JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
CLINICAL TRIAL

Linagliptin improves endothelial function in patients with type 2 diabetes: A randomized study of linagliptin effectiveness on endothelial function

Fumika Shigiyama[1], Naoki Kumashiro[1]*, Masahiko Miyagi[1], Ryo Iga[1], Yuka Kobayashi[1], Eiichiro Kanda[2,3], Hiroshi Uchino[1], Takahisa Hirose[1]

[1]Division of Diabetes, Metabolism and Endocrinology, Department of Medicine, Toho University School of Medicine, [2]Department of Nephrology, Tokyo Kyosai Hospital, and [3]Life Science and Bioethics Research Center, Tokyo Medical and Dental University, Tokyo, Japan

Keywords
Endothelial function, Linagliptin, Type 2 diabetes

***Correspondence**
Naoki Kumashiro
Tel.: +81-3-3762-4151
Fax: +81-3-3765-6488
E-mail address:
naoki.kumashiro@med.toho-u.ac.jp

J Diabetes Investig 2017; 8: 330–340

doi: 10.1111/jdi.12587

Clinical Trial Registry
University Hospital Medical Information Network Clinical Trial Registry
UMIN000012783

ABSTRACT

Aims/Introduction: The present multicenter, prospective, controlled, open and randomized three-arm parallel study was designed to compare the effects of linagliptin with those of metformin on endothelial function.

Materials and Methods: Type 2 diabetes patients treated with 750 mg of metformin (hemoglobin A1c ≥6.0% and <8.0%, $n = 96$) were randomized to continue metformin 750 mg/day (control group, $n = 29$), metformin at 1,500 mg/day (metformin group, $n = 26$) and metformin 750 mg/day supplemented with linagliptin 5 mg/day (linagliptin add-on group, $n = 29$) and treated for 16 weeks. Vascular endothelial function was evaluated by flow-mediated dilation. The primary end-point was changes in flow-mediated dilation at 16 weeks relative to baseline.

Results: Linagliptin significantly improved flow-mediated dilation from baseline (4.9 ± 2.7%) to 16 weeks (6.3 ± 2.7%, $P < 0.05$), whereas the other groups did not show any changes. Hemoglobin A1c at 16 weeks was significantly lower in the metformin and linagliptin add-on groups compared with the control (6.6 ± 0.6%, 6.5 ± 0.5% and 7.0 ± 0.6%, respectively). Single and multiple regression analyses showed that apolipoprotein B correlated significantly with change in flow-mediated dilation, and apolipoprotein B was decreased only in the linagliptin add-on group (–6.0 ± 11.3 mg/dL, $P < 0.01$).

Conclusions: Linagliptin for 16 weeks improved endothelial function with a modest improvement in glycemic control. This effect was mediated, at least in part, by reduction in apolipoprotein B. Linagliptin has a protective role on endothelial function in patients with type 2 diabetes with moderate hyperglycemia.

INTRODUCTION

Type 2 diabetes mellitus is one of the major factors for progression of atherosclerosis and development of cardiovascular diseases[1,2]. In addition to lowering blood glucose level, prevention of cardiovascular diseases is crucial for patients with type 2 diabetes mellitus. It is important to screen cardiovascular events at an early stage. Recently, it has been reported that vascular endothelial dysfunction is the initial stage of atherosclerosis, and thought to be the earliest predictor for future cardiovascular events in patients with type 2 diabetes mellitus[3]. Various non-invasive methods are currently available for early detection of atherosclerosis. Among them, the flow-mediated dilation (FMD) is an established method for evaluating vascular endothelial function[4], and the cardio-ankle vascular index (CAVI), which measures vascular stiffness, is also used as an early index of atherosclerosis[5–7].

To date, various antidiabetic oral agents with different mechanisms are available. The American Diabetes Association and the European Association for the Study of Diabetes recommend the use of metformin, which prevents cardiovascular events, as

Received 10 August 2016; revised 2 October 2016; accepted 13 October 2016

the first-line drug[8–10]. Dipeptidyl peptidase-4 (DPP-4) inhibitors, which are other antidiabetic oral agents, lower blood glucose by preventing the inactivation of incretin hormones by DPP-4. They are widely available for the treatment of type 2 diabetes mellitus, and some randomized clinical trials have shown non-inferiority of DPP-4 inhibitors on cardiovascular events in patients with type 2 diabetes mellitus compared with placebo control[11–13], however; some participants of these studies already had a history of cardiovascular diseases. To elucidate the true effect of DPP-4 inhibitors on the prevention of atherosclerosis, it seems important to start treatment at an earlier stage. The effect of linagliptin, a DPP-4 inhibitor, on cardiovascular outcomes, is currently being investigated in two studies. A meta-analysis study reported that linagliptin does not increase cardiovascular risk[14]. Other studies reported that linagliptin attenuates vascular dysfunction and has direct vasodilatory effects independent of its glucose-lowering properties[15]. Furthermore, linagliptin was reported to have an anti-inflammatory action[15,16], and is involved in the suppression of atherosclerosis progression[15]. These data suggest that DPP-4 inhibitors improve glycemic control, and seem to have some cardiovascular protective effect[14–16].

