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CLINICAL TRIAL

Effects of sitagliptin on ectopic fat contents and glucose metabolism in type 2 diabetic patients with fatty liver: A pilot study

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ABSTRACT

Aims/Introduction: Recent data have shown that ectopic fat accumulation in the liver worsens hepatic glucose metabolism, suggesting that fatty liver in patients with type 2 diabetes is a therapeutic target. Glucagon-like peptide (GLP)-1 improves fatty liver, but the effect of dipeptidyl peptidase-4 inhibitor on fatty liver is still unclear. The present pilot study determined the effects of 12-week treatment with sitagliptin, a dipeptidyl peptidase-4 inhibitor, on liver fat content in type 2 diabetes with fatty liver. We also evaluated intramyocellular lipid (IMCL) and glucose kinetics during oral glucose tolerance test (OGTT) before and after the treatment.

Materials and Methods: The study participants were seven type 2 diabetes patients with fatty liver who were studied at baseline and 12 weeks after sitagliptin treatment. Intrahepatic lipid (IHL) and IMCL were assessed by ^{1}H magnetic resonance spectroscopy. Glucose kinetics was assessed during double-tracer OGTT (U-[^{13}C]-glucose orally and 6,6-[$^{2}H_2$]-glucose intravenously).

Results: Sitagliptin significantly reduced glycated hemoglobin (from 7.1 ± 0.2 to 6.5 ± 0.3%, $P < 0.005$), but had no effects on IHL and IMCL. The glucose level diminished, whereas GLP-1 concentration increased during OGTT at the end of treatment. These changes were not accompanied by significant changes in insulin or glucagon levels. However, long-term sitagliptin treatment partially decreased the rate of appearance of oral glucose during OGTT, but did not affect endogenous glucose production or the rate of disappearance.

Conclusions: It was found that 12-week sitagliptin treatment improved glycated hemoglobin and glucose excursion during OGTT in type 2 diabetes with fatty liver, independent of changes in lipid accumulation in the liver. This trial was registered with the Japan Clinical Trials Registry (UMIN-CTR000005666).

INTRODUCTION

Accumulation of recent data highlights the importance of ectopic fat content in muscle and liver in insulin resistance in each organ[1–4]. Indeed, intramyocellular lipid (IMCL), determined by ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) or biopsy specimen, is reported to be associated with insulin resistance in skeletal muscle[1,2]. Suppression of endogenous glucose production (EGP) by insulin correlated negatively with intrahepatic lipid (IHL) content in both healthy subjects[3] and patients with type 2 diabetes mellitus[4]. In addition, our group showed that dietary

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therapy decreased IHL with increased splanchnic glucose uptake, suggesting IHL might affect splanchnic glucose uptake[5,6]. Given that increased EGP and reduced hepatic glucose uptake are considered major determinants of hyperglycemia[7], fatty liver should be recognized as a therapeutic target in type 2 diabetes.

Recently, incretin-related drugs that enhance serum glucagon-like peptide-1 (GLP-1) level have been widely used for the treatment of type 2 diabetes mellitus. GLP-1 improves the glycemic level in patients with type 2 diabetes through several mechanisms[8]. First, GLP-1 enhances insulin release and decreases glucagon secretion[8,9]. Second, independent of these hormonal changes, GLP-1 also reduces hepatic glucose production, and augments hepatic glucose uptake in dogs[10,11] and patients with type 2 diabetes[12,13]. Third, recent data have shown that a dipeptidyl peptidase-4 (DPP-4) inhibitor reduced EGP and the appearance of oral glucose (RaOral) at postprandial states, at least partly through enhanced insulin secretion and suppressed glucagon response to meal induced by elevated GLP-1 levels[14,15]. In contrast, the mechanism of the improvement of glycemic control by DPP-4 inhibitors has not been fully understood.

Several reports have suggested that GLP-1 improves fatty liver[16–20]. *In vitro* studies showed that GLP-1 directly activates adenosine monophosphate kinase and peroxisome proliferator-activated receptor-α, and thus suppresses lipogenesis and increases lipid oxidation, respectively[16,17]. At least in animal studies, DPP-4 inhibitors prevent dietary-induced fatty liver[18]. With regard to human studies, 6-month treatment with GLP-1 receptor agonists in type 2 diabetes reduced IHL by 42%, and this reduction correlated positively with reductions in glycated hemoglobin (HbA1c) levels[19]. Also, sitagliptin treatment improved liver function test in type 2 diabetes with fatty liver[20]. Therefore, improvement of fatty liver by DPP-4 inhibitors could explain, at least in part, the improvement of glycemic control observed in type 2 diabetes.

