



## Effects of a Dipeptidyl Peptidase 4 Inhibitor Sitagliptin on Glycemic Control and Lipoprotein Metabolism in Patients with Type 2 Diabetes Mellitus (GLORIA Trial)

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**Aim:** The morbidity of cardiovascular disease in patients with type 2 diabetes mellitus (DM) deteriorates in combination with dyslipidemia. The accumulation of remnant lipoproteins in patients with fasting and postprandial hypertriglyceridemia is highly atherogenic. The current study investigated whether the dipeptidyl peptidase-4 inhibitor sitagliptin ameliorates dyslipidemia and hyperglycemia.

**Methods:** We enrolled 38 patients with type 2 DM (20 males and 18 females,  $65.7 \pm 9.9$  years old, HbA1c levels  $< 8.4\%$ ), and all patients gave written informed consent. Sitagliptin (50 mg/day) was added to current antidiabetic treatments and increased to 100 mg/day to achieve low HbA1c levels ( $< 7.4\%$ ). Glucose and lipoprotein metabolism profiles were analyzed at 0, 4, and 12 weeks after sitagliptin administration.

**Results:** Sitagliptin significantly decreased fasting levels of triglyceride (TG) ( $161 \pm 90$  vs.  $130 \pm 66$  mg/dl,  $p < 0.01$ ) and non-HDL-C ( $129 \pm 29$  vs.  $116 \pm 20$  mg/dl,  $p < 0.01$ ) in combination with glucose ( $150 \pm 47$  vs.  $129 \pm 27$  mg/dl,  $p < 0.01$ ) and HbA1c ( $7.1 \pm 0.6$  vs.  $6.6 \pm 0.7$  mg/dl,  $p < 0.001$ ). Sitagliptin also significantly decreased the fasting levels of apolipoprotein (apo) B-48 ( $7.8 \pm 6.7$  vs.  $5.6 \pm 4.0$   $\mu\text{g/ml}$ ,  $p < 0.01$ ), remnant lipoprotein cholesterol ( $15.3 \pm 9.5$  vs.  $12.0 \pm 7.9$  mg/dl,  $p < 0.05$ ) and other apolipoproteins, such as apoB, apoC-II, apoC-III, and apoE. Analyses of the lipoprotein profiles of fasting sera revealed that sitagliptin significantly decreased cholesterol and TG levels of lipoprotein fractions in the size of very low density lipoprotein and low density lipoprotein.

**Conclusions:** These findings indicated that sitagliptin administration ameliorated the lipid and lipoprotein profiles in patients with diabetes, which may be due to the decrease in atherogenic remnant lipoproteins (UMIN#000013218).

**Key words:** Postprandial hypertriglyceridemia, DPP-4, Sitagliptin, Lipoprotein Profile, Remnants

**Abbreviations;** apo: apolipoprotein, ASCVD: atherosclerotic cardiovascular disease, CHD: coronary heart disease, CLEIA: chemiluminescence enzyme immunoassay, CM: Chylomicron, DPP-4: dipeptidyl peptidase-4, FFAs: free fatty acids, HPLC: high-performance liquid chromatography, IMT: intima-media thickness, LDL: low-density lipoprotein, LPL: lipoprotein lipase, PHTG: postprandial hypertriglyceridemia, RemL-C: remnant lipoprotein cholesterol, RLP-C: remnant-like particle cholesterol, TG: triglyceride, TRL: triglyceride-rich lipoprotein, VLDL: very low density lipoprotein