We hypothesized that linagliptin add-on therapy with metformin can prevent the progression of atherosclerosis and the development of cardiovascular diseases in type 2 diabetes mellitus patients. To test the hypothesis, the present study was carried out to determine the effects of 16-week treatment with linagliptin on endothelial function using FMD and CAVI in type 2 diabetes mellitus patients with moderate hyperglycemia. The present study also compared the effects of add-on linagliptin with those of metformin.

MATERIALS AND METHODS

Study design

The present study, the Randomized Study of Linagliptin Effectiveness on Endothelial Function Assessed by FMD (RELIEF), was a three-arm trial multicenter prospective, randomized open-label, blind end-point study, registered with the University Hospital Medical Information Network Clinical Trial Registry (UMIN000012783), a non-profit organization in Japan that meets the requirements of the International Committee of Medical Journal Editors. The study protocol was approved by the ethics review board of each participating institution. The present study was carried out according to the Declaration of Helsinki and current legal regulations in Japan. The processes of enrollment, randomization, data collection and management were carried out by the third-party entities for data without bias.

Study population

We recruited 96 Japanese patients with type 2 diabetes mellitus who periodically visited the outpatient clinics of 11 institutions in Japan listed in the Supporting Information. The inclusion criteria were as follows: (i) type 2 diabetes mellitus patients treated with metformin 750 mg/day or metformin 750 mg/day and another oral hypoglycemic agent (allowed dose for glimepiride was ≤2 mg/day, glimicron ≤40 mg/day) for at least 12 weeks, in addition to diet and physical therapy; (ii) hemoglobin A1c (HbA1c; National Glycohemoglobin Standardization Program) ≥6.0%, but <8.0%; (iii) age ≥20 and <80 years; (iv) patients who were willing to follow the instructions for drug intake; and (v) patients who gave written informed consent. The exclusion criteria were as follows: (i) patients with type 1 diabetes mellitus or a diabetic condition induced by other disease; (ii) patients treated with thiazolidinediones, DPP-4 inhibitors, sodium-glucose cotransporter 2 inhibitors, insulin or incretin-based drugs within 12 weeks before signing the informed consent; (iii) patients treated with more than 750 mg/day of metformin; (iv) patients who started to use/change their dose of angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, statins or antiplatelet drugs within 12 weeks before signing the informed consent; (v) patients with severe infectious disease, perioperative or severe trauma; (vi) patients with a history of myocardial infarction, angina pectoris, cerebral stroke or cerebral infarction within 12 weeks before signing informed consent; (vii) patients with atrial fibrillation, frequent atrial or ventricular extrasystoles; (viii) patients with moderate or severe heart failure (New York Heart Association stage III or more severe); (ix) Ankle Brachial Pressure Index <0.9; (x) patients with severe liver dysfunction or compromised renal function; (xi) patients with systolic blood pressure >150 mmHg, labile hypertension or labile hyperlipidemia; (xii) patients with alcohol or drug dependence; (xiii) female patients who were pregnant, lactating or possibly pregnant and those planning to become pregnant; (xiv) patients with a history of hypersensitivity to investigational drugs; and (xv) patients whom their physician considered to be ineligible for this study.

Randomization and study intervention

Participants were enrolled and randomly assigned in equal numbers into three groups as follows: control group, metformin group and linagliptin add-on group. The randomization was carried out by a computer-based dynamic allocation system with minimization procedure to balance for sex and with/without administration of statins. After the enrollment, all patients were observed for 4–6 weeks, representing the screening period, and in principle they were not permitted to change the dose of concomitant drugs or add any other drugs, such as other antidiabetic agents, antihypertension drugs, and lipid-lowering and antiplatelet agents. Four weeks or more after the enrollment, blood and urine samples were collected, and FMD and CAVI were measured (baseline data). After baseline data collection, the assigned therapies were started as follows: patients of the control group continued their therapy with metformin 750 mg/day throughout the study period. The dose of metformin in patients of the metformin group was doubled to 1,500 mg/day. Patients of the linagliptin add-on group were

treated with metformin 750 mg/day plus linagliptin 5 mg/day. The assigned treatment was continued for 16 weeks (duration of the study). The starting day represented the day of blood/urine collection for the control group; the day on which the dose of metformin was increased to 1,500 mg/day for the metformin group; and the day of addition of linagliptin for the linagliptin add-on group.