Based on the aforementioned background, we hypothesized that the improvement of glycemic control by sitagliptin is mediated in part through reduction of ectopic fat in the liver. As a first step to test this hypothesis, we carried out a pilot study to investigate the effects of 12-week sitagliptin treatment on IHL in type 2 diabetes with fatty liver. In addition, we also evaluated ectopic fat content in muscle and glucose kinetics during oral glucose tolerance test (OGTT) to elucidate the association between changes in ectopic fat and glucose kinetics. The results of the present study showed that sitagliptin treatment had no effect on ectopic fat content in liver, though it improved glycemic control and reduced RaOral during OGTT.

MATERIAL AND METHODS

Study Participants

We screened type 2 diabetic patients who regularly attended Juntendo University Hospital, Tokyo, Japan, between January 2011 and August 2011. Among them, we selected those who fulfilled all of the following criteria: (i) type 2 diabetes with fatty liver determined by ultrasonography; (ii) HbA1c between 6.9 to 8.4%; (iii) age more than 20 years; (iv) stable glycemic control with HbA1c variation <1.0% during the preceding 3 months; and (v) negative history for use of DPP-4 inhibitors or GLP-1 receptor agonists. The following exclusion criteria were applied: (i) type 1 diabetes; (ii) heavy alcohol drinking; (iii) serious liver disease and viral infection (hepatitis B or C virus); (iv) chronic renal failure; (v) apparent heart failure or those with myocardial infarction within 3 months; (vi) serious pancreatic disease; (vii) cancer; (viii) serious diabetic complications including progressive neuropathy and proliferative retinopathy; (ix) significant infection or inflammation; (x) ileus and high risk for ileus; (xi) pregnancy or planning for pregnancy during the study period or in lactation period; and (xii) treatment with pioglitazone. Five men and two women who matched the aforementioned criteria were recruited to the present study. Three participants were treated with metformin (MET) alone, two participants were treated with MET and sulfonylureas (SU), one participant was treated with SU and α-glucosidase inhibitor (α-GI) and another one was treated with the combination of MET, SU and α-GI. All participants gave written informed consent to the study, which was approved by the ethics committee of Juntendo University. This study was carried out in accordance with the principles outlined in the Declaration of Helsinki, and was registered with the Japan Clinical Trials Registry (UMIN-CTR000005666).

Study Design

The study was an open-label, non-randomized, single-arm study to investigate the effect of sitagliptin on type 2 diabetic patients with fatty liver. Participants were fasted overnight before baseline measurements. Total body fat content, IMCL in the right tibialis anterior (TA; $n = 6$) and soleus (SOL; $n = 5$) muscles, IHL of segment 6 of the liver, and intra-abdominal and subcutaneous fat were measured. Then, OGTT with double tracer (U-[^{13}C]-glucose orally and 6,6-[$^{2}H_2$]-glucose intravenously) was carried out to evaluate glucose kinetics.

After baseline evaluation, all participants were treated with 50–100 mg/day sitagliptin for 12 weeks, while other prescribed drugs were continued at the same prestudy doses throughout the study. In addition, the study participants were instructed to maintain their dietary intake and physical activity during the intervention, which were followed by a dietary (brief-type self-administered diet history questionnaire)[21] and physical activity (International Physical Activity Questionnaire)[22] questionnaire, respectively. At the end of the 12-week treatment period, all participants underwent a repeat of the same evaluations/measurements carried out at baseline.

To evaluate the relationship between changes in ectopic fat and glucose kinetics during OGTT, all patients refrained from taking any medications, including sitagliptin, on the day of OGTT to reduce the acute effect of sitagliptin treatment. The main primary outcome of the present study was the effect of

sitagliptin on fatty liver. Other primary outcomes were the effects of sitagliptin on: (i) muscle ectopic fat content; (ii) glucose metabolism during OGTT; and (iii) HbA1c level.

