Dokkyo Journal of Medical Sciences
41(3)：211～220, 2014

Original

Greater Efficacy and Improved Endothelial Dysfunction in Untreated Type 2 Diabetes with Liraglutide versus Sitagliptin

Kunihiro Suzuki. M.D., Ph.D., Seiichi Tanaka. M.D, Chie Aoki. M.D., Ph.D., Kanako Kato. M.D, Teruo Jojima. M.D., Ph.D. and Yoshimasa Aso. M.D., Ph.D.

Department of Endocrinology and Metabolism, Dokkyo Medical University School of Medicine

SUMMARY

Objective：The incretin hormone glucagon-like peptide 1 (GLP-1) and its analogs, including the glucagon-like peptide 1 receptor agonist liraglutide, use a simple once-daily regimen and can be easily introduced in the outpatient setting. We compared treatment with liraglutide monotherapy and dipeptidyl peptidase-4 (DPP-4) inhibitor monotherapy in patients with untreated type 2 diabetes (T2DM).

Methods：This study included 40 outpatients with untreated T2DM who were randomized to receive liraglutide (0.9 mg/day, n＝24) or DPP-4 inhibitors (n＝16：sitagliptin, 50 mg/day) as initial treatment for 6 months. Glycemic control, urinalysis, blood pressure, body weight, lipid levels, vascular endothelial function, and inflammatory factors were assessed before and after treatment.

Results：Significant improvement was observed in HbA_{1c} and fasting blood glucose levels after treatment in both groups；improvements in the liraglutide group were significantly better than in the sitagliptin group. Only the liraglutide group demonstrated significant improvements in blood pressure, low-density lipoprotein cholesterol levels, urinary albumin excretion, flow-mediated dilatation, and high-sensitivity C-reactive protein levels. Linear regression analysis demonstrated a significant negative relation between change in flow-mediated dilatation and high-sensitivity C-reactive protein levels.

Conclusion：Liraglutide provided significant glycemic control and improved blood pressure, lipid levels, endothelial function, and inflammatory factors in untreated T2DM. In addition to its impact on blood glucose levels, liraglutide may have beneficial effects on the cardiovascular system in patients with T2DM.

Key Words：Type 2 diabetes, Glucagon-like peptide-1 receptor agonist, Liraglutide, Dipeptidyl peptidase-4 (DPP-4) inhibitors, Flow-mediated dilatation

INTRODUCTION

The number of patients with type 2 diabetes (T2DM) is increasing rapidly worldwide, affecting an estimated 285 million patients in 2010[1]. This increase has also been seen in Japan. In Japan, the majority of 7.2 million people with diabetes mellitus are aged between 20 and 79, and the number of people with diabetes mellitus will increase to 10.15 million by 2030[2,3].

Received June 13, 2014；accepted August 5, 2014
Reprint requests to：Kunihiro Suzuki
Department of Endocrinology and Metabolism, Dokkyo Medical University Kitakobayashi, Mibu, Tochigi 321-0293, Japan

Antidiabetic pharmacotherapy has progressed significantly in recent years, and a variety of therapeutic options are currently available. In addition to insulin and conventional oral antidiabetic agents that have been used for many years[4,5], several newer antidiabetic medications have become available including dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists. These newer drugs improve glucose metabolism through the activation of GLP-1 receptor signaling, which induces insulin secretion and suppresses glucagon secretion in the pancreas[6,7].

T2DM increases the risk of atherosclerosis and cardiovascular disease. Endothelial dysfunction signals the start of the atherosclerotic process[8]. Atherosclerosis is an inflammatory disease of the arterial walls, and accumulating evidence suggests the involvement of endothelial dysfunction in all stage of atherogenesis[9]. Therefore, therapy that reduces inflammatory activity and improves endothelial function may have additional therapeutic value in the prevention of atherosclerotic diseases such as hypertension, hypercholesterolemia, and heart failure[10].

Diet and exercise therapy are the first-line treatment for T2DM, but medication to achieve normoglycemia is indicated if strict glycemic control cannot be achieved. The American Diabetes Association and the European Association for the Study of Diabetes have issued a consensus algorithm for the treatment of T2DM[11], in which biguanides are recommended as first-line drug therapy. As recommended in these guidelines, the selection of medications for patients with T2DM in Japan is dependent on the diverse conditions of individual patients[12]. In Japan, oral antidiabetic agents are often used as the first-line treatment for untreated T2DM, sometimes resulting in a complicated regimen of oral medications and poor compliance with therapy[13,14]. For patients with T2DM who cannot be adequately treated with diet and exercise, selecting a drug that will ensure good compliance may help lead to continued treatment.