## 1. Introduction

The number of Japanese patients with impaired glucose tolerance (IGT) or diabetes mellitus (DM) increased in recent decades more prominently than hypertension or hypercholesterolemia<sup>1</sup>. Diabetes is an important and independent risk factor for the development of coronary heart disease (CHD), and the mortality of CHD is higher in patients with diabetes than subjects without diabetes<sup>2</sup>. Hypertriglyceridemia is also an independent risk factor for CHD<sup>3</sup> and is a residual risk factor for CHD in dyslipidemic patients treated with cholesterol-lowering agents, such as statins<sup>4</sup>. The triglyceride (TG)-rich lipoproteins and remnant lipoproteins, named “remnants,” accumulate in patients with fasting and postprandial hypertriglyceridemia (PHTG) and diabetes<sup>3</sup>. Fasting levels and postprandial increases in remnant-like particle cholesterol (RLP-C) are also high in patients with diabetes<sup>5</sup>. In patients with type 2 DM, increased production of VLDL in combination with low lipoprotein lipase (LPL) activity leads to the accumulation of remnants, which increases the transfer of TG to LDL and HDL and a concomitant transfer of cholesteryl esters from LDL and HDL to remnants<sup>6</sup>. As a result, TG-rich remnants, small, dense LDL and small, dense HDL are increased in patients with type 2 DM<sup>6</sup>. Levels of the quantitative marker of remnants, RLP-C, correlate with the intima-media thickness (IMT) of the carotid artery independent of low-density lipoprotein (LDL)-cholesterol level<sup>7</sup> and CHD morbidity<sup>8</sup>. Remnants enhance the formation and progression of atherosclerotic plaques via direct invasion into the subendothelial space. Chylomicron (CM) remnants, produced by intestine-derived CM, are highly atherogenic remnants<sup>3</sup>. The concentration of the quantitative marker for CM remnants, fasting serum apoB-48, correlates with the prevalence of PHTG, carotid IMT, and CHD<sup>3</sup>. Fasting levels and postprandial increases in apoB-48 are high in patients with IGT or diabetes<sup>9,10</sup>. The combination of hypertriglyceridemia with diabetes is a high-risk status for CHD prevalence<sup>11</sup> and cardiovascular (CV) death<sup>12</sup>, and dual therapy to ameliorate the accumulation of remnants and hyperglycemia may strongly improve the atherosclerotic plaque and prevent CV events.

An incretin-based drug for diabetes, dipeptidyl peptidase-4 (DPP-4) inhibitor, improves glycemic control and reduces the morbidity of microvascular complications in patients with diabetes. DPP-4 inhibitors effectively decrease fasting and postprandial hyperglycemia and may exhibit anti-atherogenic effects. However, three famous studies of DPP-4 inhibitors failed to demonstrate significant effect on CV outcomes in patients with diabetes in a short-term clinical intervention<sup>13</sup>. If DPP-4 inhibitor administration would effectively improve the impaired lipoprotein metabolism in patients with type 2 DM such as increases of remnants, small, dense LDL or small, dense HDL, it would be useful for reducing risk status for atherosclerotic plaque formation and decreasing CV outcome by improving impaired metabolism of both glucose and lipoproteins. The current study evaluated whether sitagliptin treatment may ameliorate the fasting impaired lipoprotein profile as well as hyperglycemia in patients with type 2 DM, by evaluating lipoprotein change by measuring apolipoproteins, remnants, and lipid contents of lipoprotein fractions in detail.

## 2. Study Design

### 2.1 Study Population and Treatment

As a prospective cohort study, effects of sitagliptin on glucose and lipoprotein metabolism were evaluated in patients with type 2 DM under mild control via addition to the ongoing lifestyle modification and drug therapy. To perform the detailed investigation of lipoprotein change by measuring apolipoproteins, remnants, and lipid contents of lipoprotein fractions, we thought that around 40 patients are enough for this purpose in this single-arm study. The study protocol was initially reviewed and approved by the institutional review board of Osaka University and participating institutions (UMIN ID, #000013218). This study was performed in accordance with the ethical principles of the Declaration of Helsinki and the Ethical Guidelines for Clinical Research, enforced by the Ministry of Health, Labour and Welfare of Japan (2008).

Patient's background, medical history, drug doses, height, and weight were obtained during the screening period, and concentrations of serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting blood glucose (FBG), HbA1c, total cholesterol (TC), TG, and HDL-cholesterol (HDL-C) were measured according to the institutional methods. Patients with the following two clinical statuses were enrolled: (1) type 2 diabetes (FBG >126 mg/dl or HbA1c <8.4%) with an insuf-