Biochemical Tests

Serum lipids [total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, free fatty acid (FFA) and triglyceride] and liver function tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (g-GTP)] were measured by enzymatic methods and UV methods, respectively (SRL Inc., Tokyo, Japan). Plasma insulin concentrations were determined by radioimmunoassay (LINCO Research, St Charles, MO, USA). For the measurement of active GLP-1, blood was collected into tubes containing ethylenediaminetetraacetic acid-2Na, aprotinin and DPP-4 inhibitors (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), then immediately centrifuged, and plasma was separated and stored at −80°C until analysis. For measurement of active GLP-1 in plasma, solid-phase and ethanol extraction were carried out to remove interference for intact GLP-1 immunoassay[23]. Then, measurement of active GLP-1 concentration was outsourced to SRL, Inc. using the Glucagon-Like Peptide-1 (Active) ELISA KIT (Millipore, St. Charles, MO, USA).

Proton Magnetic Resonance Spectroscopy

Intramyocellular lipid and IHL were measured after overnight fast as described previously[5,6]. Briefly, IMCL of the right TA and SOL muscle, and IHL of segment 6 in the liver were measured by ^{1}H-MRS using a knee coil and a whole-body coil, respectively (VISART EX V4.40; Toshiba, Tokyo, Japan). Voxels ($1.2 \times 1.2 \times 1.2$ cm^3 for muscle and $2 \times 2 \times 2$ cm^3 for the liver) were positioned in the TA and SOL muscle or liver, avoiding visible interfascial fat and blood vessels, and the voxel sites were carefully matched at each examination. Imaging parameters were set as follows: repetition time 1500 ms, echo time 136 ms (muscle) or 10 ms (liver), acquisition numbers 192 for muscle and eight for liver, and 1,024 data points over a 1,000-kHz spectral width. After examination, resonances were quantified by reference to the methylene signal intensity (S-fat), with peaks being observed at ~1.25 p.p.m. in muscle and at ~1.3 p.p.m. in the liver. IMCL was quantified by S-fat and the creatine signal at 3.0 ppm (Cre) as the reference, and was calculated as a ratio relative to Cre (S-fat/Cre). IHL was quantified by S-fat and H_2O at ~4.7 p.p.m. as the internal reference, and calculated as a percentage of H_2O + S-fat [S-fat $\times$ 100/(H_2O + S-fat)] as described previously[5,6].

Intra-Abdominal and Subcutaneous Fat

Intra-abdominal and subcutaneous fat areas were measured as described previously using magnetic resonance imagning[6]. Briefly, T1-weighted transaxial scans were obtained to determine intra-abdominal and subcutaneous fat in a region extending from 8 cm above to 8 cm below the fourth and fifth lumbar interspaces (16 slices, field of view 370×400 mm^2, slice thickness 10 mm, breath-hold repetition time 6,000 ms, echo time 78 ms). Intra-abdominal and subcutaneous fat areas at fourth and fifth lumbar interspaces were measured as described previously[6].

OGTT with Double Tracer

After an overnight fast, an intravenous cannula was placed in the forearm and used for tracer infusion. Another catheter was placed in a vein on the contralateral hand, and the hand was warmed by a heating device for arterial blood sampling. Then, we started to infuse 6,6-[$^2H_2$]-glucose intravenously as a primed constant infusion [bolus 200 $\times$ fasting blood glucose (FBG; mg/dL)/100 mg/m^2 body surface area (BSA)], followed by constant infusion of 2 mg/m^2 BSA/min until the end of the test. To achieve isotope equilibration, we infused the isotope for 3-h before baseline determinations of plasma glucose enrichment (−180 to 0 min). After basal equilibration period, participants ingested glucose (0.7 g/kg bodyweight) containing 1% U-[^{13}C]-glucose (time 0). We carried out blood sampling at −180, −20, −10, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min for determination of tracer enrichment, insulin, C-peptide and glucagon concentration. GLP-1 concentration was also measured at 0, 30, 60 and 120 min.

