The Effect of Sodium-Glucose Cotransporter 2 Inhibitor Ipragliflozin on Insulin Resistance, Hepatic Insulin Clearance, Beta-Cell Function in the Japanese Patients with type 2 Diabetes

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Research

Keywords: Sodium-glucose cotransporter 2 inhibitor, Insulin Clearance, Type 2 Diabetes, Insulin Clamp

Posted Date: September 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-882630/v1>

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The effect of sodium-glucose cotransporter 2 inhibitor ipragliflozin on insulin resistance, hepatic insulin clearance, beta-cell function in the Japanese patients with type 2 diabetes

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Short title: The effect of SGLT2-i

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28 Word count: abstract 244 main text 2238

29 Number of figures 1

30 Number of tables 2

31

32 Abstract

33 Introduction: Sodium-glucose cotransporter 2 inhibitor (SGLT2i) is a medication for
34 type 2 diabetes mellitus (T2DM). Some reports showed SGLT2i improved insulin
35 resistance, however, the effect on insulin resistance is not well established. Hepatic
36 insulin clearance (HIC) is new pathophysiology of T2DM. The effect of SGLT2i on
37 hepatic insulin clearance and insulin resistance is not well known. We investigated the
38 effect of SGLT2i on insulin resistance, insulin secretion, incretins, body composition,
39 and hepatic insulin clearance.

40 Materials and Methods: We conducted a meal tolerance test (MTT), and the
41 hyperinsulinemic-euglycemic clamp in 9 T2DM patients. 50 mg/day ipragliflozin was
42 admitted, MTT and clamp were performed after 4 months. We calculated the
43 postprandial C-peptide AUC to insulin AUC ratio as the HIC. We also measured GLP-
44 1, GIP, and glucagon levels during MTT.

45 Results: Body weight, HbA1c, and body composition were not significantly changed
46 after 4 months of treatment. Postprandial glucose, fasting, and postprandial insulin were
47 significantly decreased. The insulin resistance of the glucose clamp was not changed,
48 but HOMA-IR and insulin sensitivity index (ISI) were significantly improved. Incretins
49 and glucagon were not changed. Hepatic insulin clearance was significantly increased,
50 but whole-body insulin clearance was not changed. Fib 4 index and fatty liver index
51 were significantly reduced. HOMA-beta and insulinogenic index was not changed but
52 the C-peptide index was significantly increased.

Conclusions: Although patients' number was small, these results suggest that SGLT2i treatment decreased hepatic insulin resistance, increased hepatic insulin clearance, and decreased hyperinsulinemia, it might protect beta-cell function.

Keywords

Sodium-glucose cotransporter 2 inhibitor; Insulin Clearance; Type 2 Diabetes; Insulin Clamp

Abbreviations

CPI, C-peptide index; eGFR, estimated glomerular filtration rate; FLI, fatty liver index; GDR, glucose disposal rate; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HIC, hepatic insulin clearance; HOMA-beta, homeostatic model assessment beta-cell function; HOMA-IR, homeostasis model assessment for insulin resistance, SGLT2i, Sodium-glucose cotransporter 2 inhibitor

Introduction

The main pathophysiology of type 2 diabetes mellitus (T2DM) is decreased insulin sensitivity and decreased insulin secretion ability (1). Sodium-glucose cotransporter inhibitor (SGLT2i) is an anti-diabetic medicine, which increases glucosuria, induces a glucose-lowering effect, and reduces body weight (2). Some studies reported SGLT2i to improve insulin resistance. Dapagliflozin improved muscle insulin resistance evaluated by the euglycemic hyperinsulinemic clamp technique (3). However, this study and other studies indicated SGLT2i increases endogenous glucose production and glucagon secretion. One study performed dual-tracer glucose administrated mixed meal, and showed SGLT2i increased insulin sensitivity and the secretion of glucagon-like peptide-1 (GLP-1), but increased endogenous glucose production, and increased glucagon secretion (4). Another study performed a glucose clamp method and oral glucose tolerance test, SGLT2i improved insulin sensitivity (5). These results suggested that SGLT2i improve muscle insulin sensitivity, but increase endogenous glucose production by glucagon secretion.

