (9.0–11.2)        | 6.5 (6.0–7.5)     | 0.000 | 9.7 (8.8–11.0)      | 6.8 (5.9–8.1)       | 0.000 | 0.286 |
| 1,5-Anhydroglucitol (µg/mL) | 2.4 (1.1–5.0)        | 9.4 (4.1–16.0)    | 0.000 | 2.2 (1.4–4.1)       | 9.1 (2.6–15.7)      | 0.000 | 0.844 |
| C-peptide immunoreactivity (ng/mL) | 2.0 (1.4–2.7) | 2.2 (1.6–3.2) | 0.062 | 2.4 (1.5–3.0) | 2.6 (1.8–3.9) | 0.008 | 0.535 |
| Bodyweight (kg)          | 65.4 (56.6–739)      | 63.1 (56.5–71.6)  | 0.010 | 70.1 (60.2–83.8)    | 67.4 (58.0–82.5)    | 0.007 | 0.375 |
| Body mass index (kg/m²)  | 25.1 (22.3–28.6)     | 24.2 (21.1–26.9)  | 0.003 | 26.2 (23.3–30.1)    | 25.3 (22.9–30.7)    | 0.004 | 0.504 |
| Waist circumference (cm) | 91.0 (82.0–101.0)    | 86.5 (81.0–94.0)  | 0.000 | 94.8 (85.9–103.6)   | 91.8 (81.0–101.0)   | 0.000 | 0.747 |
| Systolic blood pressure (mmHg) | 125.0 (114.0–139.0) | 126.0 (116.0–138.0) | 0.456 | 127.0 (118.5–138.0) | 132.0 (124.0–140.0) | 0.072 | 0.649 |
| Diastolic blood pressure (mmHg) | 76.0 (68.0–860)    | 78.0 (70.0–84.0)  | 0.729 | 76.0 (68.0–840)     | 78.0 (67.0–89.0)    | 0.184 | 0.439 |
| Heart rate (b.p.m.)      | 77.0 (69.0–860)      | 78.0 (68.0–86.0)  | 0.892 | 76.5 (69.3–87.5)    | 78.0 (70.0–85.0)    | 0.789 | 0.732 |
| White blood cell count (mL) | 6750.0 (5560.0–8010.0) | 5670.0 (4900.0–7140.0) | 0.000 | 6545.0 (5222.5–7502.5) | 5810.0 (4810.0–7180.0) | 0.017 | 0.435 |
| Aspartate transaminase (IU/L) | 21.0 (17.0–310)     | 19.0 (15.0–24.0)  | 0.061 | 22.5 (17.0–37.3)    | 21.0 (17.0–25.0)    | 0.303 | 0.608 |
| Alanine aminotransferase (IU/L) | 27.0 (18.0–490)   | 22.0 (15.0–31.0)  | 0.002 | 28.5 (18.3–360)     | 24.0 (18.0–37.0)    | 0.249 | 0.416 |
| Alkaline phosphatase (IU/L) | 262.0 (219.0–339.0) | 226.0 (186.0–291.0) | 0.004 | 249.0 (200.3–327.5) | 233.0 (187.0–271.0) | 0.014 | 0.867 |
| Serum calcium (mg/dL)     | 9.5 (9.2–9.7)        | 9.5 (8.2–9.8)     | 0.571 | 9.4 (9.1–9.7)       | 9.5 (9.3–9.9)       | 0.133 | 0.419 |
| Serum phosphorus (mg/dL)  | 3.5 (2.9–3.8)        | 3.3 (3.0–3.8)     | 0.736 | 3.2 (2.9–3.7)       | 3.5 (3.1–3.8)       | 0.050 | 0.624 |
| Bone alkaline phosphatase (IU/L) | 13.8 (11.0–179)  | 12.7 (6.3–17.0)   | 0.993 | 13.3 (11.2–16.9)    | 12.8 (10.8–15.7)    | 0.637 | 0.639 |
| Creatinine (mg/dL)        | 0.70 (0.50–0.80)     | 0.70 (0.6–0.8)    | 0.118 | 0.60 (0.5–0.7)      | 0.70 (0.50–0.80)    | 0.013 | 0.462 |
| Uric acid (mg/dL)         | 5.0 (3.9–5.8)        | 5.1 (4.2–6.2)     | 0.363 | 5.0 (3.7–6.3)       | 5.6 (4.3–7.1)       | 0.000 | 0.016 |
| Total cholesterol (mg/dL) | 175.0 (157.0–206.0) | 178.0 (151.0–207.0) | 0.480 | 192.5 (173.3–217.0) | 192.0 (168.0–223.0) | 0.760 | 0.834 |
| Triglycerides (mg/dL)     | 136.0 (94.0–241.0)  | 96.0 (78.0–161.0) | 0.039 | 170.5 (98.3–247.0)  | 112.0 (78.0–191.0)  | 0.006 | 0.953 |
| High-density lipoprotein cholesterol (mg/dL) | 420.0 (350–540) | 460.0 (420–570) | 0.001 | 450.0 (365–520) | 480.0 (380–640) | 0.015 | 0.836 |
| Urinary albumin excretion (mg/gCr) | 0.8 (0.5–2.3) | 1.1 (0.7–2.3) | 0.662 | 1.1 (0.0–3.0) | 1.8 (0.5–5.7) | 0.308 | 0.322 |
| Cystatin C (mg/L)         | 0.89 (0.80–1.10)     | 0.89 (0.80–1.13)  | 0.462 | 0.88 (0.78–1.02)    | 0.90 (0.80–1.00)    | 0.507 | 0.128 |
| Reactive hyperemia index  | 1.53 (1.35–1.87)     | 1.68 (1.46–1.99)  | 0.043 | 1.58 (1.40–2.18)    | 1.61 (1.40–2.33)    | 0.231 | 0.796 |