To determine the enrichment of 6,6-[$^2H_2$]-glucose and U-[^{13}C]-glucose in plasma, samples were deproteinized with trichloroacetic acid and derivatized with *p*-aminobenzoic acid ethyl ester. The atom percentage enrichment of glucose$_{m+2}$ and glucose$_{m+6}$ were then measured by high-performance liquid chromatography with a LTQ-XL-Orbitrap mass spectrometer (Thermo Scientific, Fremont, CA, USA). The glucose kinetics was calculated as described previously[15,24]. During the last 20 min of the tracer equilibration period, glucose concentrations and 6,6-[$^2H_2$]-glucose enrichment were stable in all participants. Therefore, EGP was calculated as the ratio of 6,6-[$^2H_2$]-glucose infusion rate to the plasma tracer enrichment [tracer-to-tracer ratio (TTR 6,6); mean of three determinations]. After glucose ingestion, the total glucose rate of appearance (RaT) was calculated from TTR 6,6 using Steele's equation[24]. The plasma glucose concentration resulting from the absorption of ingested glucose (exogenous glucose concentration) was calculated from the product of total plasma glucose concentration and the ratio of plasma U-[^{13}C]-glucose TTR to the U-[^{13}C]-glucose TTR of the ingested glucose. The plasma glucose concentration resulting from endogenous glucose release was obtained as the difference between total and exogenous glucose concentration. TTR of endogenous glucose and RaOral were calculated as described[24]. The tracer-determined rate of glucose disappearance (Rd) provided a measure of insulin-mediated total-body glucose disposal. Glucose clearance was calculated as Rd divided by plasma glucose concentration. The basal hepatic insulin resistance index was calculated as the product of the fasting plasma insulin concentration and the basal rate of EGP[24]. The logic of hepatic insulin resistance index was

described in detail previously[25]. In brief, the majority of EGP is derived from the liver at fasting state[26], and insulin is a potent inhibitor of hepatic glucose production[27]. In type 2 diabetes, it has been established that the increment in plasma insulin concentration is linearly related to the decline in EGP[27]. Thus, the product of the basal EGP and insulin concentration provides an index of hepatic insulin resistance, and its validity has been confirmed[28].

Statistical Analysis

All data are expressed as mean ± SD. Data that showed skewed distribution were log-transformed before analysis. Student's *t*-test was used for comparison of paired observations. Pearson's correlation coefficient was used to evaluate the correlation between variables. Statistical significance was set at $P < 0.05$.

RESULTS

Table 1 shows the clinical characteristics of the seven study participants before and at the end of the 12-week treatment. The mean age of the participants was 50.4 ± 11.4 years, and duration of type 2 diabetes was 12.3 ± 6.0 years. The mean alcohol intake was 6.7 ± 7.7 g/day. All participants completed the present study without evident untoward effects. HbA1c was significantly improved after the treatment (baseline 7.1 ± 0.2%, end of study 6.5 ± 0.3%), whereas changes in fasting blood glucose were not significant. This improvement was not accompanied by a decrease in IHL, IMCL or bodyweight. In addition, there were no significant changes in any other parameters listed in Table 1 after the treatment.

Plasma glucose level was significantly decreased after the treatment in the initial 0–120 min of the OGTT (Figure 1a; Table 2). This change was accompanied by an increase in GLP-1 concentration (Figure 1b; Table 2); however, there were no significant changes in insulin, C-peptide or glucagon levels (Figure S1a–c; Table 2). Glucose kinetics analysis during OGTT showed that treatment with sitagliptin decreased RaOral in 0–120 min (Figure 2; Table 2), but did not significantly improve EGP, Rd or glucose clearance (Figure S2a–c; Table 2). Thus, only the decrease in RaOral can explain the decrease in glucose level during OGTT after treatment.

To further explore the role of GLP-1 on RaOral, we investigated the relationship between area under the curve (AUC)-GLP-1 and AUC-RaOral in 0–120 min of pre- and post-treatment data, and found that AUC-GLP-1 tended to be negatively correlated with AUC-RaOral ($r = 0.49$, $P = 0.076$).

