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ORIGINAL ARTICLE

Dipeptidyl peptidase-4 inhibitor, anagliptin, alters hepatic insulin clearance in relation to the glycemic status in Japanese individuals with type 2 diabetes

Takahiro Abe[1], Yasuhiro Matsubayashi[1], Sayaka Muragishi[2], Akihiro Yoshida[1,2], Hideki Suganami[2], Kenichi Furusawa[3], Kazuya Fujihara[1], Shiro Tanaka[4], Kohei Kaku[5], Hirohito Sone[1]*

[1]Department of Hematology, Endocrinology and Metabolism, Niigata University Faculty of Medicine, Niigata, Japan, [2]Kowa Co., Ltd., Tokyo, Japan, [3]Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan, [4]Department of Clinical Biostatistics, Graduate School of Medicine Kyoto University, Kyoto, Japan, and [5]Kawasaki Medical School, Okayama, Japan

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***Correspondence**
Hirohito Sone
Tel.: +81-25-368-9026
Fax: +81-25-368-9300
E-mail address: sone@med.niigata-u.ac.jp

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ABSTRACT

Aims/Introduction: This study investigated the impact of the dipeptidyl peptidase-4 inhibitor, anagliptin, on hepatic insulin clearance (HIC) in Japanese type 2 diabetes patients and explored its relationship to glycemic status.

Materials and Methods: Data on 765 participants in anagliptin phase 2 and 3 studies were analyzed. Adjusted changes in variables during 12 weeks of anagliptin therapy were compared with a placebo. HIC was calculated as the ratio, C-peptide area under the curve 0–120 min to insulin area under the curve 0–120 min, after a meal tolerance test. To explore the effects of baseline HIC levels on variables, participants receiving anagliptin were divided according to quartiles of baseline HIC. Furthermore, multivariate analysis investigated the association between baseline HIC levels and glycemic status.

Results: Anagliptin significantly reduced glycosylated hemoglobin levels ($P < 0.001$ vs placebo) and HIC levels ($P < 0.01$). Longer duration of diabetes, lower body mass index, higher glycosylated hemoglobin and lower insulin secretion capacity were observed with increases in baseline HIC levels. Improvements in glycosylated hemoglobin, glycoalbumin and 1,5-anhydroglucitol levels were greater in the relatively higher HIC group (baseline HIC levels ≥median) than in the lower HIC group (<median).

Conclusions: Anagliptin affected HIC levels according to HIC baseline levels. Higher baseline HIC values might result in improved hyperglycemia through reduced HIC.

INTRODUCTION

Incretin hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are rapidly secreted from the intestines after meals, and enhance insulin secretion dependent on peripheral blood glucose levels[1]. Dipeptidyl peptidase-4 inhibitors (DPP-4is) inhibit the degradation of incretin hormones and reduce hyperglycemia through enhancement of insulin secretion from β-cells. Obesity in Asian people, including Japanese people, is not as prevalent as in those of European descent; therefore, type 2 diabetes mellitus among Asians occurs mainly through a decline in insulin secretion capacity rather than by enhancement of insulin resistance[2]. DPP-4is are extensively used in Japan, because they promote insulin secretion dependent on peripheral blood glucose levels without exaggerating hypoglycemic risk[3].

Insulin secreted from β-cells is first delivered to the liver, where it is degraded, thus diminishing insulin levels in the blood. Thus, blood insulin levels have already been influenced by their hepatic clearance status in the liver. The attenuation of postprandial hyperglycemia through the enhancement of incretin hormones might be attributed partially by their effects on hepatic insulin clearance (HIC)[4]. Previously, controversial results have been reported about the effects of incretin hormones (GLP-1 and GIP) on HIC[5–8]; however, little is known about the effects of DPP-4is on HIC. Furthermore, a greater

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reduction in HbA1c levels through DPP-4is was observed in Asians, who might not be as obese as white people with type 2 diabetes[9]. However, predictors of and the underlying mechanism for the improvement in hyperglycemia through DPP-4is have not been sufficiently investigated[10]. We hypothesized that a higher peripheral insulin supply by reducing HIC would be involved in the mechanism by which DPP-4is enhance the antihyperglycemic effect in Asian people with type 2 diabetes who are not obese. Therefore, we aimed to investigate the effect of a DPP-4i, anagliptin, on HIC in Japanese type 2 diabetes patients who participated in anagliptin phase II/III studies. In addition, we aimed to explore the predictors at baseline for the improvement of hyperglycemia, focusing on their relationship to HIC.

MATERIALS AND METHODS

Studies/patients

A pooled analysis of four anagliptin phase II/III studies[11–13] (Table S1) over a period of 12 weeks that enrolled people with type 2 diabetes was carried out, and various doses of anagliptin, either as monotherapy or as an adjuvant antidiabetic agent, were compared. The DP1003 study (anagliptin 200 and 400 mg monotherapy or placebo) and DP1002 and SK-0403-02 studies (anagliptin 200 mg or placebo as an add-on to other oral antidiabetic agents) were randomized, placebo-controlled, double-blind, parallel-group comparative studies. The SK-0403-01 study (anagliptin 200 mg) was a randomized, open-label, parallel-group comparative study. Meal tolerance tests (MTTs) were carried out in all studies at week 12 of anagliptin treatment. Individual-level data from the 12-week core study periods in each study were used for this analysis. Each included study was reviewed and approved by the institutional review boards of each participating center. All participants provided written informed consent before enrollment.

Measurements

Baseline values of the following laboratory variables were determined: glycosylated hemoglobin (HbA1c), fasting plasma glucose (FPG), glycoalbumin (GA), 1,5-anhydroglucitol (1,5-AG), fasting insulin, fasting C-peptide, homeostatic model assessment of insulin resistance score (HOMA-IR: fasting insulin [μU/mL] × FPG [mg/dL] / 405), homeostatic model assessment of β-cell function (HOMA-β: 360 × fasting insulin [μU/mL] / [FPG [mg/dL] − 63]) and the estimated glomerular filtration rate (eGFR) calculated from serum creatinine (eGFR [mL/min/1.73 m^2]: 194 × serum Cr $[mg/dL]^{-1.094}$ × age $[years]^{-0.287}$ × 0.739 [if female]). Furthermore, study baseline data were assessed on body mass index (BMI) and the presence of estimated fatty liver using the hepatic steatosis index: 8 × (alanine transaminase [ALT] / aspartate transaminase [AST] ratio) + BMI (+2, if female; +2, if diabetes mellitus)[14]. The presence of fatty liver might be estimated in the participants with hepatic steatosis index values >36. An MTT was carried out in the pooled studies. After a minimum fast of 10 h, participants underwent an MTT with a test meal at the medical institutions included in the clinical trials. The test meal contained 500 kcal (50–51% carbohydrate, 10–11% protein and 38–39% lipids), and the same test meal was used in all integrated studies. Insulin and C-peptide were measured during the MTT test. HIC was calculated as the ratio, C-peptide area under the curve $(AUC)_{0–120\ min}$ to insulin $AUC_{0–120\ min}$. Insulinogenic index was also calculated as the ratio, $\Delta insulin_{0–30\ min}$-to-$\Delta glucose_{0–30\ min}$. Finally, the quantitative insulin sensitivity check index (QUICKI) as an insulin sensitivity index was calculated as 1 / (log [fasting insulin] + log [FPG])[15].