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ficient response to (a) a lifestyle modification, including diet and exercise therapy only or (b) a lifestyle modification with one of the following drugs: sulfonylurea (glimepiride  $\leq 2$  mg/day, glibenclamide  $\leq 1.25$  mg/day, or gliclazide  $\leq 40$  mg/day), thiazolidine, biguanide, alpha-glucosidase inhibitor, or insulin product and (2) informed consent for participation on a voluntary basis. The following exclusion criteria were used at the time informed consent was obtained: (1) type 1 diabetes, (2) severe renal disorder, including hemodialysis (serum creatinine level  $>2.5$  mg/dl for males and  $>2.0$  mg/dl for females), (3) receiving a sulfonylurea drug at a higher dose (glimepiride  $>2$  mg, glyburide  $>1.25$  mg or gliclazide  $>40$  mg), (4) ongoing treatment with sitagliptin or other DPP-4 inhibitors, (5) ongoing treatment with a statin, fibrate, ezetimibe, or recently started probucol within 1 month, (6) women who were pregnant, lactating, may become pregnant or wish to become pregnant during the study period, (7) ongoing treatment for thyroid disease, (8) severe hepatic dysfunction (AST and ALT  $\leq 100$  IU/L), (9) participation in other clinical trials, or (10) inappropriate candidate for participation as assessed by the doctors. Height and weight were measured after informed consent, and fasting blood was drawn after an overnight fast. Serum and plasma were separated via brief centrifugation, and all samples were stored at  $-80^{\circ}\text{C}$  until analyzed. One 50-mg tablet of sitagliptin was orally administered once daily for at least 12 weeks, but the dose could be increased up to 100 mg/day to achieve a FBG of 126 mg/dl or less and HbA1c  $<7.4\%$ . The doctor could reduce the dose of antidiabetic drugs, such as sulfonylureas, in case of hypoglycemia, but changes in the dose of antihyperlipidemic or antihypertensive drugs were not permitted. Adverse side effects were examined 4 and 12 weeks after sitagliptin treatment, and blood samples were obtained and stored.

## 2.2 Measurements

Levels of serum creatinine, TC, TG, and free fatty acids were measured using enzymatic methods. HDL-C levels were measured by the direct method. HbA1c was measured using high-performance liquid chromatography (HPLC). Plasma glucose levels were measured using a hexokinase UV assay. Serum insulin levels were measured using a chemiluminescence enzyme immunoassay (CLEIA). Glucagon levels were measured using a double-antibody radioimmunoassay, and levels of apolipoproteins, such as apoA-1, apoA-2, apoB, apoC-II, apoC-III, and apoE, were measured using the immunoturbidity method (SRL, Inc., Tokyo, Japan). Non-HDL-C levels were also calculated (non-HDL-C = TC - HDL-C). ApoB-48 levels

were measured using a CLEIA method (Fujirebio Inc., Tokyo, Japan)<sup>3</sup>. Cholesterol levels of remnants were measured using a remnant lipoprotein cholesterol (RemL-C) homogenous assay (Kyowa Medex, Tokyo, Japan). Serum pre-heparin LPL mass and activity (IBL, Fujioka, Japan), adiponectin (Otsuka Pharmaceuticals, Co., Ltd., Tokyo, Japan) and omentin levels were measured using a human ELISA kit. Serum lipoproteins were analyzed using HPLC as previously described. Two CM subclasses (large and small), five VLDL subclasses (large1,2,3, medium, and small), six LDL subclasses (large, medium, small, and very small1,2,3), and six HDL subclasses (very large, large, medium, small, and very small1,2) were identified on the basis of lipoprotein particle size<sup>14</sup>.

## 2.3 Assessments and Statistical Analyses

The number of cases was set at 40 to evaluate significant differences. The patient population for statistical analyses was the full analysis set. The primary outcome was changes in apoB-48 and RemL-C concentrations at 12 weeks, and secondary outcomes were changes in glucose and lipid markers and the lipoprotein profile. Data were expressed as the means  $\pm$  standard deviations for continuous variables. Skewed variables (TG and apoB-48) were logarithmically transformed to improve data normalization. The significance of differences in all parameters was tested using paired *t*-tests. Statistical analyses were performed using JMP8 software (SAS Institute, Cary, NC, USA). Statistical significance was established at a *p* value of  $< 0.05$ .

## 3. Results

### 3.1 Baseline Characteristics

We enrolled 38 patients with type 2 DM from three hospitals (Osaka University Hospital, Osaka Central Hospital, Sousei Hospital). **Table 1** shows the baseline clinical characteristics. There was no significant difference in the average age between the 20 males and 18 females. The mean body mass index (BMI) was relatively high in Japanese patients with diabetes. Two-thirds of the enrolled patients were receiving drug treatment for DM, and sulfonylurea and insulin products were primarily used. Approximately two-thirds of the enrolled patients were diagnosed with dyslipidemia. Proper intervention using statins reduced TC and LDL-C levels in most patients with dyslipidemia to within normal levels based on Japan Atherosclerosis Society guidelines<sup>15</sup>. Hypertriglyceridemia ( $\geq 150$  mg/dl) was observed in half of the enrolled patients, and the ratio of patients who were treated with fibrates or eicosapentanoic acid was