DISCUSSION

The present study evaluated the effects of 12-week sitagliptin treatment on ectopic fat and glycemic control in seven patients with type 2 diabetes and fatty liver. The results showed that sitagliptin treatment did not alter fatty liver, but it improved postprandial glycemic control and reduced RaOral during

Table 1 | Clinical parameters at baseline and after 12-week sitagliptin treatment

	Baseline	End of study	Change from baseline
Bodyweight (kg)	86.7 ± 16.1	86.3 ± 16.2	−0.4 ± 1.2
Body mass index (kg/m^2)	32.5 ± 5.7	32.3 ± 5.6	−0.2 ± 0.4
Waist circumstance (cm)	102.6 ± 10.5	104.9 ± 13.5	2.3 ± 4.2
Fasting plasma glucose (mg/dL)	129.6 ± 18.1	119.7 ± 16.6	−9.9 ± 15.8
Fasting plasma insulin (μU/mL)	10.6 ± 5.2	14.2 ± 12.9	3.6 ± 10.0
Fasting C-peptide (ng/mL)	2.7 ± 1.2	3.2 ± 1.7	0.5 ± 1.0
Glycated hemoglobin (%)	7.1 ± 0.2	6.5 ± 0.3*	−0.6 ± 0.2
Total cholesterol (mg/dL)	164.6 ± 17.9	171.9 ± 17	7.3 ± 18.3
LDL cholesterol (mg/dL)	92.0 ± 19.4	99.9 ± 25.3	7.9 ± 15.8
HDL cholesterol (mg/dL)	43.7 ± 8.2	43.1 ± 9.3	−0.6 ± 7.6
Free fatty acid (mmol/L)	636.6 ± 156.9	615.7 ± 190.7	−20.9 ± 263.7
Aspartate aminotransferase (IU/L)	35.4 ± 13.2	38.7 ± 16.0	3.3 ± 6.7
Alanine aminotransferase (IU/L)	50.3 ± 14.2	51.6 ± 18.0	1.3 ± 13.4
γ-Glutamyl transferase (IU/L)	88.3 ± 110.9	89.0 ± 105.6	0.7 ± 7.8
Abdominal visceral fat (cm^2)	181.8 ± 56.9	191.4 ± 47.4	9.6 ± 23.2
Abdominal subcutaneous fat (cm^2)	263.8 ± 131.3	262.8 ± 135.9	−1.0 ± 20.1
Physical activity (metabolic equivalent, h/week)	16.3 ± 15.5	19.4 ± 21.7	3.1 ± 22.9
Dietary intake (kcal/day)	2127 ± 536	1825 ± 410	−302 ± 720
Intrahepatic lipid (%)	12.7 ± 4.7	13.1 ± 6.0	0.4 ± 2.4
Intramyocellular lipid in tibialis anterior (S-fat/Cre)	3.2 ± 1.1	5.4 ± 2.6	2.2 ± 2.0
Intramyocellular lipid in soleus (S-fat/Cre)	14.6 ± 5.2	20.0 ± 9.4	5.3 ± 7.9
Basal hepatic insulin resistance index (mg/kg/min·mU/mL)	21.2 ± 10.8	28.7 ± 25.5	7.5 ± 18.9

Data are mean ± SD of seven participants, except intramyocellular lipid in tibialis anterior ($n = 6$) and soleus ($n = 5$). *$P < 0.005$ vs baseline. Cre, creatine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; S-fat, methylene signal intensity.