All values are the median (interquartile range). *P-value for the intragroup comparison (baseline vs end-point). **P-value for the intergroup comparison (change from baseline between groups). GLP-1 RA first, glucagon-like peptide-1 receptor agonist first group; Insulin–GLP-1 RA, receive once-daily insulin therapy, degludec, for 12 weeks and then liraglutide for 12 weeks relay.
From per protocol set, the FPG levels at 24 weeks significantly decreased from baseline for the Insulin–GLP-1 RA relay group (219.0–115.0 mg/dL, \( P < 0.001 \)) and GLP-1 RA first group (197.0–129.0 mg/dL, \( P < 0.001 \)), with no significant differences between groups (−84.5 mg/dL in the Insulin–GLP-1 RA relay group, −48.0 mg/dL in the GLP-1 RA first group, \( P = 0.117 \)). The reduction of HbA1c in the Insulin–GLP-1 RA relay group was relatively larger than that in the GLP-1 RA first group (−3.3% in the Insulin–GLP-1 RA relay group, −2.2% in the GLP-1 RA first group, \( P = 0.055 \); Table S2). The proportion of participants who attained the HbA1c level (HbA1c <7.0%) from per protocol set is 72.3% in the Insulin–GLP-1 RA relay group and 56.5% in the GLP-1 RA first group, with no significant differences between groups (\( P = 0.173 \)).

**Metabolic profile**

For both groups, bodyweight, BMI, waist circumference, white blood cell count, and alkaline phosphatase and triglyceride levels significantly decreased, whereas the high-density lipoprotein level significantly increased. For the GLP-1 RA first group, the uric acid levels significantly increased, whereas in the Insulin–GLP-1 RA relay group, the changes were unremarkable. Alanine aminotransferase levels significantly decreased in the Insulin–GLP-1 RA relay group, but not in the GLP-1 RA first group, with no significant difference between the groups. Also, reactive hyperemia index significantly increased in the Insulin–GLP-1 RA relay group, but not in the GLP-1 RA first group, with no significant difference between the groups (Table 1).

At 12 and 24 weeks, no differences in the HbA1c across all baseline HbA1c quartiles were observed between the Insulin–GLP-1 RA relay group and the GLP-1 RA first group (Figure 1; Table 2). There was no association between HbA1c quartiles and insulin dose by the Kruskal–Wallis test (\( P = 0.230 \)). Each treatment satisfaction score of DTSQ (item 1, 4, 5, 6, 7 and 8) significantly increased for both groups, with no significant differences between the groups. The ratings for the perceived frequency of hyperglycemia or hypoglycemia were also comparable for all participants throughout the study period (Table S4). The median total DTSQ scores for the Insulin–GLP-1 RA relay group and the GLP-1 RA first group were 22.0 and 22.0, respectively, at baseline and 29.0 and 30.0, respectively, at the end of the study. The total DTSQ scores were significantly increased in both groups, with no significant differences between the groups.