Observation items and schedule

Clinical assessment and data collection were carried out at baseline and 16 weeks after the start date. All FMD and CAVI were carried out at Toho University Omori Medical Center, Tokyo, Japan. The collected blood and urine samples were sent to a central laboratory (SRL Laboratory, Tokyo, Japan) for the measurement of HbA1c, fasting plasma glucose, C-peptide, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, apolipoprotein A-I/A-II/B/C-II/C-III/E, high-sensitivity C-reactive protein (hs-CRP), adiponectin, urinary 8-hydroxy-2'-deoxyguanosin (8-OHdG) and urinary albumin.

Study outcomes

The primary end-point was a change in FMD (ΔFMD [value at week 16 – value at baseline]). The secondary end-points were changes in CAVI, HbA1c, fasting plasma glucose level, C-peptide, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, apolipoprotein A-I/A-II/B/C-II/C-III/E, hs-CRP, adiponectin, 8-OHdG and urinary albumin at week 16 relative to the values at baseline.

FMD

The FMD was measured at baseline and 16 weeks by experienced technicians, using the technique described by the International Brachial Artery Reactivity Task Force[17] and the report of Takeno *et al.*[17,18] The measurement was carried out using the UNEX EF38G (UNEX Corporation, Nagoya, Japan) after at least 10 h fasting and under no-smoking condition. After resting more than 15 min at room temperature (25°C), the cuff was applied on the forearm and inflated to acquire images of the brachial artery in the relaxed state using high-resolution ultrasound. After capturing baseline images, the cuff was inflated to 50 mmHg above the systolic blood pressure for 5 min to occlude the brachial artery. Then, the cuff was deflated and the image of the brachial artery was captured. The diameter of the brachial artery was measured on the pre- and post-hyperemia images to determine changes in %FMD (FMD [%] = [maximum diameter – diameter at rest] × 100 / diameter at rest). The average FMD coefficient of variation was sufficiently small; indicating reproducibility of the FMD measurements.

CAVI

CAVI and the ankle brachial index were measured by experienced technicians at baseline and 16 weeks using VaSera VS-1500 (Fukuda-Denshi Company, Tokyo, Japan), and the method of measurement as described in detail previously[5,19,20]. Briefly, the participant fasted for at least 10 h. While lying in the supine position, the cuffs were applied to both the upper arm and ankle, and the electrocardiogram and heart sound were monitored. After the 10-min equilibration period, the pulse wave velocity (PWV) between the heart and ankle was measured by L/T, where L is the distance from the aortic valve to the ankle, and T is the time taken for the pulse wave to propagate from the aortic valve to the ankle. The brachial and ankle pulse waves were detected with cuffs that are also used for measurement of blood pressure. The PWV was converted to CAVI digitally using the following formula: $CAVI = a([2\rho/\Delta P] \times In[Ps/Pd] \times ba\text{-}PWV^2) + b$, where ρ is blood density, Ps and Pd are systolic blood pressure and diastolic blood pressure, respectively, ba-PWV is the brachial-ankle pulse wave velocity measured between the aortic valve and the ankle, and a and b are constants. The average CAVI coefficient of variation was less than 5%, indicating the reproducibility of CAVI.

Biochemical tests

The following procedures/methods were applied: latex agglutination for HbA1c: chemiluminescence enzyme immunoassay for insulin, C-peptide and adiponectin; hexokinase ultraviolet method for blood glucose level; ultraviolet method with cholesterol dehydrogenase for total cholesterol; enzymatic method (glycerol phosphate oxidase peroxidase method without free glycerol) for triglyceride; direct method for HDL and LDL cholesterol; immunoturbidimetric method for apolipoproteins; nephelometry for hs-CRP; and high-performance liquid chromatography for 8-OHdG.

Safety and evaluation of adverse events

All adverse events observed during the study were recorded. When these were considered serious, they were reported immediately to the respective institution, the principal investigator and the administration office.

Sample size

We assumed difference in the primary end-point of ΔFMD at 2.0 ± 2.3%, based on previous studies[21,22]. Based on a two-sided *P*-value of 5% and a power of 80%, the number of cases required to detect a significant difference was estimated to be 22 cases per group. Assuming a dropout rate of 30%, the target number of enrolled patients was set to 32 cases per group for a total of 96 cases.

Statistical analysis

Analyses were carried out on the full analysis set (FAS) under an intention-to-treat approach, except for safety analysis with adverse events, which was carried out on the treated set. The FAS included all patients who showed changes in FMD at week 16, whereas the treated set included all patients who had some data on safety evaluation at week 16. All statistical