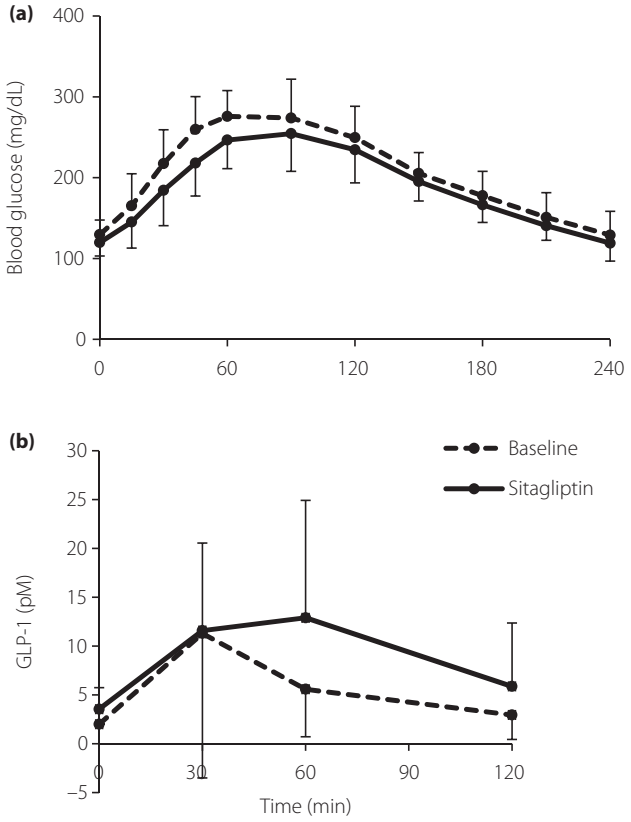

Figure 1 | Glucose and hormonal levels during oral glucose tolerance test at pre- (baseline; dashed line) and post-sitagliptin treatment (sitagliptin; solid line). (a) glucose. (b) Glucagon-like peptide (GLP)-1.

Table 2 | Metabolic parameters during oral glucose tolerance test at baseline and after 12-week sitagliptin treatment

	Baseline	End of study	Change from baseline
AUC-Plasma glucose, mg/dL h (0–240 min)	841.1 ± 77.0	769.8 ± 87.8	−71.4 ± 71.7
0–120 min	479.6 ± 48.8	430.0 ± 63.3*	−49.3 ± 34.9
120–240 min	361.6 ± 55.9	339.5 ± 44.2	−22.0 ± 44.1
AUC-plasma insulin, μU/mL h (0–240 min)	146.8 ± 87.7	173.5 ± 115.6	26.7 ± 60.8
0–120 min	92.8 ± 60.6	98.8 ± 71.8	6.0 ± 44.3
120–240 min	54.0 ± 31.1	74.7 ± 46.9	20.6 ± 24.0
AUC-C-peptide reactivity, ng/mL h (0–240 min)	23.1 ± 11.3	26.7 ± 13.6	3.6 ± 5.4
0–120 min	11.8 ± 5.8	13.0 ± 7.0	1.2 ± 3.3
120–240 min	11.3 ± 5.7	13.7 ± 6.8	2.4 ± 2.5
AUC-Glucagon, ng/mL h (0–240 min)	310.1 ± 83.1	304.0 ± 136.6	−6.1 ± 80.4
0–120 min	165.8 ± 49.8	162.7 ± 81.2	−3.1 ± 44.8
120–240 min	144.3 ± 36.4	141.3 ± 56.1	−3.0 ± 37.3
AUC-GLP-1, pmol/L h (0–120 min)	11.8 ± 12.2	19.3 ± 16.2*	7.5 ± 6.4
AUC-Endogenous glucose production, mg/kg (0–240 min)	265.5 ± 39.1	274.4 ± 68.9	8.9 ± 61.1
0–120 min	154.2 ± 27.0	161.0 ± 52.0	6.8 ± 46.1
120–240 min	111.3 ± 22.9	113.4 ± 32.0	2.1 ± 18.5
AUC-Rate of oral glucose appearance, mg/kg (0–240 min)	552.0 ± 56.3	515.4 ± 42.9	−36.6 ± 33.4
0–120 min	488.2 ± 54.6	446.4 ± 37.7*	−41.8 ± 24.6
120–240 min	63.8 ± 19.3	69.0 ± 35.0	5.2 ± 21.6
AUC-Rate of glucose disappearance, mg/kg (0–240 min)	763.7 ± 89.5	729.9 ± 90.8	−33.9 ± 64.7
0–120 min	484.1 ± 90.7	451.5 ± 76.6	−32.7 ± 71.0
120–240 min	279.6 ± 21.8	278.4 ± 27.7	−1.2 ± 21.1
AUC-Glucose clearance, mg/kg (0–240 min)	392.5 ± 52.1	407.5 ± 36.8	15.0 ± 43.0
0–120 min	204.7 ± 31.3	212.7 ± 18.3	8.0 ± 29.5
120–240 min	187.8 ± 31.8	194.8 ± 22.4	7.0 ± 20.2

Data are mean ± SD of seven participants. *$P < 0.05$ vs baseline. AUC, area under the curve; GLP-1, glucagon-like peptide.

OGTT. The present data suggested that 12-week sitagliptin treatment improved glycemic control independent of ectopic fat changes in liver.