Analysis based on quartiles of baseline HIC levels

To assess the effect of baseline HIC levels on variables, participants receiving anagliptin were divided into four groups according to quartiles of baseline HIC levels: quartile 1 (HIC <5.97 pmol·h/L/pmol·h/L), quartile 2 (5.97 pmol·h/L/pmol·h/L ≤ HIC < 7.32 pmol·h/L/pmol·h/L), quartile 3 (7.32 pmol·h/L/pmol·h/L ≤ HIC < 8.88 pmol·h/L/pmol·h/L) and quartile 4 (HIC ≥8.88 pmol·h/L/pmol·h/L). Differences across the quartiles for changes in insulin $AUC_{0–120\ min}$, C-peptide $AUC_{0–120\ min}$, HIC, HbA1c, GA and 1,5-AG were analyzed with an analysis of variance. We defined quartiles 1 and 2 (baseline HIC <the median value) as the relatively lower HIC group, and quartiles 3 and 4 (baseline HIC ≥the median value) as the relatively higher HIC group.

Multivariate analysis

To identify baseline clinical factors that might affect the change in HIC at week 12, 15 clinically significant variables were included at baseline as potential factors in a multivariable model; these were age, sex, dosage of anagliptin, duration of diabetes, HbA1c, HOMA-IR, HOMA-β, BMI, ALT, gamma-glutamyltransferase, triglyceride (TG) category (TG <150 mg/dL vs TG ≥150 mg/dL), low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, eGFR and HIC in the participants receiving anagliptin.

To identify baseline clinical factors that might affect changes in HbA1c, GA and 1,5-AG at week 12, 14 clinically significant variables were included at baseline as potential factors in a multivariable model; these were age, sex, dosage of anagliptin, duration of diabetes, HOMA-β, BMI, ALT, gamma-glutamyltransferase, TG category (TG <150 mg/dL vs TG ≥150 mg/dL), low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, eGFR, HIC (relatively lower HIC group vs relatively higher HIC group) and their baseline value.

Multivariate analysis was followed by stepwise model selection for factors with *P*-values <0.15.

Statistical analysis

Patients were analyzed according to three groupings: anagliptin versus placebo, quartiles of baseline HIC and the relatively lower HIC group versus the relatively higher HIC group in those who received anagliptin, but excluding those receiving a

placebo. In each group, demographics were summarized with appropriate descriptive statistics (means and standard deviation for continuous variables, and numerals and percentages for categorical variables). Changes and percentage changes in variables from baseline to week 12 were analyzed using a *t*-test.

To determine the differences between the placebo and anagliptin, changes in variables at week 12 were analyzed with an analysis of covariance (ANCOVA) model, with the group (placebo and anagliptin) as a fixed effect and their baseline values as covariates.

Furthermore, differences in means and proportions in the baseline assessments between groups (anagliptin vs placebo and the relatively lower HIC group vs the relatively higher HIC group) were analyzed using the Student's *t*-test and Fisher's exact test. Also, differences in means and proportions in the baseline assessments across quartiles were analyzed using analysis of variance and Fisher's exact test. In the present study, all HbA1c values are presented using National Glycohemoglobin Standardization Program and Système International units. All data were analyzed using the SAS System, Release 9.3 (SAS Institute, Cary, NC, USA). The (two-sided) significance level for each test was 0.05, unless otherwise specified.

RESULTS

A total of 765 patients with type 2 diabetes were included in this pooled analysis (Tables S1 and S2); 73% were men. The mean age, duration of diabetes, BMI, HbA1c and HIC were 57.8 years, 8.4 years, 25.1 kg/m^2, 8.1% (64.5 mmol/mol) and 7.70 pmol·h/mL/pmol·h/mL, respectively. During the 12 weeks of treatment with anagliptin, HbA1c levels were significantly reduced (least squares mean −0.63% [−6.83 mmol/mol], $P < 0.001$ vs placebo) and FPG levels were also significantly reduced (−15.5 mg/dL, $P < 0.001$ vs placebo; Table S3). The mean level of insulin $AUC_{0–120\ min}$ in the anagliptin group (mean +12.3%) was significantly increased compared with the placebo group (+4.6%); however, C-peptide $AUC_{0–120\ min}$ was not significantly increased in the anagliptin group (+6.2%) compared with the placebo group (+3.2%). The HIC levels were significantly decreased in the anagliptin group (−1.4%) compared with the placebo group (+2.9%; Table S3 and Figures 1 and 2).

Multivariate analysis showed that higher HIC levels at baseline were negatively correlated with the change in HIC at week 12 (Table 1). Participants receiving anagliptin were divided into four groups according to the quartiles of baseline HIC levels (Table 2). The duration of diabetes was longer, and