Among subanalysis of the changes in HbA1c and FPG according to baseline CPR quartile, there were no significant differences in baseline HbA1c and FPG between the groups, except for baseline HbA1c in the lowest CPR quartile (\( P = 0.022 \); Table S5). There was no association between CPR quartiles and insulin dose by the Kruskal–Wallis test (\( P = 0.432 \)). The reduction of HbA1c in the Insulin–GLP-1 RA relay group tended to be larger than that in the GLP-1 RA first group in the lowest CPR quartile (\( P = 0.072 \)).

Among subanalysis of the changes in HbA1c and FPG according to baseline BMI quartile, there were no significant differences in baseline HbA1c and FPG between the groups (Table S6). There was no association between BMI quartiles and insulin dose by the Kruskal–Wallis test (\( P = 0.290 \)). No differences in the FPG and HbA1c across all baseline BMI quartiles were observed between the Insulin–GLP-1 RA relay group and the GLP-1 RA first groups.

**Adverse events**

With regard to adverse events, gastrointestinal issues, such as nausea, abdominal distension, constipation and diarrhea, were the most common and occurred more frequently in the GLP-1 RA first group than in the Insulin–GLP-1 RA relay group (\( n = 15 \), 26% vs \( n = 6 \), 10%), followed by hypoglycemia in the Insulin–GLP-1 RA relay group (\( n = 5 \), 8%). Because of this, nine participants (\( n = 7 \) in the Insulin–GLP-1 RA relay group, \( n = 2 \) in the GLP-1 RA first group) wanted to discontinue treatment; thus, the dose of liraglutide was reduced to 0.3 mg for one participant and 0.6 mg for two participants according to the physician’s instruction. For two individuals in the Insulin–GLP-1 RA relay group who experienced pneumonia and hyperglycemia, degludec was replaced with intensive insulin therapy (Table 3).

**DISCUSSION**

The present study is the first to discover that insulin therapy before GLP-1 RA therapy does not exert an additive effect on glycemic control to the GLP-1 RA first regimen in individuals with poorly controlled type 2 diabetes.

To date, meta-analyses (with study periods of 16–156 weeks)\(^6,21\) and the clinical trials of LEAD\(^6\), DURATION-3\(^7\) and Exenatide versus Insulin Glargine in Patients with Suboptimally Controlled Type 2 Diabetes (GWAA)\(^22\) have compared the safety and efficacy of GLP-1 RAs versus insulin in participants with type 2 diabetes inadequately controlled by oral hypoglycemic agents. Evidence has shown that GLP-1 RAs are equal or superior to basal insulin in reducing HbA1c levels, although no impressive differences have been observed when stratifying participants by baseline HbA1c quartiles\(^5,7\). Although this latter outcome might seem somewhat unexpected, the average baseline HbA1c level in the aforementioned participants was low (−8%), and thus, such studies might be insufficient to elucidate the involvement of possible GLP-1 resistance among those with higher baseline HbA1c values. In the present study, the average baseline FPG and HbA1c values were 228.9 ± 113.9 mg/dL and 10.0% ± 1.8%, respectively, which were much greater than those described in previous research\(^6,7,22–25\). Furthermore, when we stratified participants with baseline HbA1c levels between 7.6% and 16.5%, both therapy regimens significantly reduced the FPG and HbA1c levels by the end of the first and second phases across all HbA1c quartiles. Taken together, these findings negate the possibility of GLP-1 resistance induced by glucose toxicity, at least in humans.
We evaluated some indirect indices for insulin secretion. As shown in Tables 1 and S2, fasting C-peptide levels were elevated, whereas bodyweight was reduced similarly in both groups. These findings suggest improved insulin secretion either in the ‘Insulin–GLP-1 RA relay regimen’ or ‘GLP-1 RA first regimen,’ which might be indirect evidence for amelioration of glucose toxicity states.