Previous reports suggested that diet, exercise and drug therapy can change ectopic fat content[5,6,29]. Previous reports showed that moderate weight reduction (6–7%) by calorie restriction can result in marked decrease in IHL, by as much as 81% in one study[29] and 36% in another[6], and that such change was associated with improved suppression of EGP by insulin[29] and splanchnic glucose uptake during hyperinsulinemic clamp study[6]. It has also been shown that a mere 2% weight reduction by calorie restriction is associated with ~25% decrease in IHL level and improved splanchnic glucose uptake[5]. Another study also showed that pioglitazone markedly decreased IHL and augmented splanchnic glucose uptake in type 2 diabetes[30]. Thus, reduced IHL is closely associated with improved glucose metabolisms in the liver. Although treatment with DPP-4 inhibitors has been shown to improve fatty liver in animal models[18], we did not observe such an effect in patients with type 2 diabetes and fatty liver. In addition, the present study also showed no significant change in IMCL level after 12-week sitagliptin treatment. It has been also reported that treatment of patients with non-alcoholic steatohepatitis (NASH) using sitagliptin improved liver histology, such as ballooning and NASH scores, and tended to improve steatosis score ($P = 0.054$)[31]. However, ~3% bodyweight reduction was observed during the 1-year treatment period, although sitagliptin is known to be weight neutral[8]. As a small weight reduction (2–7%) by calorie restriction results in marked IHL reduction (25–81%)[5,6,29], the 3% weight loss observed at the end of sitagliptin treatment could have greatly contributed to the observed improvement in NASH independent of sitagliptin. Thus, the effect of sitagliptin on IHL accumulation is still uncertain[31]. Taken together, the aforementioned data suggest that 12-week sitagliptin treatment improves

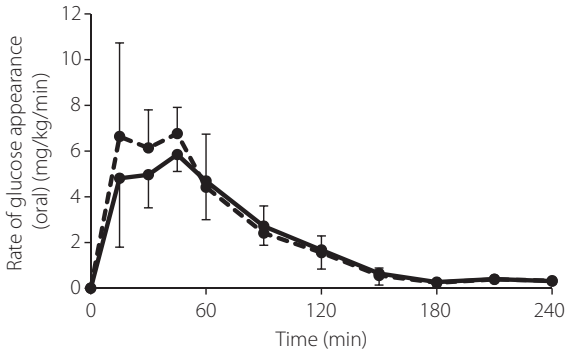

Figure 2 | Rate of oral glucose appearance during oral glucose tolerance test at pre- (baseline; dashed line) and post-sitagliptin treatment (sitagliptin; solid line).

postprandial glycemic control independent of changes in ectopic fat content in liver and muscle.

Several reports have suggested that GLP-1 administration decreases bodyweight and improves fatty liver[16–20]. In contrast, in the case of sitagliptin treatment, not only active GLP-1, but also active gastric inhibitory polypeptide (GIP) are increased[32]. GIP is shown to have an effect of enhancing lipid accumulation, thus it is likely to enhance the obesity and fatty liver[33]. Indeed, GIP level is elevated in NASH patients[34], and chronic administration of GIP antagonist improved high-fat diet-induced fatty liver in a rodent model[35]. Thus, in the case of sitagliptin treatment, it is possible that elevated GIP level might partially diminish the effect of GLP-1 on bodyweight and fatty liver.

Because the main purpose of the present study was to evaluate the effects of changes in fatty liver on glucose kinetics after chronic sitagliptin treatment, the participants did not take any medications, including sitagliptin, on the day of OGTT to reduce the acute effects of sitagliptin. It has been shown that a single dose of sitagliptin acutely suppresses DPP-4 activity by ~95%[36], and increases GLP-1 and insulin levels, and decreases glucagon level[14]. DPP-4 activity is decreased to ~80 or 30% after 24 or 48-h from final administration with 100 mg sitagliptin, respectively[36,37]. Thus, it is reasonable that the participants showed only a modest increase in GLP-1 level without significant changes in insulin and glucagon levels during OGTT. Under such conditions, it is feasible to evaluate the effects of sitagliptin on glucose kinetics during OGTT without significant changes in insulin and glucagon levels, which are known as important regulators of hepatic glucose production. This could be one of the reasons why the present results are different from previous studies, which reported that treatment with DPP-4 inhibitors resulted in decreased EGP level at fasting and postprandial states, which is accompanied by decreased glucagon and increased insulin levels[14,15].