Administration of liraglutide can be easily introduced in the outpatient setting compared to multiple daily insulin injections or exenatide, another GLP-1 receptor agonist, which is administered twice daily before meals[15]. Liraglutide is also associated with a low risk for hypoglycemia when used alone because of its glucose level-dependent hypoglycemic action, and unlike other antidiabetic drugs, is unlikely to cause weight gain[16,17]. Furthermore, previous studies in cell cultures and animal experiments demonstrated that liraglutide lowers blood pressure, suppresses arteriosclerosis, improves blood lipid profiles, and protects the vascular endothelium[18~20]. Similarly, several studies have reported the beneficial effects of DPP-4 inhibitors on endothelial function in T2DM[21,22]. The present study was conducted in patients with untreated T2DM to compare the effects of liraglutide with DPP-4 inhibitors, in particular, sitagliptin on glycemic control, urinalysis, blood pressure, body weight, lipid levels, vascular endothelial function, and inflammatory factors before and after treatment.

METHODS

Subjects

Fifty-six outpatients with T2DM who had not received any previous treatment for diabetes were enrolled. All patients had visited the Department of Endocrinology and Metabolism at Dokkyo Medical University (Tochigi, Japan) between October 2009 and August 2012. The other eligibility criteria were as follows : age>20 years and hemoglobin A1c (HbA1c)>7.5%. Use of medications for hypertension or dyslipidemia was permitted. Exclusion criteria were as follows : type 1 diabetes, severe complication of diabetes, severe renal and liver dysfunction, pregnant or nursing women and those who might be pregnant, alcoholism, a history of stroke and cardiovascular events, and any patient whom the investigator judged to be inappropriate for this study.

This study was approved by the Ethics Committee of Dokkyo Medical University. All subjects were given an explanation of the study and written informed consent was obtained. This study was designed as a prospective, non-blinded study in accordance with the principles stated in the Declaration of Helsinki.

Study Design

This study was a randomized, non-blind study, 56 outpatients were enrolled this study. Of these, 16 patients withdrew from the study and 40 completed the study. Patients were prospectively assigned to receive treatment with either liraglutide (0.9 mg/day, n = 24) or DPP-4 inhibitors, sitagliptin, (n = 16 : 50 mg/day) for 6 months. Our hospital nutritionist educated all patients regarding dietary habits in accordance with the guidelines of the Japan Diabetes Society[23].

The titration schedule for liraglutide and was determined at the discretion of the investigator. The liraglutide dose was titrated to and maintained at 0.9 mg/day within 1 month from study start. All patients were evaluated at the start of the study and 6 months after starting treatment.

Evaluation of Endothelial Function

Flow-mediated dilatation (FMD) was used to evaluate endothelial function. Monitoring took place in the outpatient setting using UNEXEF18G (UNEX, Nagoya, Japan)[24~26] after subjects rested for 30 min under fasting conditions. FMD values were calculated using the following formula[26] :

%FMD = (diameter at peak hyperemia-diameter at rest)/(diameter at rest) × 100.

Other Measurements

Blood and urine samples were collected at baseline (before treatment) and at 6 months in the early morning following an overnight fast. The following variables were assessed at the same timepoints : HbA1c level, fasting blood glucose (FBG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), urinary albumin excretion, and high-sensitivity C-reactive protein (hs-CRP). Renal function was determined from the estimated glomerular filtration rate ($mL/min^{-1} \cdot 1.73\,m^{-2}$)[27]. The C-peptide immunoreactivity (CPR) index was measured as a parameter for endogenous insulin secretion[28~30] and calculated with the following formula[29] :

(Fasting serum CPR (ng/mL)/fasting plasma glucose (mg/dL)) × 100.

Blood pressure was recorded and body weight was measured using a body composition analyzer (InBody 720 ; Biospace, Tokyo, Japan).

Statistical Analysis

Normally distributed parameters are expressed as mean ± standard deviation (SD) or as the median and interquartile range. Differences between groups were analyzed by the student paired t test or unpaired t test. Differences in non-parametric data were analyzed using the Mann-Whitney U-test and Wilcoxon's matched pairs test. We performed linear regression analysis to determine the association of each variable with change in FMD. Univariate and multivariate logistic regression analysis were used to assess if each clinical marker correlated with an improvement in endothelial dysfunction (ΔFMD or change in FMD : after 6 months %FMD-before treatment %FMD). Values of $p<0.05$ were considered statistically significant. All analyses were performed using Prism 5 (GraphPad Software, Inc., San Diego, CA, USA) or Stat mate V (Nihon 3B Scientific Inc., Niigata, Japan).