Hepatic insulin clearance (HIC) is a novel and important pathophysiology of hyperinsulinemia, metabolic syndrome, and T2DM, hepatic insulin clearance is decreased in these metabolic conditions (6). One study reported that SGLT2i increased insulin clearance after the meal tolerance test (7). However, we previously reported that poorly controlled T2DM patients showed increased hepatic insulin clearance (8).

According to these results, SGLT2i affects insulin resistance, incretins, hepatic insulin clearance, however, these effects were evaluated by different studies, therefore the total

mechanisms are not well known in the real world. We evaluated the effect of SGLT2i on insulin resistance measured by the glucose clamp method, and we also evaluated the effect on hepatic insulin clearance and beta-cell function. We also measured incretins as GLP-1, and glucose-dependent insulinotropic polypeptide (GIP), and glucagon levels in the meal tolerance test. Here, we show that SGLT2i improves hepatic insulin resistance and increases hepatic insulin clearance, improves liver function, and it might protect beta-cell function.

Materials and Methods

Subjects

Nine type 2 diabetes patients enrolled in the study at Tottori University Hospital from 2014 to 2017. We diagnosed type 2 diabetes by the World Health Organization (WHO) criteria (9). We excluded patients with malignancy, hepatitis B and C, liver cirrhosis, pancreatitis, renal dysfunction, or those who taking diabetogenic medications such as corticosteroids from the study. All patients were of Japanese ethnicity. This study had a single-arm study design. 50 mg/day ipragliflozin was admitted after meal tolerance test (MTT) and glucose clamp test, and MTT and clamp were performed after 4 months. Patients were lectured to drink water to prevent dehydration.

We performed this study according to the principles of the Declaration of Helsinki. The Ethics Committee of the Tottori University Faculty of Medicine approved this study (approval number 2439). The clinical trial number was UMIN000014521.

Trial registration: UMIN, UMIN000014521. Registered 9 July 2014 - Retrospectively registered, <https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&language=J&recptno=R000016186>

The Patient and Public Involvement

The first patient was involved on May 7, 2014. The patient was recruited from the out-patient of Tottori University hospital. The research questions were developed in the Ethics Committee and informed to all of the patients using a procedure that was approved by the Ethics Committee. This study was a single-arm and intervention study. We chose the outcome HOMA-IR and Glucose disposal rate as a marker of insulin resistance. All patients were recruited from out-patients of Tottori University hospital. We obtained informed consent from all of the patients using a procedure that was approved by the Ethics Committee.

Meal tolerance test (MTT)

The patients visited our hospital after overnight fasting. The patients took a test meal developed by the Japan Diabetes Society (JDS) (460 kcal, carbohydrate 50%, protein 15%, fat 35%, and salt 1.6 g) (10). Plasma glucose, serum insulin, and C-peptide were measured at 0, 30, 60, and 120 minutes after taking the test meal. We used the glucose oxidase method for glucose, and chemiluminescent immunoassays for insulin and C-peptide (human insulin, and C-peptide chemiluminescent immunoassay kits; Kyowa Medix,

Japan). HbA1c was measured by high-performance liquid chromatography. HbA1c was converted from the percentage values to the International Federation of Clinical Chemistry values (mmol/mol), HbA1c converter from the National Institutes of Diabetes and Digestive and Kidney Diseases was used (11).

Insulin resistance was assessed by using insulin resistance indexes calculated as follows:

Homeostatic model assessment insulin resistance (HOMA-IR) = {fasting plasma glucose (mmol/L)} × {fasting plasma insulin (pmol/L)} / 135 (12).

Insulin sensitivity index (ISI) = 10,000 / square [{fasting plasma glucose (mmol/L)} × {fasting plasma insulin (pmol/L)} × {mean glucose × mean insulin during the MTT}] (13).

Insulin secretion was assessed by using insulin secretion indexes as follows:

Homeostasis model assessment of beta cell function (HOMA-beta) = [20 × {fasting plasma insulin (pmol/L)}] / [{fasting plasma glucose (mmol/L)} - 3.5] (12).