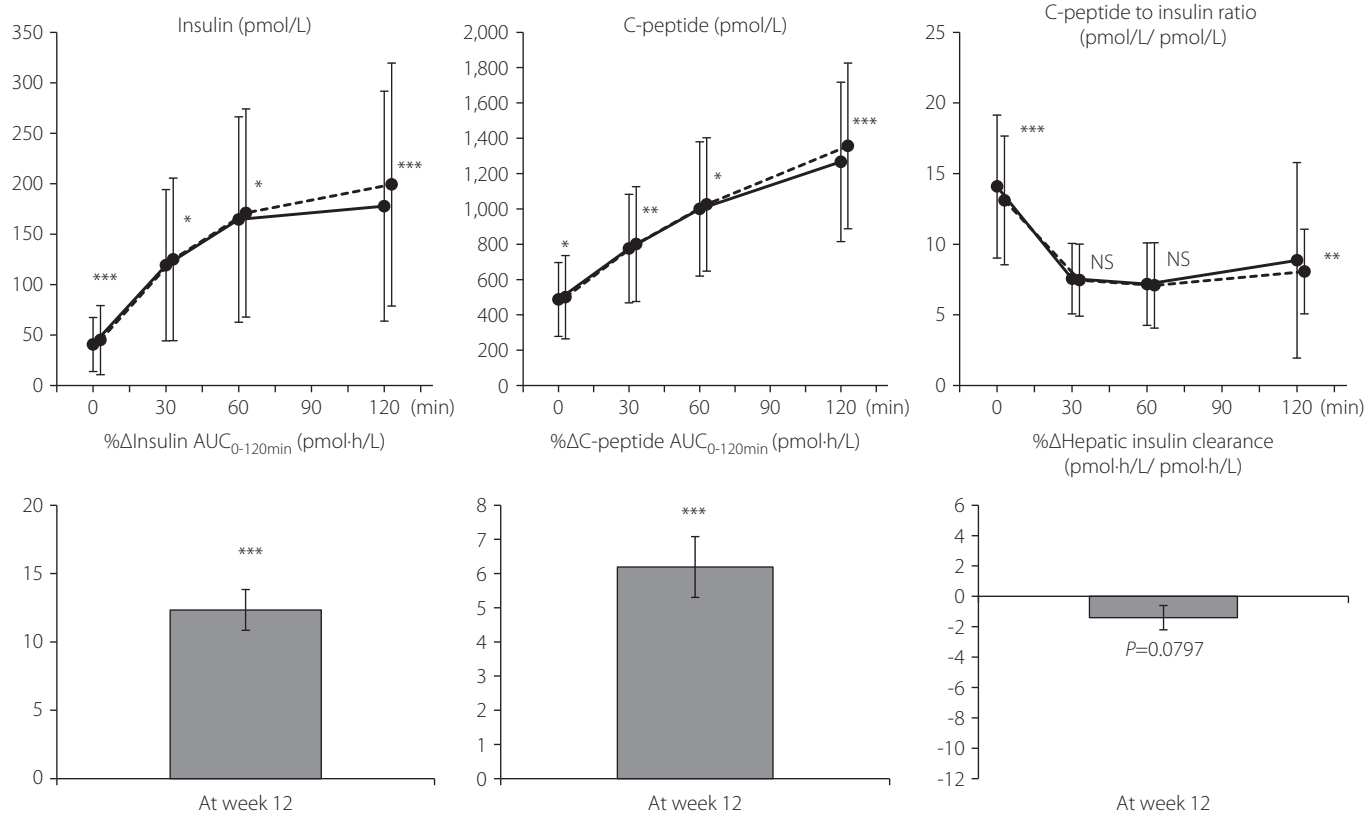

Figure 1 | Insulin, C-peptide and hepatic insulin clearance after meal tolerance test in participants receiving anagliptin. Data are expressed as the mean (standard deviation). Paired *t*-test, not significant (NS) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (vs baseline). Upper panels: ●, anagliptin; solid line, baseline; dotted line, at week 12. Lower panels: ■, anagliptin. AUC, area under the curve.

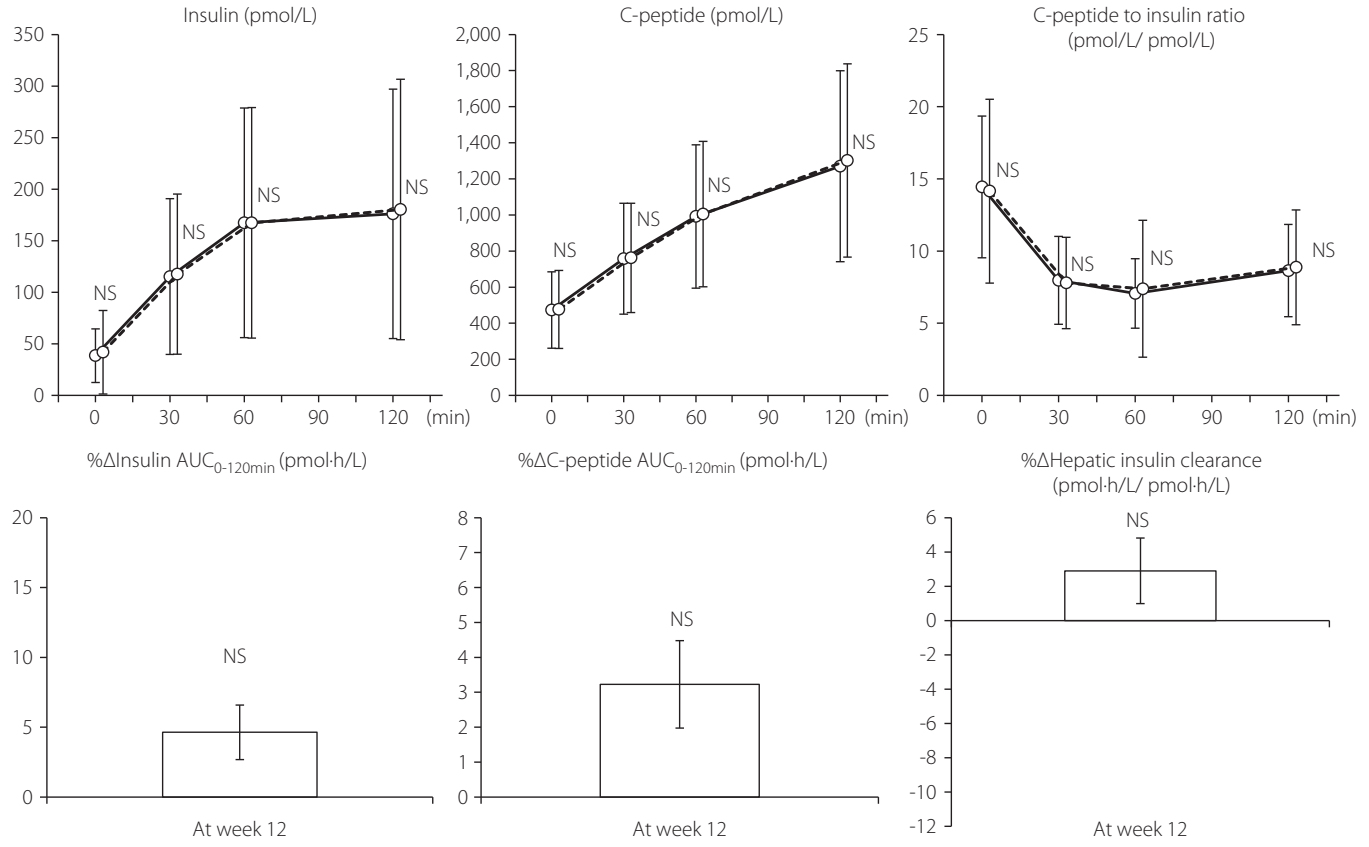

Figure 2 | Insulin, C-peptide and hepatic insulin clearance after meal tolerance test in participants receiving placebo. Data are expressed as the mean (standard deviation). Paired *t*-test, not significant (NS) (vs baseline). Upper panels: ○, placebo; solid line, baseline; dotted line, at week 12. Lower panels: □, placebo. AUC, area under the curve.

Table 1 | Baseline predictors influencing the change in hepatic insulin clearance levels at week 12

Factors	Regression coefficient	*P*
Men	0.39	0.0048
TG ≥150	0.22	0.1113
HDL-C (higher 1 mg/dL)	0.01	0.0160
HOMA-β (higher 1 unit)	−0.01	0.0382
ALT (higher 1 IU/L)	−0.01	0.0024
Hepatic insulin clearance† (higher 1 pmol·h/L/pmol·h/L)	−0.35	<0.001
Anagliptin 400 mg (vs 200 mg)	−0.58	0.0027

Selected factors for multivariate analysis: age, sex, dosage of anagliptin, duration of diabetes, glycosylated hemoglobin, homeostatic model assessment of insulin resistance score, homeostatic model assessment of β-cell function score (HOMA-β), body mass index, alanine transaminase (ALT), gamma-glutamyltransferase, triglycerides (TG) category (TG <150 vs TG ≥150), low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL-C), estimated glomerular filtration rate and hepatic insulin clearance. $P < 0.15$. †C-peptide area under the curve $(AUC)_{0–120\ min}$-to-insulin $AUC_{0–120\ min}$ ratio.