Results

Table 1 shows baseline characteristics of study subjects ; there were no significant differences between groups, including the number of patients in each group with hypertension, being treated for hypertension, with dyslipidemia, and being treated for dyslipidemia. Moreover, there were no significant differences between groups in any clinical or biochemical parameter at baseline.

After 6 months, HbA1c levels significantly decreased in both groups (Table 2). HbA1c changes in the liraglutide group were significantly greater than in the sitagliptin group ($p<0.01$). FBG levels also significantly decreased in both groups. Changes in FBG levels in the liraglutide group were significantly greater than in the sitagliptin group ($p<0.01$). CPR index significantly increased in the liraglutide group ; there were no significant changes in the sitagliptin group.

Significant reductions in systolic blood pressure and diastolic blood pressure were observed in the liraglutide group, no significant changes in these variables were seen in the sitagliptin group. Similarly, LDL-C levels decreased significantly in the liraglutide group, whereas no significant changes were seen in the sitagliptin group.

Urinary albumin excretion was significantly reduced in the liraglutide group, but not in the sitagliptin group. Mean body weight decreased significantly in the liraglutide group. Although body weight decreased in the sitagliptin group, changes did not reach statistical significance. HDL-C, TG, and eGFR levels did not

Table 1 Baseline characteristics of subjects

	Liraglutide (n=24)	Sitagliptin (n=16)	P value
Males/females	15/9	9/7	0.70
Age, yrs	58.6±15.9	56.1±15.3	0.26
Duration of diabetes, yrs	2.4±2.8	1.9±2.3	0.49
Body mass index, kg/m^2	28.2±7.2	26.3±7.2	0.39
HbA1c, %at admission	9.8±2.2	9.1±1.6	0.22
Hypertension, n (%)	12 (50.0)	6 (37.5)	0.44
Medications for hypertension			
ACEIs or ARBs, n (%)	3 (12.5)	3 (18.8)	0.59
CCB, n (%)	2 (8.3)	2 (12.5)	0.67
Dyslipidemia	13 (54.1)	6 (37.5)	0.30
Statins, n (%)	1 (3.8)	0 (0.0)	0.41
Fibrates, n (%)	0 (0.0)	0 (0.0)	0.99
Current smoking, n (%)	8 (33.3)	4 (25.0)	0.57

Data are means ±SD.
DPP-4, dipeptidyl peptidase-4 ; HbA1c, hemoglobin A1c ; ACEI, angiotensin-converting enzyme inhibitor ; ARB, angiotensin Ⅱ receptor blocker ; CCB, calcium-channel blocker.

Table 2 Comparison of clinical and biochemical parameters at baseline and 6 months

	Liraglutide (n=24)		Sitagliptin (n=16)				
	Baseline	6 Months	Baseline	6 Months	P^1 value	P^2 value	P^3 value
HbA1c, % (NGSP)	9.8±2.2	7.4±1.7	9.1±1.6	8.0±1.4	<0.001*	<0.001*	0.22
Fasting glucose, mg/dL	216.3±64.5	137.4±36.8	197.8±48.5	162.3±32.6	<0.001*	<0.01*	0.27
CPR index	1.0±0.4	2.2±1.2	1.5±1.0	1.8±1.0	<0.01*	0.06	0.34
SBP, mmHg	138.0±20.5	129.5±16.2	136.7±19.3	135.1±17.3	<0.01*	0.36	0.89
DBP, mmHg	81.6±11.8	74.2±9.5	79.8±10.4	76.8±11.3	<0.01*	0.20	0.60
LDL-C, mg/dL	137.4±42.3	117.4±32.7	139.3±26.0	140.0±26.4	<0.001*	0.63	0.83
HDL-C mg/dL	47.1±13.2	47.1±10.9	49.1±11.7	47.2±10.6	0.96	0.15	0.55
TG, mg/dL	173.5±79.5	153.4±54.3	153.1±59.6	155.0±48.0	0.16	0.82	0.3
eGFR, mL・min^{-1}・1.73 m^{-2}	73.2±13.4	72.9±14.4	73.7±12.6	72.6±13.1	0.83	0.33	0.88
Body weight, kg	82.3±19.0	79.3±19.2	81.7±25.4	80.2±25.1	<0.01*	0.19	0.94
FMD, %	6.4±1.6	8.5±1.9	6.4±1.6	6.6±1.1	<0.01*	0.44	0.90
Urinary albumin excretion, μg/g・Cre	19.0 (9.0-92.75)	9.5 (7.3-60.5)	14.0 (7.1-49.0)	15.0 (9.0-48.0)	0.02*	0.87	0.34
hsCRP, mg/L	2.3 (0.8-3.3)	0.4 (0.2-1.0)	1.3 (1.0-2.8)	1.2 (0.1-2.0)	<0.01*	0.05	0.67