Insulinogenic index = [{plasma insulin (pmol/L) at 30 min} - {plasma insulin (pmol/L) at 0 min}] / [{plasma glucose (mmol/L) at 30 min} - {plasma glucose (mmol/L) at 0 min}] 8).

C-peptide index (CPI) (14) = {fasting C-peptide (mmol/L)} / {fasting plasma glucose (mmol/L) × 100}

We calculated Hepatic insulin clearance (HIC) as follows:

HIC = { area under the curve (AUC) C-peptide 0–120 min } / { AUC insulin 0–120 min } }

(15). The AUC was calculated using the trapezoidal method.

GLP-1, GIP, and glucagon assays

We obtained the serum samples by using a blood collection tube containing DPP-4 inhibitor (BD™ P800, BD Japan, Japan). We performed ethanol and solid-phase extractions for assay (16). We measured intact GLP-1 levels by an ELISA kit (GLP-1 (active) ELISA, Merck Millipore, Germany). We also measured the active GIP by an ELISA kit (Human GIP, Active form Assay Kit, IBL, Japan). We next measured Glucagon levels by an ELISA kit (Glucagon ELISA kit, Mercodia, Sweden).

Hyperinsulinemic euglycemic clamp

The glucose clamp method was performed as we previously reported (8, 17). We performed the hyperinsulinemic-euglycemic clamp method to evaluate insulin sensitivity by an artificial endocrine pancreas (STG 55, Nikkiso, Japan). We performed the protocol as a priming constant insulin infusion (100 mU/m²/min), and we maintained the plasma glucose level at 5.2 mmol/L (95 mg/dL) during the clamp. The plasma insulin level of steady-state was achieved as 1200 pmol/L in T2DM patients based on previous studies (18). We measured the glucose infusion rate (GIR) of steady-state from 90–120 minutes, and we defined the mean GIR during this period as the glucose disposal rate (GDR). GDR is generally used as mainly reflecting peripheral insulin sensitivity. We next calculated the M/I value calculated by M value equal to the GDR, and the I value equal to the final

insulin concentration in the clamp, which is peripheral insulin sensitivity adjusted by final insulin concentration (19). The whole-body insulin clearance was calculated as the insulin infusion rate divided by the steady-state insulin concentration (19).

Fib 4 index

Liver fibrosis level was estimated by Fib 4 index = $\text{age ([yr]} \times \text{AST [U/L]} / ((\text{PLT [10}^9/\text{L]}) \times (\text{ALT [U/L]})^{1/2})$ (20).

Fatty liver index (FLI)

Fatty liver level was estimated by

$$\text{Fatty liver index (FLI)} = \left(e^{0.953 \times \log(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log(\text{GGT}) + 0.053 \times \text{waistcircumference} - 15.745} \right) / \left(1 + e^{0.953 \times \log(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log(\text{GGT}) + 0.053 \times \text{waistcircumference} - 15.745} \right) \times 100 \quad (21).$$

Body composition

Body composition was analyzed by Inbody 770 (Inbody Japan, Japan). Body water, Percent body fat, Body fat mass, Lean body mass, Skeletal muscle mass, Trunk fat mass, and Bone mineral mass were measured.

Statistical analysis

Data are expressed as the mean $\pm$ standard deviation. We assessed differences in the mean value of clinical parameters between before and after SGLT2i treatment by using the Mann–Whitney *U* test. We determined correlations analysis of non-parametric clinical variables by using Spearman's correlation analysis. Values of $P < 0.05$ were considered

to be significant. PRISM8 software (GraphPad Software, San Diego, USA) was used for all analyses.

We conducted a power analysis to compare the pretreatment and posttreatment HOMA-IR as a primary endpoint using an EZR calculator 23. The mean HOMA-IR in the pretreatment was 4.86 ± 1.14 , and the mean HOMA-IR in the posttreatment was 3.48 ± 1.53 . The difference between the mean HOMA-IR in the pre and post-treatment was 1.38 ± 0.39 . We conducted a power analysis of the statistical test that was used to compare the pre and post-treatment HOMA-IR. Assuming that the difference in the means was 1.38 and the SD was 0.39, $\alpha=0.05$, $1-\beta=0.8$, $R=0.57$, the estimated sample size was 9 patients.