In an animal study\(^{12}\), hyperglycemia downregulated the GLP-1 receptor, which was restored by glucose normalization with phlorizin, in the β-cells both in the insulinopenic diabetic model (partially pancreatectomized rats) and the obese diabetic model (db/db mice). Therefore, we carried out stratified sub-analyses in terms of fasting level of C-peptide and BMI, as shown in Tables S5 and S6, respectively.

The Insulin–GLP-1 RA relay regimen might overcome glucose toxicity-induced GLP-1 resistance in insulinopenic type 2 diabetes participants based on the HbA1c-stratified FAS findings that the reduction of HbA1c in the Insulin–GLP-1 RA

**Figure 1** | Changes in glycated hemoglobin (HbA1c) levels at (a) weeks 12 and (b) 24 weeks, assessed according to the participants’ baseline HbA1c quartile. Blue, glucagon-like peptide-1 receptor agonist first group; Red box; receive once-daily insulin therapy, degludec, for 12 weeks and then liraglutide for 12 weeks relay group.
Table 2 | Changes in glyated hemoglobin and fasting plasma glucose according to baseline glyated hemoglobin quartile

|                        | Insulin–GLP-1 RA relay |                     | GIP-1 RA first |                     |
|------------------------|------------------------|---------------------|----------------|---------------------|
|                        | Before | 12 weeks | 24 weeks | \( P^* \) | Before | 12 weeks | 24 weeks | \( P^* \) | \( p^{**} \) | \( p^{***} \) |
| HbA1c 7.6–8.7% (Q1)    | \( n = 13 \) | 15.0 (12.75–17.75) | 12.9 (8.50–14.55) | 12.0 (10.60–14.30) | 0.041 | 15.5 (14.03–18.70) | 13.6 (10.08–15.38) | 13.7 (11.50–15.33) | 0.019 | 1.000 | 0.756 |
| FPG                    |         | 8.10 (7.75–8.30) | 6.70 (5.75–7.30) | 6.40 (5.45–7.35) | 0.004 | 8.10 (7.97–8.33) | 7.25 (6.40–7.85) | 7.10 (6.45–7.83) | 0.012 | 0.220 | 0.375 |
| Insulin dose           |         | 10.0 (7.5–24.3)  |                   |                |       |                   |                   |                   |       |       |       |
| HbA1c 8.8–9.6% (Q2)    | \( n = 15 \) | 21.40 (14.80–28.00) | 13.5 (12.00–21.30) | 12.0 (98.0–139.0) | 0.001 | 16.30 (15.00–200.0) | 13.70 (106.0–159.0) | 13.60 (119.0–163.0) | 0.010 | 0.067 | 0.005 |
| FPG                    |         | 9.10 (9.00–9.50) | 7.10 (6.50–8.80) | 6.50 (6.10–7.50) | 0.001 | 9.20 (8.80–9.40) | 7.50 (6.40–8.90) | 7.50 (6.50–8.20) | 0.005 | 0.539 | 0.137 |
| Insulin dose           |         | 10.0 (8.0–18.0)  |                   |                |       |                   |                   |                   |       |       |       |
| HbA1c 9.7–10.9% (Q3)   | \( n = 16 \) | 188.5 (142.8–217.3) | 131.5 (102.8–179.3) | 146.5 (106.8–180.5) | 0.008 | 240.5 (166.0–282.0) | 133.0 (110.3–164.3) | 127.0 (111.0–163.0) | 0.006 | 0.193 | 0.139 |
| FPG                    |         | 10.25 (9.93–10.60) | 7.45 (7.03–8.35) | 7.30 (6.08–8.80) | 0.001 | 10.20 (9.88–10.83) | 6.55 (6.08–9.05) | 5.90 (5.78–8.83) | 0.002 | 0.423 | 0.448 |
| Insulin dose           |         | 14.0 (8.8–23.0)  |                   |                |       |                   |                   |                   |       |       |       |
| HbA1c 11.0–16.5% (Q4)  | \( n = 15 \) | 3480 (2620–472.0) | 1100 (910–144.0) | 131.0 (98.0–217.0) | 0.001 | 2490 (2240–407.0) | 124.0 (110.8–211.8) | 131.5 (102.8–153.8) | 0.003 | 0.277 | 0.347 |
| FPG                    |         | 11.70 (11.30–13.70) | 7.00 (6.30–9.00) | 6.40 (5.80–9.50) | 0.001 | 12.30 (11.63–12.78) | 7.10 (6.43–10.80) | 6.90 (6.23–9.10) | 0.005 | 0.867 | 0.683 |
| Insulin dose           |         | 190 (10.5–33.0)  |                   |                |       |                   |                   |                   |       |       |       |