HbA1c and FPG levels were higher, whereas BMI, insulin secretion capacity levels and the proportion of the presence of estimated fatty liver were lower based on increased baseline HIC levels. At week 12 a greater increase in insulin $AUC_{0–120\ min}$ was observed according to increasing baseline HIC levels across quartiles ($P < 0.001$), whereas C-peptide $AUC_{0–120\ min}$ did not change across the quartiles. The HIC levels in quartile 1 were significantly increased from baseline to week 12, whereas in both quartiles 3 and 4, they were significantly decreased. The changes in HIC levels were significantly different across the quartiles ($P < 0.001$; Table 3). QUICKI levels significantly increased in quartile 1 (mean [standard error], +0.005 [0.002], $P < 0.001$ vs baseline).

Baseline characteristics in the relatively lower and higher HIC group, respectively, are shown in Table S4. In the relatively higher HIC group, peripheral blood insulin levels were significantly increased from baseline to week 12; also, C-peptide-to-insulin ratios were significantly decreased at all periods during the MTT (Fig. 3). Insulin $AUC_{0–120\ min}$ was significantly increased (mean +21.6%, $P < 0.001$ vs baseline) in the relatively

Table 2 | Baseline characteristics according to quartiles of baseline hepatic insulin clearance

	Hepatic insulin clearance				*P*
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
n	140	140	140	141	
Age (years)	56.0 (10.7)	58.5 (9.9)	57.8 (9.2)	58.0 (9.8)	0.159
Sex, *n* (men/women)	79 (56.4)/61 (43.6)	95 (67.9)/45 (32.1)	102 (72.9)/38 (27.1)	128 (90.8)/13 (9.2)	<0.001
Anagliptin, *n* (200/400 mg)	123 (87.9)/17 (12.1)	124 (88.6)/16 (11.4)	121 (86.4)/19 (13.6)	135 (95.7)/6 (4.3)	0.049
Mono/α-GI/Met/SU/TZD, *n*	70 (50.0)/8 (5.7)/24 (17.1)/20 (14.3)/18 (12.9)	75 (53.6)/14 (10.0)/16 (11.4)/20 (14.3)/15 (10.7)	72 (51.4)/14 (10.0)/19 (13.6)/19 (13.6)/16 (11.4)	54 (38.3)/26 (18.4)/9 (6.4)/30 (21.3)/22 (15.6)	–
Duration of diabetes (years)	6.3 (4.1)	8.7 (6.2)	8.5 (5.6)	9.8 (6.3)	<0.001
BMI (kg/m^2)	27.1 (4.5)	25.5 (3.7)	24.6 (3.7)	23.7 (3.7)	<0.001
eGFR (mL/min/1.73 m^2)	86.4 (18.9)	81.8 (17.3)	85.1 (17.4)	85.3 (16.9)	0.143
HbA1c (%)	7.8 (0.8)	8.0 (0.9)	8.2 (1.0)	8.3 (1.1)	<0.001
HbA1c (mmol/mol)	62.2 (8.3)	63.4 (9.6)	65.8 (11.1)	67.5 (12.5)	<0.001
FPG (mg/dL)	153.7 (29.2)	155.9 (31.9)	164.1 (35.9)	174.7 (44.6)	<0.001
GA (%)	20.3 (3.7)	21.7 (4.0)	23.1 (4.1)	24.2 (5.2)	<0.001
1,5-AG (μg/mL)	6.8 (5.2)	5.7 (4.3)	5.5 (4.5)	4.7 (4.1)	0.002
Glucose AUC (mg·h/dL)	425.0 (77.7)	435.2 (81.8)	446.7 (98.5)	474.5 (105.1)	<0.001
Bodyweight (kg)	71.0 (14.6)	68.1 (12.6)	67.2 (11.3)	65.5 (12.6)	0.004
HOMA-β	45.1 (26.2)	29.8 (17.4)	20.1 (9.3)	14.0 (8.2)	<0.001
Insulinogenic index	0.59 (0.42)	0.45 (0.83)	0.31 (0.37)	0.15 (0.39)	<0.001
HOMA-IR	4.1 (2.3)	2.8 (1.6)	2.3 (1.5)	1.8 (1.5)	<0.001
Fasting insulin (pmol/L)	63.5 (32.2)	42.5 (21.6)	32.3 (16.6)	24.1 (14.9)	<0.001
Fasting C-peptide (pmol/L)	598.6 (235.6)	499.1 (196.7)	436.9 (172.4)	412.0 (178.9)	<0.001
Insulin $AUC_{0–120\ min}$ (pmol·h/L)	469.9 (166.2)	298.4 (98.8)	212.0 (62.4)	148.1 (62.2)	<0.001
C-peptide $AUC_{0–120\ min}$ (pmol·h/L)	2344.7 (687.3)	1963.7 (611.7)	1697.0 (486.8)	1564.6 (574.9)	<0.001
Hepatic insulin clearance (pmol·h/L/pmol·h/L)	5.10 (0.60)	6.62 (0.38)	8.04 (0.45)	10.95 (2.06)	<0.001
AST (IU/L)	26.6 (10.0)	24.4 (7.6)	24.1 (7.8)	23.3 (8.3)	0.008
ALT (IU/L)	32.7 (18.0)	28.4 (14.5)	27.3 (14.6)	25.7 (13.9)	0.001
GGT (IU/L)	44.3 (34.8)	42.4 (32.5)	40.4 (35.0)	50.6 (46.1)	0.124
Estimated fatty liver, *n* (%)	76 (54.3)	58 (41.4)	49 (35.0)	33 (23.4)	<0.001
LDL-C (mg/dL)	124.4 (30.5)	125.2 (27.7)	119.3 (24.9)	122.4 (30.5)	0.310
HDL-C (mg/dL)	53.1 (11.4)	53.6 (14.2)	59.2 (16.0)	59.0 (16.3)	<0.001
TG (mg/dL)	158.8 (119.2)	158.2 (127.0)	142.9 (158.8)	146.9 (199.8)	0.770

Participants receiving anagliptin were divided into four groups according to the quartile of baseline hepatic insulin clearance levels: quartile 1 (hepatic insulin clearance < 5.97 pmol·h/L/pmol·h/L), quartile 2 (5.97 pmol·h/L/pmol·h/L ≤ hepatic insulin clearance < 7.32 pmol·h/L/pmol·h/L), quartile 3 (7.32 pmol·h/L/pmol·h/L ≤ hepatic insulin clearance < 8.88 pmol·h/L/pmol·h/L) and quartile 4 (hepatic insulin clearance ≥ 8.88 pmol·h/L/pmol·h/L). Data are expressed as the mean (standard deviation). Analyses were carried out by analysis of variance and Fisher's exact test. α-GI, alpha-glucosidase inhibitor; 1,5-AG 1,5-anhydroglucitol; ALT, alanine transaminase; AST, aspartate transaminase; AUC, area under the curve; BMI, body mass index; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; GA, glycoalbumin; GGT, gamma-glutamyltransferase; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostatic model assessment of β-cell function score; HOMA-IR, homeostatic model assessment insulin resistance; LDL-C, low-density lipoprotein cholesterol; Met, metformin; Mono, monotherapy; SU, sulfonylurea; TG, triglycerides; TZD, thiazolidinedione