Results

The mean age of subjects was 54.1 ± 9.1 years old, and 2 males, 7 females. Duration of Diabetes was 5.0 ± 4.1 years. Participant characteristics are shown in Table 1. Seven patients were only diet therapy, 2 patients take DPP4 inhibitor and alpha-glucosidase inhibitor, and 1 patient also take metformin. Table 1 shows 4 months' effects of SGLT2i. Bodyweight, HbA1c, and body composition were not significantly changed after 4 months of treatment. AST, fib 4 index, and fatty liver index were significantly decreased. Postprandial glucose, fasting and postprandial insulin were decreased. Figure 1 shows MTT results. Glucose 120 minutes after MTT was significantly decreased. Fasting and 60, 120 minutes insulin levels were significantly decreased. C-peptide, GLP-1, GIP, and glucagon levels were not changed. The insulin resistance of the glucose clamp was not changed, but HOMA-IR and insulin sensitivity index were significantly improved. Incretins were not changed. Hepatic insulin clearance was significantly increased. The whole-body insulin clearance was not changed. HOMA-beta and insulinogenic index was not changed but the C-peptide index was significantly increased.

Table 2 shows the correlation between delta C-peptide index and delta clinical parameters from pretreatment to after treatment. The hepatic insulin clearance was significantly correlated with CPI. Creatinine and eGFR were also correlated with CPI.

231 Discussion

232 Our study indicated that SGLT2i ipragliflozin improved HOMA-IR, liver function, and
233 C-peptide index, but did not change significantly the muscle insulin resistance evaluated
234 by glucose clamp method, and body weight, body composition. SGLT2i increased hepatic
235 insulin clearance, but not whole-body insulin clearance. A recent study indicated that
236 SGLT2i improved insulin resistance evaluated by glucose clamp (22). Dapagliflozin
237 reduces liver fat but did not affect tissue insulin sensitivity of the liver, muscle, and
238 adipocyte (23). Recent another study also reported that SGLT2i increased hepatic insulin
239 clearance (24). SGLT2i increases glucagon secretion from alpha cells (25). We did not see
240 the significant glucagon inhibition by SGLT2i, but it may depend on the number of small
241 patients number, and 2 patients were already taking DPP4i.

242 According to these results, SGLT2i decreased postprandial glucose levels and insulin
243 levels. HOMA-IR mainly reflects liver insulin resistance, and the glucose clamp method
244 mainly reflects muscle insulin resistance (26). Although the insulin resistance of muscle
245 evaluated by the glucose clamp method was not improved, liver insulin resistance
246 evaluated by HOMA-IR was considered as improved. Hyperinsulinemia was decreased
247 after SGLT2i treatment, this insulin lowering effect was induced by increased hepatic
248 insulin clearance. These effects induced improved liver function. Finally, the C-peptide
249 index was increased, which might mean SGLT2i has the effect of beta-cell protection.
250 Although further basic research of animals and cellular are needed for understanding
251 molecular mechanisms, our results contribute to understanding the effects of SGLT2i. A
252 recent American study showed similar results that SGLT2i canagliflozin improved liver
253 insulin sensitivity and beta-cell function, and increased hepatic insulin clearance (27).

However, the pathophysiology of diabetes between American, European and Asian people are very different, Asian shows low insulin secretion ability and insulin resistance with mild obesity (28, 29). All patients in our study are Japanese, therefore, our results contribute to the daily clinical work in the Asian population.

Our study has some limitations, the major limitation is the very small patients' number of nine. However, the glucose clamp method and meal tolerance test including incretins are very complicated and expensive studies. We need a larger study. We evaluated insulin resistance by the glucose clamp method. The previous report evaluated GDR by subtracting the urinary glucose excretion (3). We measured the urinary glucose extraction in only 2 patients, therefore we cannot evaluate the urinary glucose excretion, we must consider this point. We did not evaluate hepatic insulin resistance by tritium-glucose, because of the difficulty of using tritium-glucose in Japan. We could evaluate hepatic insulin resistance by using only HOMA-IR, we need furthermore study. We used the MTT to avoid hyperglycemia by using OGTTs in patients with severe diabetes. However, the previous study used the HIC obtained from MTT, therefore we think our study was acceptable (7). Although our main purpose was to evaluate hepatic insulin clearance, whole-body and peripheral insulin clearance are also important as previously reported (30). Unfortunately, the steady-state C-peptide levels in the glucose clamp were not measured in this study, we need to investigate the peripheral insulin clearance evaluated during the glucose clamp test. The recent article stated that the post-load CPR / IRI molar ratio did not accurately evaluate hepatic insulin clearance (31). We must be careful to evaluate our results. We evaluated beta-cell function by C-peptide index. C-peptide index is affected by renal function shown as correlation analysis. We excluded renal disorder