All values are median (interquartile range). \( P^* \)-value for the intragroup comparison (baseline vs 24 weeks); \( p^{**} \)-value for the intergroup comparison (change from baseline between groups, 12 weeks); \( p^{***} \)-value for the intergroup comparison (change from baseline between groups, 24 weeks). FPG, fasting plasma glucose; GLP-1 RA first, glucagon-like peptide-1 receptor agonist first group; HbA1c, glyated hemoglobin; Insulin–GLP-1 RA, receive once-daily insulin therapy, degludec, for 12 weeks and then liraglutide for 12 weeks relay.
Table 3 | Adverse events

|                      | Insulin–GLP-1 (n = 59) | GLP-1 RA (n = 57) |
|----------------------|------------------------|-------------------|
| 0–12 weeks           |                        |                   |
| Nausea               | 0                      | 9 (2)             |
| Hypoglycemia         | 4 (1)                  | 0                 |
| Hyperglycemia        | 1 (1)                  | 0                 |
| Pneumonia            | 1 (1)                  | 0                 |
| Abdomen distension   | 0                      | 2                 |
| Constipation         | 0                      | 1                 |
| Appetite loss        | 0                      | 1                 |
| Diarrhea             | 0                      | 1                 |
| Palpitation without hypoglycemia | 0 | 1 |
| 12–24 weeks          |                        |                   |
| Nausea               | 4 (1)                  | 1                 |
| Hypoglycemia         | 1 (1)                  | 0                 |
| Injection site wheal and flare | 1 (1) | 0 |
| Constipation         | 1 (1)                  | 1                 |
| Diarrhea             | 1                      | 0                 |
| Dizziness            | 1                      | 1                 |
| Appetite loss        | 1                      | 0                 |
| General malaise      | 1                      | 0                 |

Number of participants who discontinued the study medication. GLP-1 RA relay group tended to be bigger than that in the GLP-1 RA first group in the lowest CPR quartile. These findings are consistent with the previous observation study by Usui et al. However, because baseline HbA1c was significantly higher in the Insulin–GLP-1 RA relay group in the lowest baseline CPR quartile, we should carefully conclude whether glucose toxicity-induced GLP-1 resistance exists in the insulinopenic participants. In contrast, no differences in the FPG and HbA1c across all baseline BMI quartiles were observed between the Insulin–GLP-1 RA relay group and the GLP-1 RA first groups, suggesting that BMI might not be involved in the hyperglycemia-induced GLP-1 resistance.

Dosing algorithms are important in evaluating the efficacy of insulin formulations. In the present study, the initial dosing of degludec was selected according to the doses used in clinical trials. However, by week 12, the average daily dose was lower than that observed in predominantly Western populations, but similar to that reported in Japanese populations. Furthermore, the FPG levels in our Insulin–GLP-1 RA relay group were congruent with those detected in the investigations using basal insulin. In the per protocol set, the mean achieved FPG levels were 127.5 ± 47.1 mg/dL, similar to the previous studies, such as the DURATION-3 (mean achieved FPG 124.2 mg/dL) and the LEAD-5 (mean achieved FPG 131.6 mg/dL).

Interestingly, the incidence of hypoglycemia in the Insulin–GLP-1 RA relay group was lower than that of prior research aiming to improve the levels of basal insulin. At the present study’s midway point, the GLP-1 RA first group showed a significantly greater reduction in bodyweight, BMI and waist circumference compared with the Insulin–GLP-1 RA relay group (Tables S2 and S3), which is consistent with the findings from meta-analyses. However, by 24 weeks, the changes between the groups were not appreciable, as liraglutide was found to significantly decrease the aforementioned variables in the Insulin–GLP-1 RA relay group during the study’s second phase. Indeed, among Japanese participants with type 2 diabetes, it is well established that switching from insulin therapy to liraglutide decreases glycemic levels and bodyweight; thus, the administration of a GLP-1 RA might be more ideal, as it prevents the development of hypoglycemia and excess adiposity.