Table 3 | Changes in variables from baseline to week 12

	Hepatic insulin clearance				*P*
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Insulin $AUC_{0–120\ min}$ (pmol·h/L)					
n	137	135	137	140	
Mean (SE)	−14.2 (7.7)	23.7 (7.7)**	39.8 (7.7)***	27.9 (7.6)***	<0.001
C-peptide $AUC_{0–120\ min}$ (pmol·h/L)					
n	137	135	137	140	
Mean (SE)	35.5 (34.7)	103.5 (34.9)**	124.5 (34.6)***	63.2 (34.3)**	0.264
Hepatic insulin clearance (pmol·h/L/pmol·h/L)					
n	137	135	137	140	
Mean (SE)	0.42 (0.12)***	0.06 (0.12)	−0.41 (0.12)***	−1.14 (0.12)***	<0.001
HbA1c (%)					
n	138	136	137	140	
Mean (SE)	−0.51 (0.05)***	−0.59 (0.05)***	−0.71 (0.05)***	−0.72 (0.05)***	0.005
GA (%)					
n	138	136	137	140	
Mean (SE)	−2.2 (0.2)***	−2.6 (0.2)***	−3.4 (0.2)***	−3.6 (0.2)***	<0.001
1,5-AG (μg/mL)					
m	138	136	137	140	
Mean (SE)	3.6 (0.3)***	3.6 (0.3)***	4.1 (0.3)***	3.6 (0.3)***	0.505

Participants receiving anagliptin were divided into four groups according to quartiles of baseline hepatic insulin clearance levels: quartile 1 (hepatic insulin clearance < 5.97 pmol·h/L/pmol·h/L), quartile 2 (5.97 pmol·h/L/pmol·h/L ≤ hepatic insulin clearance < 7.32 pmol·h/L/pmol·h/L), quartile 3 (7.32 pmol·h/L/pmol·h/L ≤ hepatic insulin clearance < 8.88 pmol·h/L/pmol·h/L) and quartile 4 (hepatic insulin clearance ≥ 8.88 pmol·h/L/pmol·h/L). Mean (standard error) for variables. ANOVA across the quartiles. Paired-*t* vs baseline. 1,5-AG, 1,5-anhydroglucitol; AUC, area under the curve; GA, glycoalbumin; HbA1c, glycosylated hemoglobin; SE, standard error. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$ vs baseline.

higher HIC group, whereas there was no significant increase in the percentage change in insulin $AUC_{0–120\ min}$ (+3.0%, not significant) in the relatively lower HIC group. Additionally, c-peptide $AUC_{0–120\ min}$ was significantly increased in both the relatively higher HIC group (+7.7%, $P < 0.001$) and the relatively lower HIC group (+4.7%, $P = 0.0001$). As a result, HIC was significantly reduced (−7.4%, $P < 0.001$) in the relatively higher HIC group, whereas it was significantly increased (+4.7%, $P < 0.001$) in the relatively lower HIC group (Fig. 3). Multivariate analysis showed that greater reductions in HbA1c and glycoalbumin levels, and a greater increase in 1,5-anhydroglucitol levels were observed in the relatively higher HIC group (Table 4).

DISCUSSION

The present study is the first to find that a DPP-4i significantly reduced HIC, which allowed an additional supply of insulin into peripheral blood. Multivariate analysis showed that a greater reduction in HIC was observed in participants with higher baseline HIC levels. Also, larger improvements in glycemic status were shown among participants with higher baseline HIC levels. Taken together, baseline HIC status might be a predictor related to the improvement in hyperglycemia through the DPP-4i, anagliptin. Evaluation of baseline HIC levels before the initiation of anagliptin administration might provide information about the population that would be expected to achieve greater reductions in hyperglycemia through anagliptin-induced HIC reductions in clinical settings.

In the present analysis, we examined the ratio of C-peptide AUC-to-insulin AUC as a surrogate index to estimate HIC status. The definition of the ratio of C-peptide AUC-to-insulin AUC to show HIC has been a matter of debate. In the current study, the difference in the 30-min value of the C-peptide-to-insulin ratio after the administration of anagliptin was small, and that difference tended to be greater at 90 and 120 min (Figure 3). The possibility that the $AUC_{0–120\ min}$ ratio includes not only HIC, but also systemic insulin clearance, cannot be completely ruled out. However, in many reports, HIC was calculated using the $AUC_{0–120\ min}$ ratio[16–18]. Furthermore, that index was reported to be closely correlated with HIC ($r = 0.74$, $P < 0.001$) measured by the euglycemic hyperinsulinemic clamp method[19]. Therefore, the index, the C-peptide $AUC_{0–120\ min}$ to insulin $AUC_{0–120\ min}$ ratio, might indicate mainly HIC and was used in the present study.

Higher HIC might reduce peripheral insulin levels and require postprandial hypersecretion of insulin from β-cells to provide sufficient peripheral insulin[20]. In contrast, the reduced HIC might cause an excessive postprandial insulin supply, which could cause hyperinsulinemia, obesity, metabolic syndrome and insulin resistance[7,16]. The present study