**Table 1.** Patient backgrounds and profiles

Age (years)	65.7 ± 9.9
Sex (m, f)	(20, 18)
Body mass index (BMI) (kg/m <sup>2</sup> )	25.1 ± 4.4
< 25 ( <i>n</i> )	18
25-30 ( <i>n</i> )	18
> 35 ( <i>n</i> )	2
Fasting glucose (mg/dl)	150.3 ± 57.3
HbA1c (%)	7.1 ± 0.7
Creatinine (mg/dl)	0.8 ± 0.2
Drugs for diabetes treatment	
none (%)	31.5
sulfonylurea (%)	21.1
thiazolidine (%)	2.6
BG (%)	18.4
αGI (%)	7.9
insulin (%)	18.6
Morbidity of dyslipidemia (%)	68.4
TC (mg/dl)	179.5 ± 32.5
TG (mg/dl)	141.1 ± 78.1
HDL-C (mg/dl)	58.0 ± 15.7
LDL-C (mg/dl)	100.2 ± 24.6
Drugs for dyslipidemia (%)	
statins (%)	44.7
fibrates (%)	7.9
EPA (%)	18.4
Morbidity of hypertension (%)	57.9
Prior ischemic heart disease (%)	21.2

Morbidity of dyslipidemia was based on clinical diagnosis using the guidelines of the Japan Atherosclerosis Society (ref. 15) or patients who were treated with anti-hyperlipidemic drugs prior to the study. The morbidity of hypertension was based on the number of patients who were treated with anti-hypertensive drugs prior to the study. HbA1c: Hemoglobin A1c; BG: Biguanide; αGI: alpha-glucosidase inhibitor; TC: total cholesterol; TG: triglyceride; EPA: eicosapentaenoic acid.

low.

### 3.2 Changes in Glucose and Lipid Metabolism

Sitagliptin treatment for 12 weeks decreased many biomarkers of glucose and lipoprotein metabolism (**Table 2**). No significant difference in body weight or waist circumference was observed after 12 weeks of treatment. Sitagliptin decreased fasting glucose levels (150 ± 47 vs. 129 ± 27 mg/dl,  $p < 0.01$ ) and HbA1c levels (7.1% ± 0.6% vs. 6.6% ± 0.7%,  $p < 0.001$ ). There was no significant difference in fasting levels of insulin, adiponectin, omentin, or glucagon and no significant change in homeostasis model assessment of insulin resistance. No adverse side effects, including hypoglycemia, were observed. Sitagliptin significantly decreased levels of fasting TG, LDL-C, non-HDL-C at 4 and 12 weeks of treatment. Sita-

gliptin decreased TC and LDL-C levels significantly at 4 weeks of treatment, but there was no significant difference in these levels at 12 weeks of treatment.

### 3.3 Changes in Lipoprotein Metabolism

Sitagliptin significantly decreased clinical markers for remnants, fasting apoB-48 (7.8 ± 6.7 vs. 5.6 ± 4.0 μg/ml,  $p < 0.01$ ) and RemL-C levels (15.3 ± 9.5 vs. 12.0 ± 7.9 mg/dl,  $p < 0.05$ ; **Table 2**). Sitagliptin treatment decreased other apolipoproteins, such as apoB, apoC-II, apoC-III, and apoE and increased the ratio of preheparin LPL activity/LPL mass. HPLC analyzed changes in lipoprotein profile during sitagliptin treatment. Sitagliptin decreased TC and total TG levels, and decreases in the cholesterol and TG concentrations of lipoprotein fractions were primarily observed in the lipoproteins with VLDL and LDL size (**Fig. 1A**). Decreases in cholesterol and TG concentration were observed in lipoproteins with large and small VLDL size fractions as well as small to very small LDL size fractions (**Fig. 1B** and **C**). In contrast, cholesterol and TG concentration in lipoproteins with very small HDL size fractions were decreased (**Fig. 1D**). There was no significant difference in changes of fasting glucose, HbA1c, lipid, and lipoprotein profiles between in patients with BMI > 25 kg/m<sup>2</sup> and those with BMI < 25 kg/m<sup>2</sup>.

## 4. Discussion

### 4.1 Effect of Sitagliptin on Lipid and Lipoprotein Metabolism

Sitagliptin significantly decreased fasting levels of glucose and HbA1c in patients with controlled type 2 DM (**Table 2**). Hyperglycemia exacerbates thrombogenicity, endothelial dysfunction and inflammation of the arterial wall, and these changes may cause microvascular and macrovascular events<sup>16</sup>. Sitagliptin treatment did not produce significant changes in body weight or levels of insulin, omentin, or glucagon, likely because two-thirds of the patients were already treated with antidiabetic drugs, and the hyperglycemia at the baseline was not too severe (HbA1c levels; 7.1% ± 0.6%). The significant decreases in fasting glucose and HbA1c levels were observed without any adverse side effects, including hypoglycemia, during the intervention period for 12 weeks. Therefore, the effective dose of sitagliptin is likely safe. Dyslipidemia, including hypertriglyceridemia, is an important complication in patients with diabetes<sup>3, 6</sup>. Therefore, if hyperglycemia and dyslipidemia were improved by sitagliptin, its administration may be suitable and effective for the amelioration of atherogenic status in these patients. In this study, sitagliptin markedly