Data are mean±SD or median and interquartile range.
CPR, C-peptide immunoreactivity ; SBP, systolic blood pressure ; DBP, diastolic blood pressure ; LDL-C, low-density lipoprotein cholesterol ; HDL-C, high-density lipoprotein cholesterol ; TG, triglycerides ; eGER, estimated glomerular filtration rate ; FMD, flow-mediated dilatation ; hsCRP : high-sensitivity C-reactive protein.
*P^1 value : <0.05, comparison of respective data between baseline and after 6 months with liraglutide.
*P^2 value : <0.05, comparison of respective data between baseline and after 6 months with Sitagliptin.
P^1 value : <0.05, comparison of baseline data between liraglutide and Sitagliptin gloups.

change significantly in either group at 6 months.

%FMD significantly improved from 6.4%±1.6% to 8.5%±1.9% in the liraglutide group, while in the sitagliptin group, % FMD slightly increased from 6.4%±1.6% to 6.6%±1.1%. although changes were not significant. The change in % FMD in the liraglutide group was significantly better than in the sitagliptin group ($p<0.05$; Figure 1A).

The level of hsCRP significantly decreased from 2.3 [0.8-3.3] mg/L to 0.4 [0.2-1.0] mg/L ($p<0.01$) in the

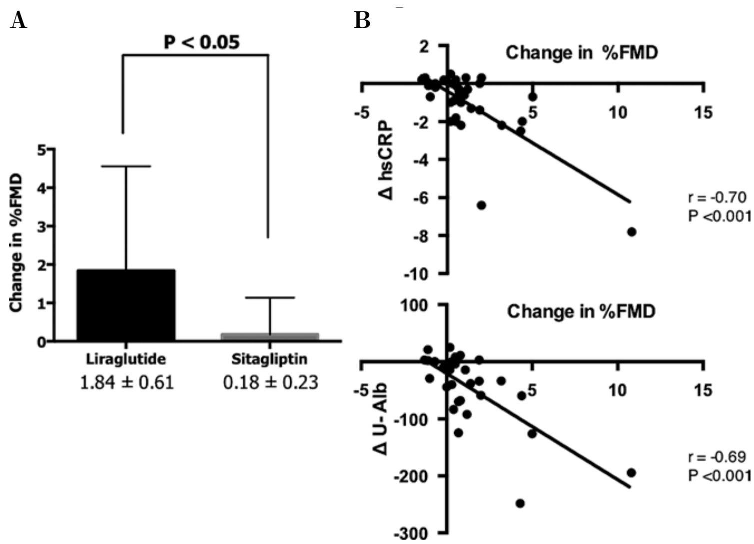

Figure 1 Change in flow-mediated dilatation (FMD) : Univariate analysis for improvement in endothelial function. **A**) Change in FMD after 6 months. **B**) Correlation between changes in FMD and changes in high-sensitive C-reactive protein (hs-CRP) and urinary albumin excretion (U-Alb excretion). Linear regression analysis demonstrated a significant correlation in liraglutide group between changes in % FMD and hs-CRP ($r = -0.70$, $p < 0.001$), urinary albumin excretion (ΔU-Alb excretion) ($r = 0.69$, $p < 0.001$).

liraglutide group, but did not change significantly in the sitagliptin group (1.3 [1.0-2.8] mg/L to 1.2 [0.1-2.0] mg/L).

According to linear regression analysis, changes in hs-CRP (Δhs-CRP) and urinary albumin excretion (ΔU-Alb) were significantly negatively correlated with changes in %FMD in the liraglutide group. (Figure 1B). Furthermore, multivariate logistic regression analysis identified the decrease of Δhs-CRP due to the treatment of liraglutide as a significant and independent determinant of improvement in endothelial function (Table 3).

DISCCUSION

The present study compared the efficacy of liraglutide with DPP-4 inhibitors, in particular, sitagliptin for untreated T2DM. Although both agents lead to improvements in glycemic control, changes were significantly better with liraglutide. In addition, liraglutide provided significantly better improvement in peripheral endothelial dysfunction compared with DPP-4 inhibitors. This is the first report that compares treatment with liraglutide with DPP-4 inhibitors for untreated T2DM.