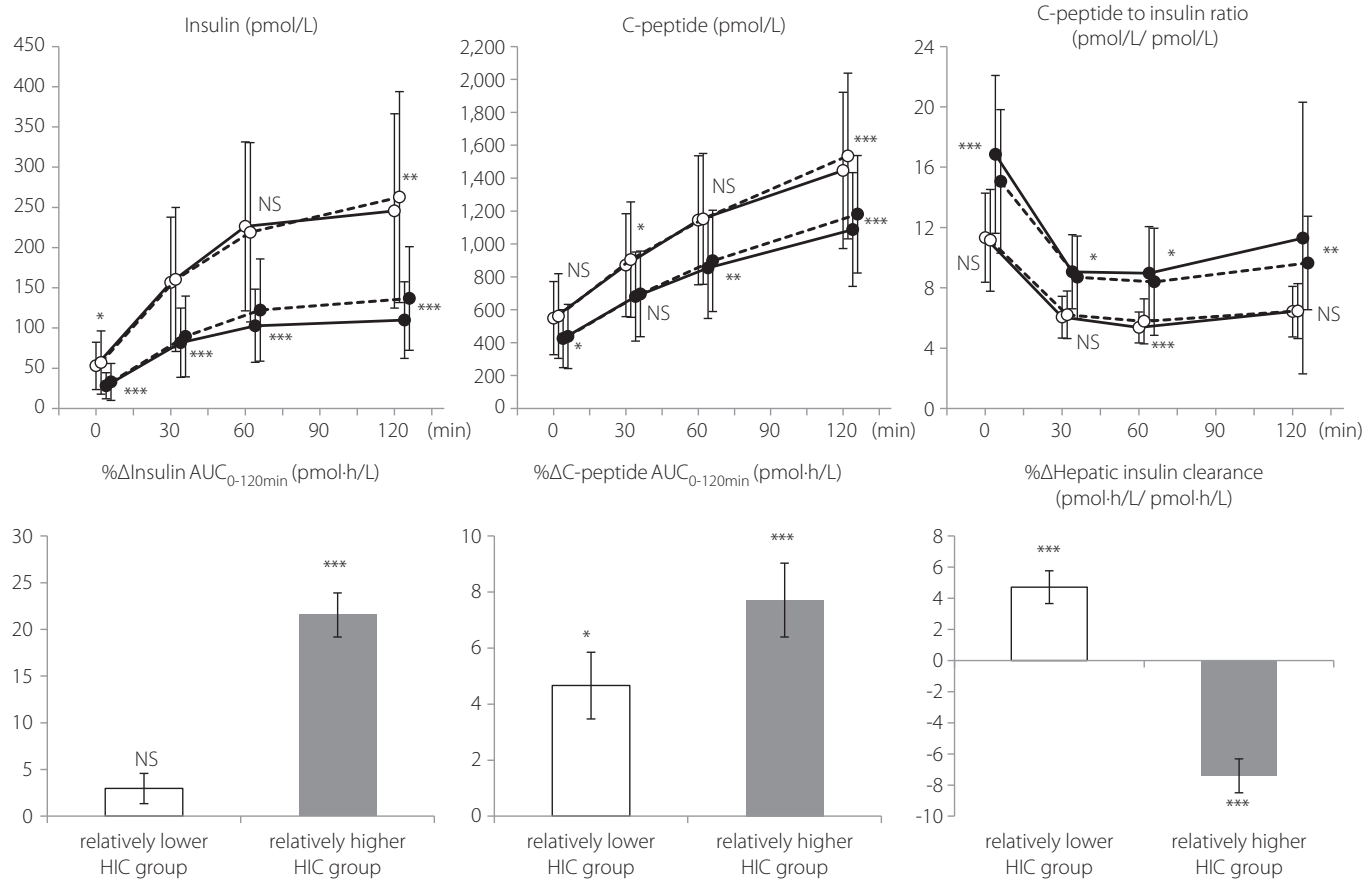

Figure 3 | Insulin, C-peptide and hepatic insulin clearance after meal tolerance test according to baseline hepatic insulin clearance (HIC; relatively lower HIC group vs relatively higher HIC group). Participants receiving anagliptin were divided into four groups according to the quartiles of baseline HIC levels: quartile 1 (HIC < 5.97 pmol·h/L/pmol·h/L), quartile 2 (5.97 pmol·h/L/pmol·h/L ≤ HIC < 7.32 pmol·h/L/pmol·h/L), quartile 3 (7.32 pmol·h/L/pmol·h/L ≤ HIC < 8.88 pmol·h/L/pmol·h/L) and quartile 4 (HIC ≥8.88 pmol·h/L/pmol·h/L). Quartiles 1 and 2 were defined as the relatively lower HIC group, and quartiles 3 and 4 were defined as the relatively higher HIC group. Data are expressed as the mean (standard deviation). Paired *t*-test, not significant (NS), **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (vs baseline). Upper panels: ○, relatively lower HIC group; ●, relatively higher HIC group; solid line, baseline; dotted line, at week 12. Lower panels: □, relatively lower HIC group; ■, relatively higher HIC group. AUC, area under the curve.

showed that anagliptin might promote an additional postprandial insulin supply in participants with a lower peripheral insulin supply by reducing HIC levels. This study also suggested that an excessive insulin supply might not be elicited in participants with sufficient postprandial insulin levels, which provides support that DPP-4is might not exaggerate hyperinsulinemia, which would be likely to cause weight gain and hypoglycemia[3]. HIC levels were significantly decreased from baseline to week 12 in the quartile 3 and 4 groups, which had baseline HIC levels that were relatively higher (Table 3). In contrast, HIC levels were significantly increased in quartile 1, but were not significantly changed in quartile 2 (Table 3). In quartiles 3 and 4, the reduction in HIC might contribute to the improvement in blood glucose, whereas the excessive peripheral insulin supply due to the reduction in HIC might lead to the risk of various metabolic disorders in the future. In contrast, in patients with low baseline HIC, anagliptin does not alter or elevate HIC, so the benefit of glycemic control is relatively small. Also, the risk of contributing to the development of metabolic abnormalities is considered to be low.

Some investigations of the effects of incretin hormones on HIC have produced controversial results. An exogenously administered incretin hormone and a DPP-4i, sitagliptin, did not influence HIC in healthy volunteers[8,21], whereas the exogenously administered GIP reduced HIC in first-degree relatives of type 2 diabetes patients[7]. Basic experiments showed that exogenously administered GLP-1 reduced HIC in a mouse model[6], and that the double knockout mouse model for GIP and GLP-1 receptor showed increased HIC[5]. Furthermore,

Table 4 | Baseline predictors influencing the change in glucose-related indices

Change in HbA1c			Change in GA			Change in 1,5-AG		
(A) Factors	β	*P*	(B) Factors	β	*P*	(C) Factors	β	*P*
eGFR (higher 1 mL/min/1.73m^2)	0.01	<0.001	eGFR (higher 1 mL/min/1.73 m^2)	0.02	0.0004	eGFR (higher 1 mL/min/1.73 m^2)	−0.02	0.0260
TG (≥150 mg/dL)	0.08	0.1192	Duration of diabetes (higher 1 year)	0.04	0.0176	ALT (higher 1 IU/L)	0.02	0.0374
GGT (higher 1 IU/L)	0.001	0.1006	GA (higher 1%)	−0.31	<0.001	Anagliptin 400 mg (vs 200 mg)	0.004	0.0547
Anagliptin 400 mg (vs 200 mg)	−0.11	0.1446	Hepatic insulin clearance (vs relatively lower HIC group)	−0.38	0.0281	1,5-AG (higher 1 μg/mL)	0.28	<0.001
HbA1c (higher 1%)	−0.21	<0.001				Hepatic insulin clearance (vs relatively lower HIC group)	0.67	0.0111
Hepatic insulin clearance (vs relatively lower HIC group[†])	−0.10	0.0267						

Selected factors for the multivariate analysis: (A) age, sex, dosage of anagliptin, duration of diabetes, glycosylated hemoglobin (HbA1c), homeostatic model assessment of β-cell function (HOMA-β), body mass index (BMI), alanine transaminase (ALT), gamma-glutamyltransferase (GGT), triglycerides category (TG <150 vs TG ≥150), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), estimated glomerular filtration rate (eGFR) and hepatic insulin clearance (relatively lower hepatic insulin clearance [HIC] group vs relatively higher HIC group); (B) age, sex, dosage of anagliptin, duration of diabetes, glycoalbumin (GA), HOMA-β, BMI, ALT, GGT, TG category (TG <150 vs TG ≥150), LDL-C, HDL-C, eGFR and hepatic insulin clearance (relatively lower HIC group vs relatively higher HIC group); (C) age, sex, dosage of anagliptin, duration of diabetes, 1,5-anhydroglucitol, HOMA-β, BMI, ALT, GGT, TG category (TG < 150 vs TG ≥ 150), LDL-C, HDL-C, eGFR and hepatic insulin clearance (relatively lower HIC group vs relatively higher HIC group). $P < 0.15$. β, Regression coefficient.
[†]Baseline HIC levels <the median value.

backgrounds of study participants with reference to race and ethnicity were likely to influence HIC levels[22,23]. In the present study, baseline HIC was negatively correlated with the effect of anagliptin on HIC at week 12 from the result of multivariate analysis; thus, baseline HIC status might have an important impact on the effects of incretin hormones on HIC. The impact on HIC through the DPP-4i might be attributed to the baseline HIC status; therefore, consistent results might not be observed through the intervention of DPP-4i and exogenous incretin hormones.