**Table 2.** Changes in glucose and lipid profiles by sitagliptin treatments

	before treatment	4 weeks		12 weeks	
glucose (mg/dl)	150.3 ± 47.2	126.1 ± 28.9	0.002	129.4 ± 27.1	0.011
HbA1c (%)	7.1 ± 0.6	6.8 ± 0.6	0.000	6.6 ± 0.7	0.000
insulin (μU/L)	11.9 ± 12.6	11.2 ± 14.2	0.453	12.2 ± 13.0	0.957
adiponectin (mg/dl)	6.6 ± 3.4	5.9 ± 3.2	0.096	5.4 ± 3.1	0.093
omentin (mg/dl)	486.8 ± 140.9	476.2 ± 161.5	0.620	470.7 ± 155.0	0.463
glucagon (mg/dl)	84.3 ± 24.8	85.6 ± 22.7	0.892	87.2 ± 26.5	0.517
creatinine (mg/dl)	1.1 ± 0.3	1.1 ± 0.4	0.439	1.1 ± 0.5	0.733
TC (mg/dl)	185.2 ± 30.5	169.2 ± 27.9	0.037	172.0 ± 24.9	0.057
TG (mg/dl)	160.7 ± 89.6	142.8 ± 87.7	0.026	129.7 ± 66.5	0.004
HDL-C (mg/dl)	55.8 ± 12.1	54.4 ± 12.4	0.043	55.9 ± 12.5	0.407
LDL-C (mg/dl)	89.1 ± 23.5	80.2 ± 17.6	0.014	81.0 ± 19.3	0.039
non-HDL-C (mg/dl)	126.0 ± 35.6	105.8 ± 38.9	0.021	85.6 ± 20.0	0.001
total FFA (mEq/l)	521.2 ± 303.1	465.5 ± 235.4	0.224	612.1 ± 309.2	0.020
apoB-48 (μg/ml)	7.8 ± 6.7	5.9 ± 5.5	0.047	5.6 ± 4.0	0.009
RemL-C (mg/dl)	15.3 ± 9.5	12.5 ± 10.0	0.086	12 ± 7.9	0.034
apoA-I (mg/dl)	151.3 ± 20.2	145.0 ± 21.4	0.000	148.1 ± 20.1	0.119
apoA-II (mg/dl)	30.8 ± 6.3	29.2 ± 6.1	0.001	29.7 ± 5.6	0.000
apoB (mg/dl)	88.8 ± 20.2	79.7 ± 16.9	0.004	80.9 ± 14.9	0.032
apoC-II (mg/dl)	5.2 ± 2.3	4.3 ± 2.1	0.000	4.3 ± 1.6	0.000
apoC-III (mg/dl)	11.9 ± 5.0	10.1 ± 4.4	0.000	10.2 ± 3.2	0.001
apoE (mg/dl)	4.4 ± 1.6	4.0 ± 1.5	0.068	4.0 ± 1.4	0.191
LPL mass	95.5 ± 32.1	80.4 ± 24.5	0.007	80.5 ± 33.2	0.017
LPL activity	11.8 ± 1.3	11.9 ± 2.2	0.612	15.0 ± 2.0	0.000
LPL activity/mass	0.140 ± 0.060	0.161 ± 0.055	0.009	0.214 ± 0.124	0.000

Blood was drawn after an overnight fast at registration and after 12 weeks of sitagliptin treatment. Sera were separated via brief centrifugation and stored at  $-80^{\circ}\text{C}$ . LDL-C levels were measured by the HPLC method. The data are expressed as the means  $\pm$  standard deviations (SDs) for continuous variables, and skewed variables (TG and apoB-48) were logarithmically transformed to improve data normalization. Measured parameters at 4 and 12 weeks of sitagliptin treatment were compared to those before treatments and analyzed using paired Student's *t*-test. Statistical significance was set at  $p < 0.05$ .