Cr > 1.3 mg/ml according to a past report (31), and eGFR did not show significant change therefore we consider our data are reliable. Despite these limitations, our study performed glucose clamp and meal tolerance test and incretins measurement in the Japanese patients with T2DM in the real-world setting, we consider that our study might contribute to our daily clinical work, especially in the Asian population.

In conclusion, SGLT2i decreased postprandial glucose and insulin levels, increased hepatic insulin clearance, and improved liver function and liver insulin resistance, it might protect beta-cell function.

Acknowledgments

We thank Ms. Satomi Endo for her excellent technical assistance.

Contributors: All authors contributed significantly. TO participated in the design of the study and performed the statistical analysis. YF, RN, SK, YI, MA, KM, SK, KM, SI, HO, and EU collected the data. MK, TI, ST, and KY conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval

296 This study was approved by the Ethics Committee of the Faculty of Medicine, Tottori
297 University (approval number 2439). Clinical trial number was UMIN000014521

298

299 **Funding:** This study was funded by Astellas Pharma Inc. No grant number.

300 The company was not involved in study design, patient selection, data
301 aggregation/analysis, interpretation of results, or preparation of the manuscript.

302

303 **Competing interests:** None declared.

304

305 **Data sharing statement:** No additional data available.

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425 **Figure Legends**

426 Figure 1. Figure 1 shows the meal tolerance test (MTT) results. Figure 1(a) shows
427 glucose levels after the MTT, (b) insulin, (c) C-peptide, (d) GLP-1, (e) GIP, and (f)
428 glucagon. White circles represent the data from pre-treatment, and black squares
429 indicate post treatment. *, $p < 0.05$

430

431 **Table 1. Comparison between before and after SGLT2 treatment**

Parameters	before	after	P value
Body weight (kg)	71.5 ± 9.1	70.2 ± 9.8	0.12
BMI (kg/m ²)	28.2 ± 4.0	27.7 ± 4.0	0.11
Waist circumference (cm)	96.9 ± 9.2	96.5 ± 9.6	0.39
eGFR	86.2 ± 23.9	85.6 ± 24.3	0.84
AST (U/L)	28.1 ± 7.7	24.2 ± 8.7	<0.05
ALT (U/L)	32.3 ± 14.6	28.5 ± 13.5	0.12
Gamma GTP (U/L)	42.5 ± 47.3	37.3 ± 41.0	0.11
Fib 4 index	0.92 ± 0.30	0.77 ± 0.32	<0.05
Fatty liver index	59.8 ± 23.2	51.3 ± 27.9	<0.05
HbA1c (%)	7.4 ± 1.1	6.8 ± 0.5	0.09
HbA1c (mmol/mol)	57.4 ± 12.0	50.8 ± 5.8	
Insulin secretion			
Fasting Plasma Glucose (mmol/L)	7.28 ± 1.35	6.83 ± 1.26	0.41
Postprandial Plasma Glucose 2h (mmol/L)	9.94 ± 2.50	8.25 ± 2.30	<0.05
Fasting Plasma insulin (mmol/L)	90.4 ± 20.9	69.9 ± 32.6	<0.01
Postprandial Plasma insulin 2h (mmol/L)	285.3 ± 122.9	175.1 ± 111.2	<0.05
Glucose AUC (0-120 min)	362.4 ± 62.8	331.1 ± 61.5	0.17
Insulin AUC (0-120 min)	568.1 ± 250.9	370.9 ± 293.9	0.08
CPR AUC (0-120 min)	3.28 ± 1.33	2.90 ± 0.81	0.35
Hepatic insulin clearance	6.03 ± 1.46	9.96 ± 4.26	<0.05
HOMA-beta (%)	87.4 ± 32.7	78.5 ± 46.3	0.30
Insulinogenic Index	0.62 ± 0.25	0.63 ± 0.55	0.98
CPI	1.92 ± 0.79	2.25 ± 0.66	<0.05
Insulin resistance			
HOMA-IR	4.86 ± 1.14	3.48 ± 1.53	<0.05
Insulin sensitivity index	2.70 ± 0.81	4.67 ± 2.56	<0.05
GDR (mg/kg/min)	5.52 ± 1.66	5.68 ± 2.63	0.77
M/I	4.97 ± 1.60	6.07 ± 4.38	0.65
Whole-body insulin clearance	1.37 ± 0.28	1.36 ± 0.39	0.95
Incretins			
GLP-1 AUC	14.8 ± 9.1	15.8 ± 11.2	0.41
GIP AUC	126.1 ± 75.6	106.3 ± 27.5	0.30
Glucagon AUC	13.3 ± 5.1	12.7 ± 5.4	0.44
Body composition			
Body water (L)	34.3 ± 4.2	33.7 ± 4.9	0.09
Percent body fat (%)	34.2 ± 7.6	34.5 ± 7.7	0.49
Body fat mass (kg)	24.7 ± 7.8	24.6 ± 7.9	0.72
Lean body mass (kg)	43.9 ± 5.5	43.2 ± 6.4	0.1
Skeletal muscle mass (kg)	25.6 ± 3.5	25.1 ± 4.1	0.1
Trunk fat mass (kg)	14.6 ± 5.5	15.2 ± 5.5	0.53
Bone mineral mass (kg)	2.6 ± 0.3	2.5 ± 0.4	0.34