Liraglutide is a GLP-1 analog derived from native GLP-1 (7-37) by introducing an arginine substitution at position 34 and attaching *N*-palmitoyl-glutamate to the e-amino group of the lysine residue at position 26[31]. Native GLP-1 has a short-lasting action because it is rapidly inactivated via enzymatic degradation by DPP-4. Liraglutide has several clinically relevant features such as a once-daily regimen due to its long terminal half-life of approximately 13 h ; its 97% homogeneity to human GLP-1, and a lack of elevating antibody titers when administered to humans[15,32,33]. In addition, GLP-1 supplementation is considered important because its secretion is generally suppressed in T2DM diabetes[34].

Both GLP-1 receptor agonists and DPP-4 inhibitors, which are incretin-related drugs, have a protective effect on pancreatic β-cell dysfunction[33,35]. Given the long-term perspective, pancreatic β-cell dysfunction in patients with diabetes might be prevented or delayed by relatively early intensive therapy with these drugs. Therefore, in Japan, DPP-4 inhibitors are often used as first-line treatment for early stage T2DM. In this

Table 3 Univariate and multivariate analysis for association with each variable with improvement endothelial function

Variable	Univariate		Multivariate	
	r	P value	β	P value
Age, yrs	−0.18	0.23	—	—
Sex	−0.12	0.43	—	—
ΔHbA1c, per%	−0.22	0.14	—	—
ΔFasting glucose per mg/dL	−0.432	<0.05	−0.01	0.88
ΔCPR index	0.22	0.13	—	—
ΔSBP, per mmHg	−0.44	<0.01	−0.03	0.23
ΔDBP, per mmHg	−0.25	0.13	—	—
TC				
ΔLDL-C, per mg/dL	−0.5	<0.001	−0.13	0.45
ΔHDL-C, per mg/dL	0.01	0.96	—	—
ΔTG, per mg/dlL	−0.07	0.63	—	—
ΔeGFR, per $mL \cdot min^{-1} \cdot 1.73\,m^{-2}$	−0.23	0.18	—	—
ΔBody weight, per kg	−0.08	0.59	—	—
ΔhsCRP, per mg/L	−0.7	<0.001	−5.56	0.01
ΔUrinary albumin excretion	−0.69	<0.001		
R^2			0.64	

HbA1c, hemoglobin A1c ; CPR, C-peptide immunoreactivity ; SBP, systolic blood pressure ; DBP, diastolic blood pressure ; DBP, diastolic blood pressure ; LDL-C, low-density lipoprotein cholesterol ; TC, total cholesterol ; HDL-C, high-density lipoprotein cholesterol ; TG, triglycerides ; eGER, estimated glomerular filtration rate ; hsCRP, high-sensitivity C-reactive protein.

study, HbA1c and FBG levels showed significantly greater improvement in the liraglutide treatment group compared with the DPP-4 inhibitors group. These data showed greater glycemic improvement than what was reported in the LEAD3 trial (approximately 1.1% reduction after 52 weeks of treatment with 1.8 mg liraglutide)[36] and a domestic phase III clinical trial (1.74% reduction after 24 weeks of treatment with 0.9 mg liraglutide)[37]. The favorable results in our study may be attributed to differences in patient populations ; our study included patients with untreated T2DM, and the proportion of obese patients was high in this study.

While the LEAD3 trial only reported a significant reduction in systolic blood pressure[36], our study demonstrated significant improvements in both systolic and diastolic blood pressure. Although these effects could be due to weight reduction, we also evaluated vascular endothelial function by FMD, which showed a greater improvement of % FMD than what was reported with DPP-4 inhibitor monotherapy[21]. Accumulating evidence supports the involvement of endothelial dysfunction in all stages of atherosclerosis[38,39]. DeFronzo et al. reported that a GLP-1 receptor agonist enhanced GLP-1 concentration after 2 weeks administration (15.1 pM vs. 63.8 pM)[40] compared DPP-4 inhibitors. The effect of GLP-1 receptor agonists and DPP-4 inhibitors on endothelial function could reflect increased phosphorylation of endothelial nitric oxidase synthase (eNOS)[20,40]. Nitric oxide, produced by eNOS, plays an important role in vascular homeostasis, coordination of endothelial cell function via GLP-1 receptors, and atheroprotective effects[41]. Although we could not measure active GLP-1 levels in this study, the effect of DPP-4 inhibitors in this area might be weaker than that exhibited by GLP-1 receptor agonists. Gaspari et al. reported that liraglutide improved endothelial function via GLP-1 receptors, increased eNOS levels, and reduced ICAM-1 expression in the aortic endothelium in mice[42]. On the other hand, Hopkins et al. reported that exedin-4 and liraglutide did not improve the FMD response in obese patients with T2DM[43]. These findings remain controversial because none of the studies that showed that GLP-1 receptor agonists improved endothelial function were randomized trials. However, subjects in the present study were all untreated and drug-naive patients with type 2 diabetes, which is similar to subjects included in GLP-1 receptor clinical trials, and this might have influenced our findings regarding the protective effects of liraglutide on endothelial function.