analyses were carried out under the condition of two-sided *P*-value of <5%. Comparisons of continuous variables among the groups were carried out using ANOVA or Kruskal–Wallis test depending on the results of Shapiro–Wilk test and Bartlett test. We applied the Tukey–Kramer method multiple comparisons when a significant difference was detected in ANOVA or Kruskal–Wallis test among the three groups. The paired *t*-test or Wilcoxon signed-rank test was used for comparisons between the continuous variables at baseline and at week 16 in each group, depending on the result of the Shapiro–Wilk test. Comparisons of categorical data were conducted by the χ^2-test or Fisher's exact test. Furthermore, we carried out several subgroup analyses for ΔFMD and ΔCAVI, including subgroups of sex (male/female), age at baseline (<65/≥65 years), body mass index (BMI) at baseline (<25/≥25 kg/m^2), HbA1c at baseline (<7/≥7%) and change in HbA1c (<median/≥median). We also carried out simple and multiple linear regression analyses with ΔFMD as the dependent variable after single linear regression analysis. Simple regression analysis was carried out with each of all items (clinical parameters at baseline and changes at end of study, and background variables) as the independent variable. Multiple linear regression analysis was carried out with the following independent variables: age, sex and all variables with a *P*-value of regression coefficient in the simple regression analysis of <0.1. In order to avoid multicollinearity, we calculated the variance inflation factor and chose one variable from the correlated variables. All statistical analyses were carried out on SAS 9.3 (SAS Institute Inc., Cary, NC, USA) under instruction of an independent biostatistician.

RESULTS

Differences between the participants of the three groups

A total of 96 patients were enrolled in the study between January 2014 and March 2015, and randomized to the three groups (Figure 1). At the end of the study, the FAS included 29, 26 and 29 patients in the control group, metformin group and linagliptin add-on group, respectively. The baseline clinical characteristics of the patients are shown in Table 1. As significant differences were detected in diastolic blood pressure and the use of α-glucosidase inhibitor ($P < 0.05$, each) among the three groups, we applied multiple comparisons for these two variables. The results showed that only diastolic blood pressure was significantly different between the control and linagliptin groups. There were no differences in all the clinical characteristics, except for diastolic blood pressure, among the three groups.

Significant improvement in FMD in the linagliptin add-on group

Table 2 and Figure S1 show the FMD values at baseline, week 16 and the ΔFMD values. ΔFMD value was significantly better in the linagliptin add-on group ($P < 0.05$), but not in the other two groups. FMD were not different among the three groups at baseline, week 16 and ΔFMD. Subgroup analyses for ΔFMD showed that FMD improved significantly only in the linagliptin add-on group, with HbA1c <7.0% at baseline ($P < 0.05$; Table 2).

CAVI and other clinical parameters

Table S1 shows the results of CAVI. No significant differences were observed at baseline, week 16, and ΔCAVI in the same group and among the groups. CAVI improved significantly in only the linagliptin add-on group in patients aged <65 years at baseline (Table S1). In addition, patients of the linagliptin add-on group with BMI ≥25 kg/m^2 at baseline showed significant improvement in CAVI compared with the metformin group.

Improvement of HbA1c in the metformin and linagliptin add-on groups, whereas insulin resistance improves only in the metformin group

HbA1c improved significantly in both the metformin group and the linagliptin add-on group ($P < 0.01$ and $P < 0.001$, respectively; Table 3). Notably, the mean baseline HbA1c was <7% in the participants of the present study. Significant differences were also detected in HbA1c at week 16 and the ΔHbA1c among the three groups. Fasting plasma glucose was significantly reduced only in the linagliptin add-on group ($P < 0.001$), but not the other two groups (Table 3). C-peptide and HOMA-IR were significantly reduced only in the metformin group ($P < 0.05$, each; Table 3).

Significant reduction in apolipoprotein B in the linagliptin add-on group

Tables S2 and S3 show changes in clinical parameters related to atherosclerosis and cardiovascular events, such as lipid metabolism, inflammation, oxidative stress and urinary albumin. Total cholesterol at week 16 and the delta change were significantly different among the three groups ($P < 0.01$, each; Table S2). The Δtotal-cholesterol was significantly larger in the control group and lower in the linagliptin add-on group. HDL cholesterol was significantly reduced in the metformin group, and the Δchange in this parameter was significantly different among the three groups. The Δchange in LDL cholesterol was significantly different among the three groups. Apolipoprotein A-I was significantly reduced in both the metformin and linagliptin add-on groups ($P < 0.05$, each), and the Δchange in this variable was also significantly different among the three groups. Apolipoprotein A-II increased in the control group, and Δapolipoprotein A-II was significantly different among the three groups. The apolipoprotein B level at week 16 and Δapolipoprotein B were significantly different among the three groups ($P < 0.05$, each), and apolipoprotein B was significantly reduced only in the linagliptin add-on group ($P < 0.01$). Apolipoprotein C-III at week 16 was significantly different among the three groups, and was significantly reduced only in the metformin group. Apolipoprotein E was significantly reduced only in the linagliptin add-on group. No significant changes were observed in hs-CRP, adiponectin, 8-OHdG and