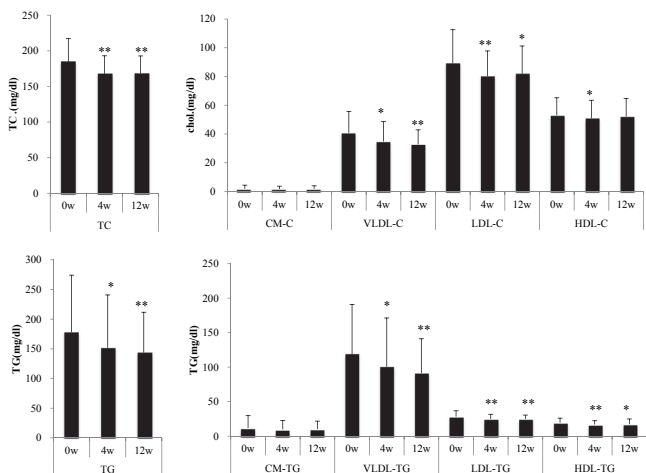
decreased fasting levels of non-HDL-C, LDL-C, and apo B, which suggests that RemL-C levels decreased in combination with LDL-C levels (**Table 2**). Sitagliptin markedly decreased fasting levels of apoB-48 and RemL-C, which are related to the accumulation of remnants (**Table 2**). Since the increase in pre-heparin LPL activity/mass suggests the enhancement of the clearance of remnants, it was suggested that sitagliptin decreased the accumulation of remnants by enhancing LPL activity. HPLC analysis revealed that sitagliptin decreased cholesterol and TG concentrations of lipoprotein fractions in VLDL and LDL, especially from large VLDL to very small LDL fractions (**Fig. 1A–D**). These results suggest that sitagliptin efficiently reduced lipoproteins in the size of large VLDL to very small LDL. In our former study, apoB-48-containing lipoproteins such as CM and CM remnants were existed from large VLDL to very small LDL fractions when serum samples were subfractioned by HPLC<sup>3</sup>.

Sitagliptin reduced apoB-48 concentrations, which suggests that sitagliptin treatment ameliorated the accumulation of CM remnants. In contrast, sitagliptin decreased TG and cholesterol concentrations in lipoproteins with very small HDL size fractions (**Fig. 1D**). These fractions contain small, dense HDL particle, which is increased in patients with type 2 DM<sup>6</sup>. The decrease of small, dense LDL is suitable for preventing ASCVD as well as that of remnants in patients with type 2 DM. Moreover, there was no difference in changes of lipid and lipoprotein profiles between in obese (BMI  $> 25 \text{ kg/m}^2$ ) and lean (BMI  $< 25 \text{ kg/m}^2$ ) patients, sitagliptin may be useful for improving impaired lipid metabolism independent of the obesity.

#### 4.2 Effect of Sitagliptin on Ameliorating the Accumulation of Remnants in Comparison with Other Incretin-based Drugs

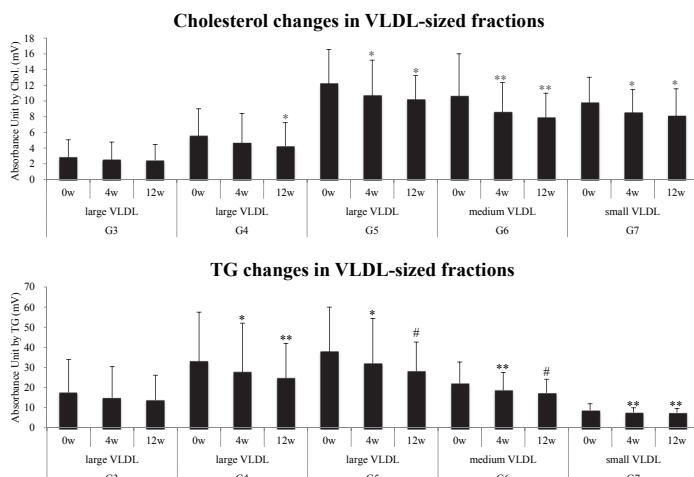
Sitagliptin treatments decreased apoC-III levels