Specific effects of DPP-4 inhibitors on endothelial function could occur within other substrates of DPP-4. Inhibition of DPP-4 enzymatic activity could also modulate the activity of several proteins, such as stromal cell-derived factor-1 alpha, which stimulates bone marrow mobilization of endothelial cells and brain natriuretic peptide. DPP-4 inhibition may repair endothelial cells and improve cardiovascular function, thus resulting in an indirect improvement of endothelial function[44]. These results suggest that DPP-4 inhibitors improve endothelial function by increasing the number of endothelial cells through augmentation of stromal cell-derived factor-1 alpha. Although we could not demonstrate the effect of DPP-4 inhibitors on endothelial function in the present study, we believe that this is an interesting hypothesis for future study.

We also observed a significant improvement in the hs-CRP and urinary albumin excretion. Although GLP-1 exerts its effects on the vessels through nitric oxide-mediated action[45] as well as via direct action[46], what we observed is likely due to direct anti-inflammatory actions that can preserve endothelial dysfunction and inhibit diabetic complications. In terms of blood lipid profiles, we observed a significant reduction in LDL-C levels but not in TG or HDL-C levels after liraglutide treatment. However, GLP-1 receptor agonists have been reported to reduce TG levels[47], and further evaluation of the effect on TG in a larger population is required.

The body weight of patients in the DPP-4 inhibitors group did change significantly before and after treatment, but in the liraglutide group, body weight significantly decreased. The underlying mechanisms of liraglutide that mediate weight loss are not clear, but it is a general belief that it is likely a combination of effects on the gastrointestinal tract and central GLP-1 receptor expressing neurons[48]. It is generally accepted that GLP-1 receptor agonists increase satiety, reduce food intake, and promote weight loss, whereas DPP-4 inhibitors are weight neutral. Recently, it was shown that chronic stimulation of GLP-1 receptor leads to desensitization of gastric inhibitory effects, and the effects of liraglutide on gastric emptying were markedly reduced following 14 days of dosing[49] ; similar findings were seen in an acute study by Nauck et al[50]. However, we do not believe that gastric emptying is responsible for the long-term reductions in body weight seen with liraglutide. Jelsing et al. reported that chronic GLP-1 receptor exposure by liraglutide is needed to desensitize the gastric inhibitory effects of GLP-1 receptor agonists and that gastric inhibition did not contribute to weight loss. Body weight loss elicited by liraglutide is thought to be mediated by either brainstem or hypothalamic GLP-1 receptors[49]. Our results indicate that, at least, that body weight loss induced by liraglutide continued for 6 months.

This study has several limitations. First, this was a randomized, non-blind study. However the characteristics of patients were well matched between groups. Second, the number of subjects enrolled was relatively small. We expect that large-scale, randomized, prospective, clinical studies will provide more concrete evidence of whether incretin therapy provides clinical benefits of vascular protection for patients with T2DM. Third, we did not assess levels of GLP-1 and oxidative stress factors. Fourth, it should be noted that the maximum dosage of liraglutide used in Japan (0.9 mg/day) is much lower than that used in western countries. Further studies are needed to determine the correlation between changes in endothelial function and changes in active GLP-1, glucose-dependent insulinotropic polypeptide (GIP), and oxidative stress.

CONCLUSION

We compared the efficacy and benefit of liraglutide with DPP-4 inhibitors, sitagliptin, in patients with early-stage, untreated T2DM. Our results indicate that liraglutide could provide additional benefits to blood glucose lowering, subsequently protecting against vascular endothelial dysfunction.

Acknowledgements All authors thank the nursing staff of the Department of Endocrinology and Metabolism, Dokkyo Medical University, and all of the participants in this study. Honyaku Center Inc., a member of the Japan Translation Federation provided assistance with English language editing and preparation of the manuscript for submission.

Conflicts of Interest Kunihiro Suzuki, Seiich Tanaka, Chie Aoki, Kanako Kato, Teruo Jojima and Yoshimasa Aso declare that they have no conflicts of interest. No funding was received for this study or publication of this article.

REFERENCES

1) Shaw JE, Sicree RA, Zimmet PZ : Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract **87** : 4-14, 2010.

2) Whiting DR, Guariguata L, Weil C, Shaw J : IDF diabetes atlas : global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract **94** : 311-321, 2011.