In the current study, a longer duration of diabetes and lower baseline BMI, fasting and postprandial insulin, and insulin secretion capacity and a smaller proportion of estimated fatty liver were observed according to increased baseline HIC levels. Also, baseline HIC levels were significantly associated with the baseline parameters that were previously reported. Baseline HIC levels were negatively associated with fat accumulation in the whole body[24], liver[25] and visceral areas[26]. The characteristics of participants with high baseline HIC levels might be similar to those in Asian people with type 2 diabetes who are not obese.

This is the first study to explore potential clinical factors, including HIC status, that might influence the degree of reductions in HbA1c and GA, and the increase in 1,5-AG through anagliptin. Previously, age[27], duration of diabetes[28], BMI[29], glycemic status[10], insulin secretion capacity, insulin resistance[30], racial differences[9,10] and nutrients[31,32] before the start of treatment with DPP-4i were proposed as independent factors that might influence a reduction in HbA1c levels. A greater lowering effect on HbA1c through DPP-4i was observed in Asian participants with type 2 diabetes than in non-Asians[9]. Differences in insulin secretion capacity[33], postprandial GLP-1 secretin[34] and nutrients[31] between those participants were proposed as the underlying mechanism. Also, higher BMI levels in type 2 diabetes participants were thought to weaken the degree of reduction in HbA1c[29], because serum activity of DPP-4 was positively correlated with BMI values[35], and lower levels of postprandial GLP-1 secretion were seen in non-diabetic participants with obesity[36]. The current study suggested that a high baseline HIC level might be one of the predictors of the antihyperglycemic effects through anagliptin.

Sulfonylureas were reported to decrease HIC, but sulfonylurea-induced changes in HIC have not been investigated according to an association with baseline HIC values[37–39]. Similarly, there have been investigations of the effect of incretin (GLP-1, GIP) on HIC, but the results were controversial and not evaluated by baseline HIC[5–8]. Thus, it is unclear whether this effect is a DPP-4 inhibitor-specific effect of insulin secretagogues. However, in the current study, a similar degree of increases in C-peptide AUC among quartiles after anagliptin treatment was observed, with HIC significantly reduced in quartiles 3 and 4 and relatively higher baseline HIC groups (Table 3). These results suggested that changes in HIC were not simply affected by insulin secretion alone. To further support the DPP-4 inhibitor-induced unique effects on HIC,

further investigations of participants with similar clinical backgrounds (baseline HIC levels, race and ethnicity) to those in the current study using the other insulin secretagogues will be required.

Tolerance tests that enhance secretion of incretin hormones might be preferred when the effects of DPP-4is are investigated. The secretion of incretin hormones was more greatly enhanced in the mixed meal (glucose, fat and protein) tolerance test than in tests with only glucose, protein and fat in a previous report[4]. Therefore, incretin effects by DPP-4is might be less with the oral glucose tolerance test than with the MTT. Similarly, these effects might be smaller with single fat or protein loading than with the MTT. Nutritional differences in tolerance tests might influence DPP-4is-induced effects on HIC. Thus, further study will be required.

The present study had several limitations. The current study was a post-hoc study using the integrated analysis of four prospective studies focusing on HIC. Also, the participants' backgrounds were heterogeneous. Furthermore, the number of participants who had received each concomitant antidiabetic agent was limited and was not balanced. Thus, we did not clearly evaluate the effects of concomitant antidiabetic agents on HIC. A further prospective study will be required to address this issue. We evaluated the C-peptide AUC-to-insulin AUC ratio as the surrogate index for HIC, which might be an indirect estimate for HIC. A direct approach to calculate HIC through the measurements of hormone levels in the portal vein was shown[40]; however, this approach might be difficult to apply to large human cohorts. Additionally, concentrations of GLP-1, GIP, glucagon and anagliptin were not measured and monitored during the study period. An MTT was not carried out until the 12th week of treatment with anagliptin in all examined studies. Further prospective long-term placebo-controlled studies on larger cohorts are required to confirm our presumed explanation. There are many factors that determine HIC, and those factors that had been examined in the current study were limited. Thus, it is unclear whether the relationship between DPP4i and HIC is direct or indirect.

In conclusion, the DPP-4i, anagliptin, significantly reduced HIC and its reduction was independently influenced by baseline HIC status. The reduced HIC might contribute to an additive supply of peripheral blood insulin followed by a greater reduction in hyperglycemia in participants with higher HIC status who were not obese and had lower insulin secretion capacity. The effects on HIC might be associated with the antihyperglycemic effects through DPP-4is.

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REFERENCES

1. Ahren B, Landin-Olsson M, Jansson PA, *et al*. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 2004; 89: 2078–2084.
2. Yabe D, Seino Y, Fukushima M, *et al*. beta cell dysfunction versus insulin resistance in the pathogenesis of type 2 diabetes in East Asians. *Curr Diab Rep* 2015; 15: 602.
3. Seino Y, Kuwata H, Yabe D. Incretin-based drugs for type 2 diabetes: focus on East Asian perspectives. *J Diabetes Investig* 2016; 7(Suppl 1): 102–109.
4. Alsalim W, Tura A, Pacini G, *et al*. Mixed meal ingestion diminishes glucose excursion in comparison with glucose ingestion via several adaptive mechanisms in people with and without type 2 diabetes. *Diabetes Obes Metab* 2016; 18: 24–33.
5. Tura A, Bizzotto R, Yamada Y, *et al*. Increased insulin clearance in mice with double deletion of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide receptors. *Am J Physiol Regul Integr Comp Physiol* 2018; 314: R639–R646.
6. Ahren B, Thomaseth K, Pacini G. Reduced insulin clearance contributes to the increased insulin levels after administration of glucagon-like peptide 1 in mice. *Diabetologia* 2005; 48: 2140–2146.
7. Rudovich NN, Rochlitz HJ, Pfeiffer AF. Reduced hepatic insulin extraction in response to gastric inhibitory polypeptide compensates for reduced insulin secretion in normal-weight and normal glucose tolerant first-degree relatives of type 2 diabetic patients. *Diabetes* 2004; 53: 2359–2365.
8. Meier JJ, Holst JJ, Schmidt WE, *et al*. Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans. *Am J Physiol Endocrinol Metab* 2007; 293: E849–E856.