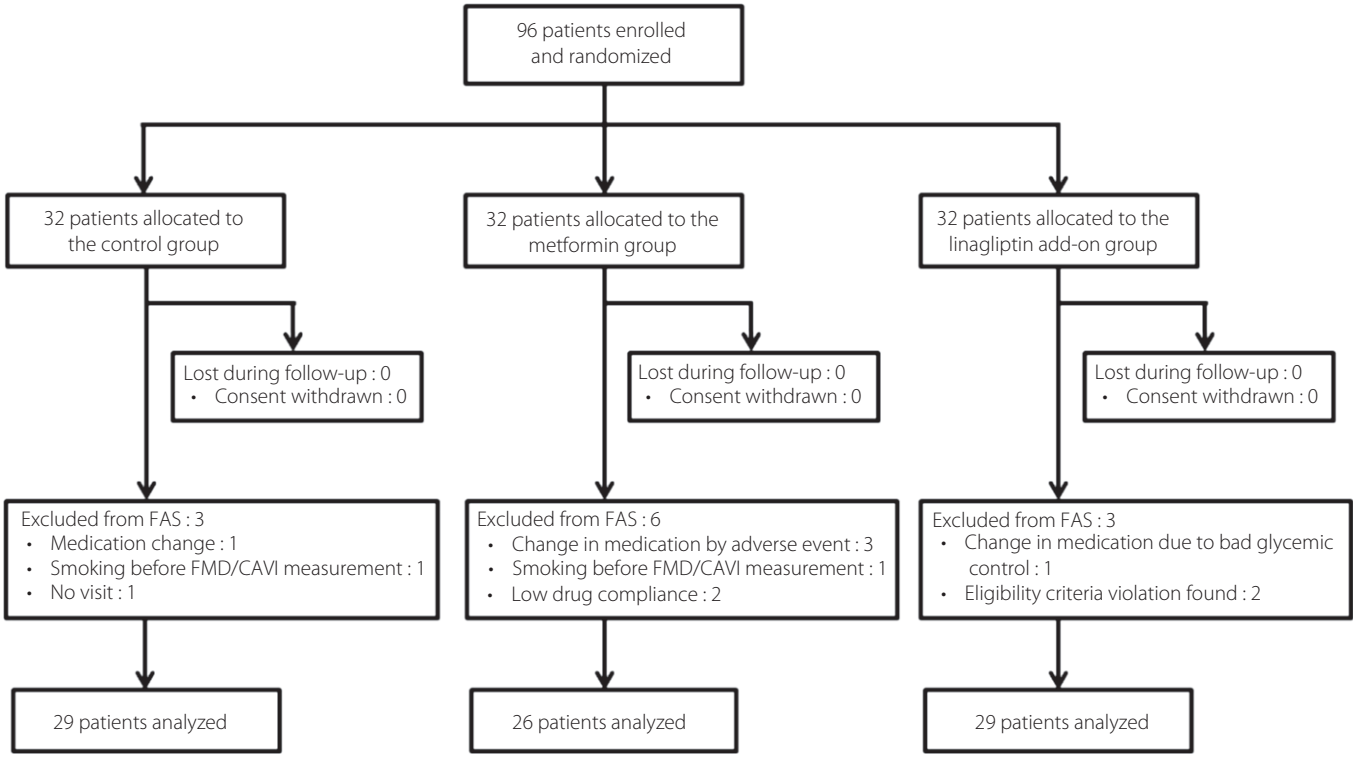

Figure 1 | Flow diagram of patient enrollment in the present study. A total of 96 patients were enrolled in the study and divided at random into three groups (32 patients in each group). A total of 12 patients were excluded from full analysis set (FAS) analysis due to changes in medications ($n = 5$); no visit to the hospital ($n = 1$); smoking before flow-mediated dilation (FMD)/cardio-ankle vascular index (CAVI) measurement ($n = 2$); eligibility violation ($n = 2$); and low-drug compliance ($n = 2$). Accordingly, FAS included 29, 26 and 29 patients for the control group, metformin group and linagliptin add-on group, respectively.

urinary albumin at the end of the study in any group, and the Δchanges were not significantly different among the groups (Table S3).

Association of ΔFMD with ΔApolipoprotein B, baseline FMD and sex

Simple regression analyses were carried out between ΔFMD as the dependent variable and each of the evaluated factors as independent variables. Significant regression coefficient was detected for seven independent variables (Table S4). Multiple linear regression analysis that included the aforementioned variables identified Δapolipoprotein B ($\beta = -0.0507$, $P < 0.05$), FMD at baseline ($\beta = -0.7890$, $P < 0.001$) and sex ($\beta = 1.2295$, $P < 0.05$) as significant associations with ΔFMD (Table 4).

Adverse events

Table S5 lists the reported/observed adverse events during the study. The observed adverse events per groups were one for the control group, four for the metformin group and one for the linagliptin add-on group. There was no significant difference in the incidence of adverse events among the groups. Only a single serious adverse event was noted in the present study; pancreatic cancer in a patient of the metformin group.