The present study suggests some beneficial effects in the Insulin–GLP-1 RA relay regimen. First, alanine aminotransferase levels significantly decreased in the Insulin–GLP-1 RA relay group, but not in the GLP-1 RA first group. Second, peripheral endothelial function assessed by reactive hyperemia index significantly increased in the Insulin–GLP-1 RA relay group, but not in the GLP-1 RA first group. Third, creatinine and uric acid levels significantly increased in the GLP-1 RA first group, but not in the Insulin–GLP-1 RA relay group. These changes were observed under similar glycemic control and weight reduction between the groups. The possible beneficial significance of initial insulin therapy under poor glycemic status should be examined in the future.

The overall treatment satisfaction scores assessed by DTSQ significantly increased in both groups, with no significant differences between the groups. These findings might be attributable to significant improvement in glycemic control in both groups with no significant changes between the groups.

The present study had some limitations. First, this study had a relatively higher dropout rate than we had expected before the study, which might cause insufficient statistically significant differences in the analyses and difficulty in subanalyses. Second, incretin effects might be more profound in the Asian and far east populations. Hence, further large-scale clinical studies including other populations are required to confirm our conclusion. Third, a once-daily insulin regimen might be insufficient to eliminate glucose toxicity before using GLP-1 RA. However, we were afraid that multiple injections themselves become a bias to affect glycemic control compared with the once-daily GLP-1 RA regimen. Fourth, we could not calculate the achieved time to the target glucose levels in the present study. However, we already reported in a past study that the time to achieve the target glucose levels were 9.4 ± 3.2 days with once-daily basal insulin.

In conclusion, in poorly controlled participants with endogenous insulin secretion-preserved type 2 diabetes, insulin therapy before GLP-1 RA therapy does not exert an additive effect on glycemic control to the GLP-1 RA first regimen in individuals with poorly controlled type 2 diabetes. Hence, once-daily liraglutide is an effective alternative to once-daily insulin therapy.
degludec in glucose toxicity states, avoiding weight gain and hypoglycemia. However, in participants with insulinopenic type 2 diabetes, prior glycemic control with insulin might overcome glucose toxicity-induced GLP-1 resistance.

**DISCLOSURE**

The authors declare no conflict of interest.

Approval of the research protocol: the ethics committee of Kanazawa University Hospital in Japan (approval number; 2013-081 (1538), 16 April 2014).

Informed consent: Before participation, all eligible individuals provided.

Registry and the registration no. of the study/trial: This trial was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN 000014140: 2 June 2014).

Animal studies: N/A.

**ACKNOWLEDGMENTS**

This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and supported by Novo Nordisk Pharma Ltd. The funders had no role in the design, data collection, data analysis, data interpretation, decision to publish or writing the manuscript. T. Takamura received grants from Astellas, Novo Nordisk, Ono Pharmaceutical Co. Ltd., Takeda, Mitsubishi Tanabe Pharma Corp., Sumitomo Dainippon Pharma, Novartis, Eli Lilly and MSD, and lecture fees from MSD, Astellas, AstraZeneca, Ono Pharmaceutical Co. Ltd., Eisai, Sanwa Kagaku Kenkyusho Co., Ltd., Kowa Company Ltd., Kyowa Kirin Co., Ltd., Sanofi, Taisyo Pharma Co., Sumitomo Dainippon Pharma, Takeda, Mitsubishi Tanabe Pharma Corp., Novo Nordisk, Novartis and Eli Lilly.

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**SUPPORTING INFORMATION**

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

**Table S1** | Baseline characteristics of the study participants.

**Table S2** | Changes in the participants’ characteristics between baseline and 24 weeks (per protocol set).

**Table S3** | Changes in the participants’ characteristics between baseline and 12 weeks (full analysis set).

**Table S4** | Changes in the treatment satisfaction scores.

**Table S5** | Changes in glycated hemoglobin and fasting plasma glucose according to baseline CPR quartile.

**Table S6** | Changes in glycated hemoglobin and fasting plasma glucose according to baseline body mass index quartile.

**Table S7** | Changes in the participants' characteristics between baseline and 24 weeks with and without antidiabetes medications.

**Figure S1** | Study protocol.

**Figure S2** | Flowchart of the study participants.