3) Guariguata L : By the numbers : new estimates from the IDF Diabetes Atlas Update for 2012. Diabetes Res Clin Pract **98** : 524-525, 2012.

4) de Heer J, Rasmussen C, Coy DH, et al : Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. Diabetologia **51** : 2263-2270, 2008.

5) Balas B, Baig MR, Watson C, et al : The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single-dose administration in type 2 diabetic patients. J Clin Endocrinol Metab **92** : 1249-1255, 2007.

6) Drucker DJ, Nauck MA : The incretin system : glucagon-like peptide-1 receptor agonist and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet **368** : 1696-1705, 2006.

7) Kolterman OG, Buse JB, Fineman MS, et al : Synthetic exedin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subject with type 2 diabetes. J Clin Endocrinol Metab **88** : 3082-3089, 2003.

8) Davignon J, Ganz P : Role of endothelial dysfunction in atherosclerosis. Circulation **109** : 27-32, 2004.

9) Libby P : Inflammation in atherosclerosis. Nature **420** : 868-874, 2002.

10) Szabo C : Role of nitrosative stress in the pathgenesis of diabetic vascular dysfunction. Br J Pharmacol **156** : 713-727, 2009.

11) Inzucchi SE, Bergenstal RM, Buse JB, et al : Management of hyperglycemia in type 2 diabetes : A patient-centered approach : position statement of the American Diabetes Association (ADA) and the European Association for the study of Diabetes (EASD). Diabetes Care **35** : 1364-1379, 2012.

12) Treatment Guide for diabetes 2012-2013, Japan Diabetes Society, Bunkodo, P 17-18.

13) Yoshioka N, Ishii H, Tajima N, et al : Curr Med Res Opin **30** : 177-183, 2013.

14) Hertz RP, Unger AN, Lustik MB : Adherence with pharmacotherapy for type 2 diabetes : a retrospective cohort study of adults with employer-sponsored health insurance. Clin Ther **27** : 1064-1073, 2005.

15) Buse JB, Rosenstock J, Sesti G, et al : Liraglutide once a day versus exenatide twice a day for type 2 diabetes : a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet **374** : 39-47, 2009.

16) Drucker DJ, Sherman SI, Gprelick FS, et al : Incretin based therapies for the treatment of type 2 diabetes : evaluation of the risks and benefits. Diabetes Care **33** : 428-433, 2010.

17) Astrup A, Carraro R, Finer N, et al : Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. Int Obes (Lond) **36** : 843-854, 2012.

18) Liu H, Dear AE, Knudsen LB, et al : A long-acting glucagon-like peptide-1 analogue attenuates induction of plasminogen activator inhibitor type-1 and vascular adhesion molecules. J Endocrinol **201** : 59-66, 2009.

19) Arakawa M, Mita T, Azuma K, et al : Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. Diabetes **59** : 1030-1037, 2010.

20) Han L, Yu Y, Wang B : Exendin-4 directly improves endothelial dysfunction in isolated aortas from obese rats through the cAMP or AMPK-eNOS pathways. Diabetes Res Clin Pract **97** : 453-460, 2012.

21) van Poppel PC, Netea MG, Smits P, et al : Vildagliptin improves endothelium-dependent vasodilatation in type 2 diabetes. Diabetes Care **34** : 2072-2077, 2011.

22) Matsubara J, Sugiyama S, Akiyama E, et al : Dipepti-

dyl peptidase-4 inhibitor, sitagliptin, improves endothelial dysfunction in association with its anti-inflammatory effects in patients with coronary artery disease and uncontrolled diabetes. Circ J **77**：1337-1344, 2013.

23) Treatment Guide for diabetes 2012-2013, Japan Diabetes Society, Bunkodo, P 25-26.

24) Voidonikola PT, Stamatelopoulos KS, Alevizaki M, et al：The association between glycemia and endothelial function in nondiabetic individuals：the importance of body weight. Obesity **12**：2658-2662, 2008.

25) Nochioka K, Tanaka S, Miura M, et al：Ezetimibe improves endothelial function and Rho-kinase activity associated with inhibition of cholesterol absorption in humans. Circ J **76**：2023-2030, 2012.

26) Corretti MC, Anderson TJ, Benjamin EJ, et al：Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery：a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol **39**：257-265, 2002.

27) Matsuo S, Imai E, Horio M, et al：Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis **53**：982-992, 2009.

28) Meier JJ, Menge BA, Breuer TG, et al：Functional assessment of pancreatic beta-cell area in humans. Diabetes **58**：1595-1603, 2009.