DISCUSSION

Treatment of type 2 diabetes mellitus encompasses two important goals; adequate glycemic control, and prevention of complications including atherosclerosis and cardiovascular events. The main result of the present study was the beneficial effects of linagliptin add-on therapy compared with those of metformin in Japanese type 2 diabetes mellitus patients with moderate hyperglycemia. Although there was no significant difference among the three groups, only linagliptin add-on therapy for 16 weeks significantly improved endothelial function, as reflected by FMD.

A subpopulation analysis based on baseline HbA1c (using a cut-off value of 7%) found conspicuous improvement in FMD in patients of the linagliptin add-on group with low baseline HbA1c level (Table 2). This finding shows that linagliptin could potentially improve endothelial function in the earlier stage of type 2 diabetes mellitus. Another subanalysis based on ΔCAVI also showed significant reduction in CAVI in patients of the linagliptin add-on group aged less than 65 years, compared

Table 1 | Baseline demographic and clinical characteristics of patients of the three groups

	Control group (n = 29)	Metformin group (n = 26)	Linagliptin add-on group (n = 29)	P-value
Age (years)	62.1 ± 11.4	60.3 ± 12.3	60.4 ± 9.0	0.789
Male	17 (58.6)	14 (53.8)	18 (62.1)	0.821
Height (cm)	161.5 ± 8.4	161.4 ± 8.0	163.4 ± 9.5	0.628
Weight (kg)	64.3 ± 15.2	68.2 ± 12.0	67.2 ± 13.2	0.538
BMI (kg/m^2)	24.8 ± 5.4	26.2 ± 4.0	25.3 ± 4.4	0.482
SBP (mmHg)	127.0 ± 11.8	129.1 ± 13.1	130.1 ± 12.3	0.620
DBP (mmHg)	72.9 ± 9.5‡	75.2 ± 8.5	79.6 ± 7.9†	0.015
HbA1c (NGSP%)	6.9 ± 0.6	6.8 ± 0.7	6.9 ± 0.6	0.859
Drinking	9 (31.0)	10 (38.5)	7 (24.1)	0.452
Current smoking	7 (24.1)	6 (23.1)	4 (13.8)	0.748
Medical history	7 (24.1)	9 (36)	7 (24.1)	0.562
Angina	2 (6.9)	0 (0)	0 (0.0)	0.327
Myocardial infarction	1 (3.4)	1 (4.0)	0 (0.0)	0.753
Cerebral infarction	0 (0.0)	0 (0.0)	0 (0.0)	–
Cerebral hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	–
Others	5 (17.2)	8 (32.0)	7 (24.1)	0.453
Complications				
Macroangiopathy	0 (0.0)	0 (0.0)	0 (0.0)	–
Ischemic heart disease	0 (0.0)	0 (0.0)	0 (0.0)	–
Cerebrovascular disease	0 (0.0)	0 (0.0)	0 (0.0)	–
Arteriosclerosis obliterans	0 (0.0)	0 (0.0)	0 (0.0)	–
Microangiopathy	8 (29.6)	10 (40)	9 (32.1)	0.731
Diabetic retinopathy	3 (11.1)	3 (12.0)	3 (10.7)	1.000
Diabetic nephropathy	3 (11.1)	1 (4.0)	3 (10.7)	0.691
Diabetic neuropathy	5 (18.5)	6 (24.0)	4 (14.3)	0.676
Others	27 (93.1)	21 (84.0)	25 (86.2)	0.590
Renal disease	1 (3.4)	0 (0.0)	0 (0.0)	1.000
Liver disease	3 (10.3)	3 (12.0)	3 (10.3)	1.000
Cerebrovascular disease	0 (0.0)	0 (0.0)	0 (0.0)	–
Heart disease	2 (6.9)	1 (4.0)	0 (0.0)	0.515
Hypertension	15 (51.7)	9 (36.0)	14 (48.3)	0.532
Dyslipidemia	22 (75.9)	13 (52.0)	16 (55.2)	0.146
Others	13 (44.8)	14 (56.0)	10 (34.5)	0.292
Antidiabetic drugs	29 (100)	26 (100)	29 (100)	–
Biguanides	29 (100)	26 (100)	29 (100)	–
α-Glucosidase inhibitors	5 (17.2)	0 (0.0)	1 (3.4)	0.044
Sulfonylureas	6 (20.7)	6 (23.1)	5 (17.2)	0.942
Glinides	1 (3.4)	0 (0.0)	2 (6.9)	0.771
Antihypertensive drugs	13 (44.8)	9 (34.6)	13 (44.8)	0.755
Diuretic drugs	1 (3.4)	1 (3.8)	2 (6.9)	1.000
Calcium channel blockers	6 (20.7)	7 (26.9)	8 (27.6)	0.857
ACE inhibitors	1 (3.4)	3 (11.5)	1 (3.4)	0.435
Angiotensin II receptor blockers	11 (37.9)	6 (23.1)	11 (37.9)	0.420
Direct renin inhibitor	0 (0)	0 (0)	0 (0)	–
β-Blockers	1 (3.4)	1 (3.8)	2 (6.9)	1.000
α-Blockers	0 (0.0)	0 (0.0)	1 (3.4)	1.000
Others	1 (3.4)	1 (3.8)	0 (0)	0.759
Lipid-lowering agents	16 (55.2)	11 (42.3)	14 (48.3)	0.680
Statins	10 (34.5)	9 (34.6)	9 (31.0)	1.000
Fibrates	1 (3.4)	2 (7.7)	4 (13.8)	0.384
Resins	1 (3.4)	0 (0.0)	0 (0.0)	1.000
Ezetimibe	1 (3.4)	1 (3.8)	0 (0.0)	0.759
EPA	4 (13.8)	1 (3.8)	2 (6.9)	0.494
Others	2 (6.9)	0 (0.0)	0 (0.0)	0.326