432 Data are presented as the mean ± standard deviation.

433 The comparison of parameters between before and after treatment was performed using
434 the Mann–Whitney *U* test. The chi-squared test was used for categorical comparisons of
435 the sex data.

436

437 AUC, area under the curve; CPI, C-peptide index; GDR, glucose disposal rate; GIP,
438 glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1; HbA1c,
439 glycated hemoglobin; HOMA-beta, homeostatic model assessment beta cell function;
440 HOMA-IR, homeostasis model assessment for insulin resistance; N.S., not significant

441

442

443 **Table 2. Correlation coefficients between the Δ CPI and clinical parameters**

Parameters	r	p
Δ Fasting plasma glucose	-0.334	NS
Δ Postprandial plasma glucose 120min	-0.366	NS
Δ Fasting insulin	-0.133	NS
Δ Postprandial insulin 120min	0.158	NS
Δ Matsuda Index	0.533	0.07
Δ HOMA-IR	-0.466	0.106
Δ Insulinogenic Index	0.266	NS
Δ HIC=AUC CPR/INS	0.833	<0.005
Δ TG(0)	-0.400	NS
Δ AST	0.252	NS
Δ ALT	-0.218	NS
Δ γ GTP	-0.292	NS
Δ Fatty liver index	0.274	NS
Δ Fib 4 index	0.144	NS
Δ HDL	0.033	NS
Δ LDL	0.133	NS
Δ HbA1c	-0.235	NS
Δ eGFR	0.733	<0.05
Δ GDR	0.567	0.06
Δ M/I	0.250	NS
Δ Whole body insulin clearance	0.410	NS
Δ BMI	-0.117	NS
Δ Waist circumference	-0.516	0.08
Δ Total body fat	0.183	NS
Δ GLP-1 AUC120	-0.100	NS
Δ GIP AUC120	-0.100	NS
Δ Glucagon AUC120	-0.100	NS

444

445 Correlation coefficients were determined using Spearman's product-moment correlation
 446 coefficient test.

447 AUC, area under the curve; GDR, glucose disposal rate; GIP, glucose-dependent
 448 insulintropic polypeptide; GLP-1, glucagon-like peptide-1; HbA1c, glycated
 449 hemoglobin; HOMA-beta, homeostatic model assessment beta-cell function; HOMA-IR,
 450 homeostasis model assessment for insulin resistance; N.S., not significant

451 Figure 1

452