### Changes in Lipoprotein Profile



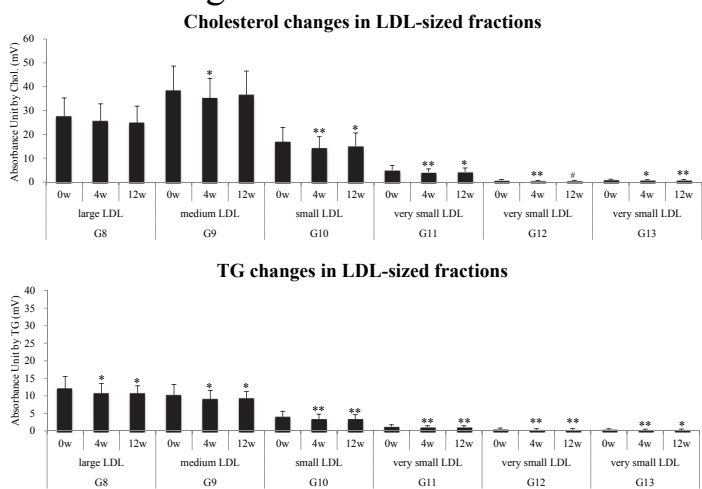
(Figure 1A) (\* p<0.05. \*\* p<0.01. # p<0.001 by paired Student's t-test vs. 0 w)

### Changes in VLDL-sized fractions



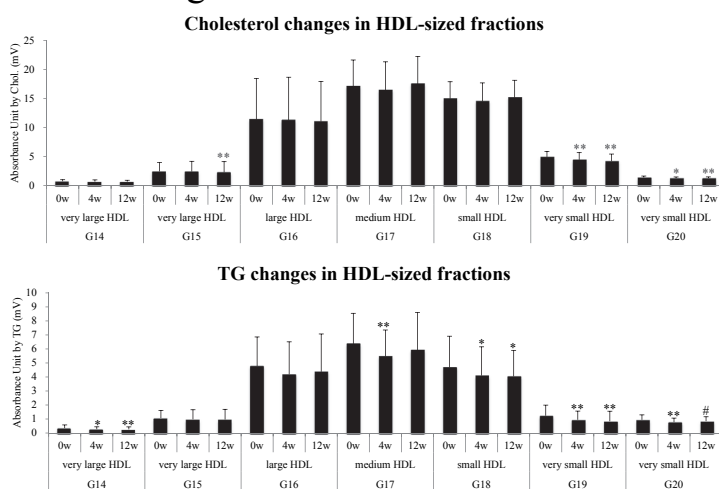
(Figure 1B) (\* p<0.05. \*\* p<0.01. # p<0.001 by paired Student's t-test vs. 0 w)

### Changes in LDL-sized fractions



(Figure 1C) (\* p<0.05. \*\* p<0.01. # p<0.001 by paired Student's t-test vs. 0 w)

### Changes in HDL-sized fractions



(Figure 1D) (\* p<0.05. \*\* p<0.01. # p<0.001 by paired Student's t-test vs. 0 w)

**Fig. 1.** Blood was drawn at registration after an overnight fast and after 12 weeks of sitagliptin treatment

Sera were separated via brief centrifugation and stored at  $-80^{\circ}\text{C}$ . Lipoprotein profiles were analyzed using HPLC, as described in reference 24. TG and cholesterol concentrations of the following separated subfractions were measured on the basis of lipoprotein particle size: two CM subclasses (large and small), three VLDL subclasses (large, medium, and small), four LDL subclasses (large, medium, small, and very small), and five HDL subclasses (very large, large, medium, small, and very small). Changes in these two levels were compared at 0, 4, and 12 weeks after sitagliptin treatment. Total contents and the four main fractions (Fig. 1A), VLDL-sized fractions (Fig. 1B), LDL-sized fractions (Fig. 1C), and HDL-sized fractions (Fig. 1D). \* $p < 0.05$ . \*\* $p < 0.01$ . # $p < 0.001$  by paired Student's  $t$ -test vs. 0 week data.

and increased LPL activity in the current study (Table 2). ApoC-III increases TG concentrations via inhibition of LPL activity<sup>17)</sup> and decreases the hepatic uptake of remnants<sup>18)</sup>, causing the accumulation of remnants. However, apoC-III gene expression is downregulated by insulin and upregulated by glucose, and insulin resistance and hyperglycemia induce an overproduction of apoC-III<sup>19, 20)</sup>. Elevated apoC-III levels lead to vascular dysfunction and the develop-

ment of atherosclerosis by impairing insulin-mediated stimulation of endothelial NO production and inducing endothelial dysfunction<sup>21)</sup>. Therefore, the effects of sitagliptin in decreasing apoC-III levels and enhancing LPL activity may ameliorate hypertriglyceridemia and the accumulation of remnants in conditions with DM.