Table 1 (*Continued*)

	Control group (n = 29)	Metformin group (n = 26)	Linagliptin add-on group (n = 29)	P-value
Antithrombotic agents	5 (17.2)	1 (3.8)	2 (6.9)	0.265
Antiplatelet agents	5 (17.2)	1 (3.8)	2 (6.9)	0.265
Anticoagulants	0 (0.0)	0 (0.0)	0 (0.0)	–
Thrombolytic agents	0 (0.0)	0 (0.0)	0 (0.0)	–
Others	0 (0.0)	0 (0.0)	0 (0.0)	–
Other drugs	12 (41.4)	16 (61.5)	11 (37.9)	0.178

Data are expressed as mean ± standard deviation or number of patients (%). P-values are results of ANOVA or Kruskal–Wallis test for continuous data, and Fisher's exact test for categorical data; when significant difference was detected, multiple comparisons were applied, the results of which are marked by superscripts as follows: $^{\dagger}P < 0.05$ versus the control group; $^{\ddagger}P < 0.05$ versus the linagliptin add-on group. ACE inhibitor, angiotensin-converting enzyme inhibitor; BMI, body mass index; DBP, diastolic blood pressure; EPA, eicosapentaenoic acid; HbA1c, hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program; SBP, systolic blood pressure.

Table 2 | Changes in flow-mediated dilation (%)

	Control group	Metformin group	Linagliptin add-on group	P-value*
FAS population				
Baseline	5.66 ± 2.46 (29)	5.33 ± 2.41 (26)	4.93 ± 2.71 (29)	0.549
Week 16	5.60 ± 2.01 (29)	6.60 ± 2.93 (26)	6.26 ± 2.71 (29)	0.344
Change	–0.06 ± 2.75 (29)	1.27 ± 3.33 (26)	1.33 ± 3.45 (29)	0.168
P-value within group	0.909	0.130	0.047	
Subgroup analysis for FMD change				
Males	0.45 ± 2.49 (17)	0.93 ± 3.18 (14)	1.58 ± 3.79 (18)	0.583
P-value within group	0.464	0.294	0.095	
Females	–0.78 ± 3.04 (12)	1.67 ± 3.6 (12)	0.91 ± 2.92 (11)	0.094
P-value within group	0.392	0.233	0.326	
Age at baseline <65 years	–0.85 ± 2.68 (13)	1.51 ± 3.47 (16)	1.04 ± 3.26 (17)	0.173
P-value within group	0.277	0.159	0.207	
Age at baseline ≥65 years	0.58 ± 2.72 (16)	0.88 ± 3.22 (10)	1.73 ± 3.81 (12)	0.640
P-value within group	0.406	0.410	0.143	
BMI at baseline <25 kg/m^2	0.15 ± 3.36 (15)	1.06 ± 2.5 (14)	1.51 ± 3.59 (13)	0.323
P-value within group	0.862	0.334	0.156	
BMI at baseline ≥25 kg/m^2	–0.08 ± 1.94 (13)	1.51 ± 4.2 (12)	0.89 ± 3.59 (14)	0.627
P-value within group	0.878	0.240	0.369	
HbA1c at baseline <7%	–0.22 ± 3.17 (17)	2.03 ± 3.90 (16)	1.89 ± 2.54 (15)	0.098
P-value within group	0.781	0.056	0.012	
HbA1c at baseline ≥7%	–0.15 ± 1.92 (11)	0.06 ± 1.65 (10)	0.72 ± 4.23 (14)	0.946
P-value within group	0.807	0.911	0.534	
HbA1c change <median	0.53 ± 1.24 (6)	1.14 ± 3.76 (15)	1.63 ± 3.72 (21)	0.780
P-value within group	0.341	0.259	0.058	
HbA1c change ≥median	–0.39 ± 2.98 (22)	1.45 ± 2.81 (11)	0.54 ± 2.67 (8)	0.186
P-value within group	0.549	0.240	0.587	

Data are mean ± standard deviation. *P-value for comparisons among groups. P-values within groups are results of paired t-test or Wilcoxon signed-rank test. P-values among groups are results of ANOVA or Kruskal–Wallis test applied for comparisons among groups. BMI, body mass index; FAS, full analysis set; FMD, flow-mediated dilation; HbA1c, hemoglobin A1c.