Our protocol incidentally induced unique hormonal changes, a moderate increase in GLP-1, but no significant changes in area under the curves of insulin and glucagon when tested after sitagliptin treatment. In such a situation, we observed decreased RaOral during OGTT. In agreement with these findings, Muscelli *et al.*[15] reported recently that sitagliptin treatment in type 2 diabetes decrease not only EGP, but RaOral during the meal test. These data suggest that long-term treatment with DPP-4 inhibitor could have the potential to improve hepatic glucose uptake. In fact, injection of GLP-1 directly in the portal vein increased hepatic glucose uptake in dogs[11]. In addition, administration of DPP-4 inhibitor in dogs infused with low-dose GLP-1 into the portal vein ameliorated hepatic glucose uptake[10]. In these studies, insulin and glucagon levels were fixed by pancreatic clamp, and the GLP-1 concentration in the portal vein was increased within the physiological range. Although hepatic glucose uptake is, at least in part, regulated by glucokinase (GK) activity in the liver[38], recent data showed that treatment by exendin-4, a potent GLP-1 receptor agonist, increased hepatic GK activity in *db*/*db* mice and enhanced glycogen content in hepatocyte[39]. Thus, increased hepatic GK activity might be involved in the mechanism of decreased RaOral after sitagliptin treatment. Altogether, these data suggest that DPP-4 inhibitor might increase hepatic glucose uptake through increased GLP-1 level. In the present study, AUC-GLP-1 tended to be negatively correlated with AUC-RaOral ($r = 0.49$, $P = 0.076$). However, at least in part because of the small sample size, we cannot conclude the mechanism of increased hepatic glucose uptake by DPP-4 inhibitor. Thus, further study with increasing samples size is definitely essential to clarify the mechanism.

In addition, it is also possible that improved glycemic control by long-term sitagliptin treatment contributes to increased splanchnic glucose uptake, based on previous findings suggesting that improvement of glycemic control by lifestyle modification[5,6], insulin treatment[40] or pioglitazone[30] is associated with increased splanchnic glucose uptake. The hepatic GK expression level is increased by insulin[41], and GLP-1 has been reported to directly activate insulin signaling[42]. We did not observe significant changes in insulin levels during OGTT after sitagliptin treatment; however, sitagliptin treatment might enhance both insulin and GLP-1 levels during daily food intake, and contribute to an increase in hepatic GK expression as a chronic effect.

One limitation of the present study was the small sample size. In addition, we recruited type 2 diabetic patients with fatty liver. Although BMI level was not used as inclusion criteria, most of the included patients were obese. A recent meta-analysis study has shown that lower BMI predicts greater reduction of HbA1c after treatment with DPP-4 inhibitor[43], thus, the participants in the present study might be relatively low responders to treatment with sitagliptin.

In addition, patients were treated with different oral hypoglycemic agents and continued during sitagliptin treatment. Of note, most patients were treated with metformin, and we cannot exclude the possibility that metformin treatment could potentially influence the results, whereas a recent meta-analysis

study showed that metformin did not improve steatosis in non-alcoholic fatty liver disease (NAFLD)/NASH patients[44]. Because we did not have a control group, we cannot exclude the possibility that lifestyle changes during intervention might have an influence on the result. Another limitation was the short duration of treatment with sitagliptin. Thus, we cannot conclude that treatment with sitagliptin is not effective on ectopic fat and other parameters of glucose kinetics analysis.

In conclusion, the beneficiary effects of 12-week sitagliptin treatment in type 2 diabetics with fatty liver on glycemic control (i.e., reduced postprandial hyperglycemia and RaOral) do not encompass changes in liver fat content, at least in the present pilot study. Further randomized control trials in a large number of participants are required to confirm the data presented.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | Glucose and hormonal levels during oral glucose tolerance test at pre- (baseline; dashed line) and post-sitagliptin treatment (sitagliptin; solid line). (a) Insulin. (b) C-peptide. (c) Glucagon. CPR, C-peptide immunoreactivity; IRI, immune reactive insulin.

Figure S2 | Glucose kinetics during oral glucose tolerance test at pre- (baseline; dashed line) and post-sitagliptin treatment (sitagliptin; solid line). (a) Endogenous glucose production. (b) Rate of disappearance. (c) Glucose clearance.