29) Saisho Y, Kou K, Tanaka K, et al：Postprandial serum C-peptide to plasma glucose ratio as a predictor of subsequent insulin treatment in patients with type 2 diabetes. Endocr J **8**：315-322, 2011.

30) Okuno Y, Komada H, Sakaguchi K, et al：Postprandial serum C-peptide to plasma glucose concentration ratio correlates with oral glucose tolerance test- and glucose clamp-based disposition indexes. Metabolism **13**：1470-1476, 2013.

31) Knudsen LB：Glucagon-like peptide-1：the basis of a new class of treatment for type 2 diabetes. J Med Chem **47**：4128-4134, 2004.

32) Rollin B, Larsen MO, Gotfredsen CF, et al：The long-acting GLP-1 derivate NN2211 ameliorates glycemia and increases beta-cell mass in diabetic mice. Am J Physiol Endocrino Metab **283**：E745-752, 2002.

33) Bregenholt S, Møldrup A, Blume N, et al：The long-acting glucagon-like peptide-1 analogue, liraglutide, inhibits beta-cell apoptosis in vitro. Biochem Byophys Res Commun **330**：577-584, 2005.

34) Vilsbøll T, Krarup T, Deacon CF, et al：Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes **50**：609-613, 2001.

35) Xu L, Man CD, Charbonnel B, et al：Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on beta-cell function in patients with type 2 diabetes：a model-based approach. Diabetes Obes Metab **10**：1212-1220, 2008.

36) Gabor A, Henry R, Ratner R：Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono)：a randomised, 52-week, phase III, double-blind, parallel-treatment trial. Lancet **373**：473-481, 2009.

37) Seino Y, Rasmussen MF, Nishida T, et al：Efficacy and safety of the once-daily human GLP-1 analogue, liraglutide, vs glibenclamide monotherapy in Japanese patients with type 2 diabetes. Curr Med Res Opin **26**：1013-1022, 2010.

38) van Poppel PC, Netea MG, Smits P, et al：Vildagluptin improves endothelium-dependent vasodilatation in type 2 diabetes. Diabetes Care **34**：2072-2077, 2011.

39) Vita JA, Keaney JF Jr：Endothelial function：a barometer for cardiovascular risk. Circulation **6**：640-642, 2002.

40) DeFronzo RA, Okerson T, Viswanathan P, et al：Effect of exenatide versus sitagliptin on postprandial glucose, insulin and glucagon secretion, gastric emptying and caloric intake：a randomized, cross-over study. Curr Med Res Opin **24**：2943-2952, 2008.

41) Shah Z, Pineda C, Kampfrath T, et al：Acute DPP-4 inhibition modulates vascular tone through GLP-1 independent pathways. Vascul Pharmacol **55**：2-9, 2011.

42) Gaspari T, Liu H, Welungoda I, et al：A GLP-1 receptor agonist liraglutide inhibits endothelial cell dysfunction and vascular adhesion molecule expression in an ApoE-/- mouse model. Diab Vasc Dis Res **8**：117-124, 2011.

43) Hopkins ND, Cuthbertson DJ, Kemp GJ, et al：Effects of 6 months glucagon-like peptide-1 receptor agonist treatment on endothelial function in type 2 diabetes mellitus patients. Diabetes Obes Metab **15**：770-773, 2013.

44) Fadini GP, Agostini C, Sartore S, et al : Endothelial progenitor cells in the natural history of atherosclerosis. Atherosclerosis **194** : 46-54, 2007.

45) Golpon HA, Puechner A, Welte T, et al : Vasorelaxant effect of glucagon-like peptide-(7-36) amide and amylin on the pulmonary circulation of the rat. Regul Pept **102** : 81-86, 2003.

46) Ozyazgan S, Kutluata N, Afsar S, et al : Effect of glucagon-like peptide-1(7-36) and exendin-4 on the vascular reactivity in streptozotocin/nicotinamide diabetic rats. Pharmacology **74** : 119-126, 2005.

47) Vilsbøll T, Zdravkovic M, Le-Thi T, et al : Liraglutide, a long-acting human glucagon-like peptide-1 analog, given as monotherapy significantly improves glycemic control and lower body weight without risk of hypoglycemia in patients with type 2 diabetes. Diabetes Care **30** : 1608-1610, 2007.

48) Jelsing J, Vrang N, Hansen G, et al : Liraglutide : short-lived effect on gastric emptying — long lasting effects on body weight. Diabetes Obes Metab **14** : 531-538, 2012.

49) Nauck MA, Kemmeries G, Holst JJ, et al : Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes **60** : 1561-1565, 2011.