9. Kim YG, Hahn S, Oh TJ, *et al*. Differences in the glucose-lowering efficacy of dipeptidyl peptidase-4 inhibitors between Asians and non-Asians: a systematic review and meta-analysis. *Diabetologia* 2013; 56: 696–708.
10. Fujita K, Kaneko M, Narukawa M. Factors related to the glucose-lowering efficacy of dipeptidyl peptidase-4 inhibitors: a systematic review and meta-analysis focusing on ethnicity and study regions. *Clin Drug Investig* 2017; 37: 219–232.
11. Kaku K. Efficacy and safety of long-term monotherapy with anagliptin in Japanese patients with type 2 diabetes. *Jpn Pharmacol Ther* 2012; 40: 733–744.
12. Kaku K. Efficacy and safety of anagliptin add-on therapy in Japanese patients with type 2 diabetes. *Jpn Pharmacol Ther* 2012; 40: 745–770.
13. Kaku K. Dose-ranging study of anagliptin in Japanese patients with type 2 diabetes. *Jpn Pharmacol Ther* 2012; 40: 973–984.
14. Lee JH, Kim D, Kim HJ, *et al*. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; 42: 503–508.
15. Katz A, Nambi SS, Mather K, *et al*. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; 85: 2402–2410.
16. Pivovarova O, Bernigau W, Bobbert T, *et al*. Hepatic insulin clearance is closely related to metabolic syndrome components. *Diabetes Care* 2013; 36: 3779–3785.
17. Semnani-Azad Z, Johnston LW, Lee C, *et al*. Determinants of longitudinal change in insulin clearance: the Prospective Metabolism and Islet Cell Evaluation cohort. *BMJ Open Diabetes Res Care* 2019; 7: e000825.
18. Bril F, Lomonaco R, Orsak B, *et al*. Relationship between disease severity, hyperinsulinemia, and impaired insulin clearance in patients with nonalcoholic steatohepatitis. *Hepatology* 2014; 59: 2178–2187.
19. Rudovich N, Pivovarova O, Fisher E, *et al*. Polymorphisms within insulin-degrading enzyme (IDE) gene determine insulin metabolism and risk of type 2 diabetes. *J Mol Med* 2009; 87: 1145–1151.
20. Tamaki M, Fujitani Y, Hara A, *et al*. The diabetes-susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance. *J Clin Invest* 2013; 123: 4513–4524.
21. Ohlsson L, Alsalim W, Carr RD, *et al*. Glucose-lowering effect of the DPP-4 inhibitor sitagliptin after glucose and non-glucose macronutrient ingestion in non-diabetic subjects. *Diabetes Obes Metab* 2013; 15: 531–537.
22. Piccinini F, Polidori DC, Gower BA, *et al*. Hepatic but not extrahepatic insulin clearance is lower in African American than in European American women. *Diabetes* 2017; 66: 2564–2570.
23. Goff LM, Ladwa M, Hakim O, *et al*. Ethnic distinctions in the pathophysiology of type 2 diabetes: a focus on black African-Caribbean populations. *Proc Nutr Soc* 2020; 79: 184–193.
24. Yki-Jarvinen H, Koivisto VA, Karonen SL. Influence of body composition on insulin clearance. *Clin Physiol* 1985; 5: 45–52.
25. Kotronen A, Juurinen L, Tiikkainen M, *et al*. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. *Gastroenterology* 2008; 135: 122–130.
26. Kabir M, Catalano KJ, Ananthnarayan S, *et al*. Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. *Am J Physiol Endocrinol Metab* 2005; 288: E454–E461.
27. Monami M, Cremasco F, Lamanna C, *et al*. Predictors of response to dipeptidyl peptidase-4 inhibitors: evidence from randomized clinical trials. *Diabetes Metab Res Rev* 2011; 27: 362–372.
28. Nomiyama T, Akehi Y, Takenoshita H, *et al*. Contributing factors related to efficacy of the dipeptidyl peptidase-4 inhibitor sitagliptin in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract* 2012; 95: e27–e28.
29. Yagi S, Aihara K, Akaike M, *et al*. Predictive factors for efficacy of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes mellitus. *Diabetes Metab J* 2015; 39: 342–347.
30. Dennis JM, Shields BM, Hill AV, *et al*. Precision medicine in type 2 diabetes: clinical markers of insulin resistance are associated with altered short- and long-term glycemic response to DPP-4 inhibitor therapy. *Diabetes Care* 2018; 41: 705–712.
31. Iwasaki M, Hoshian F, Tsuji T, *et al*. Predicting efficacy of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes: association of glycated hemoglobin reduction with serum eicosapentaenoic acid and docosahexaenoic acid levels. *J Diabetes Investig* 2012; 3: 464–467.
32. Kuwata H, Okamoto S, Seino Y, *et al*. Relationship between deterioration of glycated hemoglobin-lowering effects in dipeptidyl peptidase-4 inhibitor monotherapy and dietary habits: retrospective analysis of Japanese individuals with type 2 diabetes. *J Diabetes Investig* 2018; 9: 1153–1158.
33. Kodama K, Tojjar D, Yamada S, *et al*. Ethnic differences in the relationship between insulin sensitivity and insulin response: a systematic review and meta-analysis. *Diabetes Care* 2013; 36: 1789–1796.
34. Yabe D, Kuroe A, Watanabe K, *et al*. Early phase glucagon and insulin secretory abnormalities, but not incretin secretion, are similarly responsible for hyperglycemia after ingestion of nutrients. *J Diabetes Complications* 2015; 29: 413–421.
35. Kirino Y, Sei M, Kawazoe K, *et al*. Plasma dipeptidyl peptidase 4 activity correlates with body mass index and the plasma adiponectin concentration in healthy young people. *Endocr J* 2012; 59: 949–953.
36. Carr RD, Larsen MO, Jelic K, *et al*. Secretion and dipeptidyl peptidase-4-mediated metabolism of incretin hormones after a mixed meal or glucose ingestion in obese

compared to lean, nondiabetic men. *J Clin Endocrinol Metab* 2010; 95: 872–878.
37. Thulé PM, Umpierrez G. Sulfonylureas: a new look at old therapy. *Curr Diab Rep* 2014; 14: 473.
38. Groop L, Groop PH, Stenman S, *et al*. Comparison of pharmacokinetics, metabolic effects and mechanisms of action of glyburide and glipizide during long-term treatment. *Diabetes Care* 1987; 10: 671–678.
39. Scheen AJ, Lefebvre PJ, Luyckx AS. Glipizide increases plasma insulin but not C-peptide level after a standardized breakfast in type 2 diabetic patients. *Eur J Clin Pharmacol* 1984; 26: 471–474.
40. Meier JJ, Veldhuis JD, Butler PC. Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. *Diabetes* 2005; 54: 1649–1656.

SUPPORTING INFORMATION

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

Table S1 | Integrated clinical studies.

Table S2 | Baseline characteristics.

Table S3 | Change in variables in comparison with placebo at week 12.

Table S4 | Baseline characteristics according to medians of baseline hepatic insulin clearance.