There is a close relationship between apoB-48-containing lipoprotein metabolism and insulin resis-

tance. A postprandial accumulation of CM and CM remnants exists in a state of insulin resistance, which is due to an overproduction of CM from the intestine<sup>22-24</sup>. The DPP-4 inhibitor sitagliptin enhances incretin function, and the receptor of incretins was reported to be essential for CM synthesis and secretion in hamsters and mice<sup>25</sup>. One incretin, glucagon-like peptide 1 (GLP-1), reduces intestinal lymph flow, TG absorption, and apolipoprotein production in rats<sup>26</sup>, and a GLP-1 analog may affect gastric emptying rate<sup>27</sup>, but there is a controversial result<sup>28</sup>. Incretin-based drugs improve intestine-derived CM metabolism during the postprandial state. We examined the effect of sitagliptin on glucose and lipoprotein metabolism during a fasting state, but a previous report demonstrated that sitagliptin improves postprandial increases in TG and apoB-48 concentrations in patients with diabetes<sup>29</sup>. Vildagliptin also suppresses postprandial increases in TG and CM remnants, suppresses lipid oxidation, and activates sympathetic responses<sup>30, 31</sup>. The mechanism of incretin-based drugs on improving PHTG has not been clarified yet, but incretin-based treatments may reduce the CV risk in DM patients and the accumulation of remnant lipoproteins during fasting and postprandial states. Further studies are needed to delineate the mechanism for the improvement of accumulation of remnants in DM by incretin-based drugs.

#### 4.3 Effect of Sitagliptin and Incretin-based Drugs on CV Outcomes

Recent mega-trials demonstrated that the DPP-4 inhibitors saxagliptin (SAVOR-TIMI 53 trial), alogliptin (EXAMINE trial), and sitagliptin (TECOS trial) did not improve CV outcomes in patients with diabetes<sup>13</sup>. The observation periods of these studies were several years (3–5 years), and the enrolled patients with type 2 DM were not stratified by the presence or absence of dyslipidemia. In contrast, recent investigation from Taiwan using a national health insurance research database showed that significant low incidences of CVD, CHD, ischemic cerebral stroke, and all-cause death were observed in sitagliptin users of type 2 diabetic population compared with nonusers over a mean of 14 months' observation after adjustment for covariates<sup>32</sup>. Other incretin-based drug, an analog of human GLP-1, has been approved for the treatment of type 2 diabetes. One of a GLP-1 analog, lixisenatide, did not alter the rate of major CV events or other serious adverse events in patients with type 2 diabetes and a recent acute coronary syndrome<sup>33</sup>; however, another GLP-1 analog, liraglutide, significantly decreased CV-related deaths in patients with type 2 DM and high CV risk<sup>34</sup>. The enrolled

patients in this LEADER trial exhibited diabetes and CV disease or risks, which suggest that these patients had a clustering of other metabolic abnormalities, such as dyslipidemia. CV outcomes in the clinical subclass analysis of the LEADER trial were reduced in patients with severe obesity (BMI > 30) more effectively than those with moderate or nonobesity (BMI ≤ 30)<sup>34</sup>. Serum DPP-4 level was positively and specifically associated with the accumulation of visceral fat and the presence of metabolic syndrome in men with type 2 diabetes<sup>35</sup>. Therefore, the anti-atherogenic effect of incretin-based drugs may be more useful and effective in obese rather than nonobese subjects. Taken together, DPP-4 inhibitor and GLP-1 administration may decrease remnant lipoproteins by improving hyperglycemia and lipid metabolism, thereby reducing the CV risk in patients with DM. The proper selection of patients with accumulated remnants prior to sitagliptin use is strongly needed. Furthermore, an appropriate measurement of markers for accumulated remnants, such as RemL-C or apoB-48 concentrations, should identify these patients. Further studies are needed to improve PHTG in incretin-based treatments.

There are some limitations in the present study. First, the sample size was relatively small compared with similar studies. Second, all subjects were Japanese, and different findings may be obtained in other ethnic groups. Third, we cannot set the control placebo group as we examined the outpatients of the clinic.

## 5. Conclusions

The current study revealed that the administration of DPP-4 inhibitor sitagliptin improved the fasting lipid and lipoprotein profiles in patients with type 2 DM. Sitagliptin treatment may ameliorate the impaired accumulation of atherogenic remnants.

## Conflicts of Interest

DM, TO, YS and SY received research funds from Ono Pharmaceutical Company Ltd. as joint researchers. YS received honoraria from Ono Pharmaceutical Company Ltd. SY and DM received lecture fees in 2010 from FUJIREBIO Inc. The measurement of apoB-48 concentration was supported by FUJIREBIO Inc. as a joint research effort, and RemL-C concentration was supported by Kyowa Medex Co., Ltd. The other authors have nothing to disclose. This study was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 15K01713, grant-in-aid for Scientific Research (C).

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