with marginal reduction in those with BMI of ≥25 kg/m^2 or >25 kg/m^2 (Table S1). Although improvement of CAVI by linagliptin was obvious in participants of young age and high BMI, interestingly, an increase of ΔFMD by linagliptin was observed in old age and low BMI (Table 2). According to previous reports, no association was observed between CAVI and FMD[23,24]. These data might partially explain the discrepancy between FMD and CAVI change in the linagliptin add-on group. Linagliptin seems to be more effective in patients with low HbA1c and young age with high BMI with regard to the prevention of atherosclerosis. These findings suggest that treatment with linagliptin is more beneficial than metformin

Table 3 | Changes in parameters of glycemic control

Parameters	Control group	Metformin group	Linagliptin add-on group	*P*-value*
HbA1c (%)				
Baseline	6.86 ± 0.60 (28)	6.85 ± 0.70 (26)	6.93 ± 0.63 (29)	0.859
Week 16	6.98 ± 0.56 (29)‡,§	6.55 ± 0.59 (26)†	6.46 ± 0.50 (29)†	0.001
Change	0.13 ± 0.40 (28)‡,§	−0.29 ± 0.46 (26)†	−0.48 ± 0.45 (29)†	<0.001
P-value within group	0.097	0.004	<0.001	
Fasting plasma glucose (mg/dL)				
Baseline	143.6 ± 28.4 (28)	149.1 ± 43.5 (26)	137.9 ± 30.4 (29)	0.669
Week 16	141.6 ± 32.4 (29)§	132.3 ± 27.1 (25)	119.4 ± 16.9 (29)†	0.007
Change	−2.2 ± 32.2 (28)	−14.6 ± 35.7 (25)	−18.5 ± 25.3 (29)	0.086
P-value within group	0.724	0.078	<0.001	
C-peptide (ng/mL)				
Baseline	2.1 ± 1.1 (28)	2.8 ± 2.2 (26)	2.0 ± 0.9 (29)	0.338
Week 16	1.9 ± 1.0 (29)	2.5 ± 2.2 (25)	1.8 ± 0.7 (29)	0.801
Change	−0.2 ± 0.6 (28)	−0.4 ± 1.0 (25)	−0.2 ± 0.5 (29)	0.522
P-value within group	0.422	0.038	0.259	
HOMA-IR				
Baseline	3.16 ± 2.78 (28)	6.70 ± 13.23 (26)	2.41 ± 2.07 (29)	0.288
Week 16	2.83 ± 3.32 (29)	4.97 ± 10.65 (25)	1.87 ± 1.17 (29)	0.638
Change	−0.34 ± 2.31 (28)	−1.92 ± 6.46 (25)	−0.54 ± 1.72 (29)	0.491
P-value within group	0.550	0.0496	0.101	

Data are mean ± standard deviation (*n*). **P*-value for comparisons among groups. *P*-values within groups are results of paired *t*-test or Wilcoxon signed-rank test. *P*-values among groups are results of ANOVA or Kruskal–Wallis test. When significant difference was detected, multiple comparisons were applied, the results of which are shown by superscripts as follows: †$P < 0.05$ versus the control group; ‡$P < 0.05$ versus the metformin group; §$P < 0.05$ versus the linagliptin add-on group. HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

Table 4 | Results of multiple linear regression analysis for change in flow-mediated dilation

Independent variables	Regression coefficient (SE)	*P*-value
Age at baseline (years)	−0.0416 (0.0283)	0.145
Sex	1.2295 (0.6053)	0.046
FMD at baseline (%)	−0.7890 (0.1231)	<0.001
Use of α-blockers at baseline	−3.8063 (2.5737)	0.144
Erythrocyte count at baseline ($\times 10^4/\mu L$)	0.0065 (0.0074)	0.388
Leukocyte count at baseline (/μL)	0.0002 (0.0002)	0.230
ΔApolipoprotein B (mg/dL)	−0.0507 (0.0246)	0.043
ΔPlatelet count ($\times 10^4/\mu L$)	0.0609 (0.1151)	0.598
Adjusted R^2	0.4635	

Multiple linear regression analysis was carried out with the following parameters as independent variables: age at baseline, sex and variables with *P*-values of single regression analysis of <0.1. To avoid the multicollinearity, one variable from correlate variables was selected and the variance inflation factor was calculated. FMD, flow-mediated dilation; SE, standard error.

supplementation in terms of the suppression of atherosclerosis progression.

We used metformin therapy as the control because it is one of the most widely used oral antidiabetic agents for type 2 diabetes mellitus[9,10], and is known to prevent cardiovascular events[8]. In addition, because insulin resistance is considered to be involved in the pathogenesis of cardiovascular disease, metformin, which improves whole-body insulin resistance, is considered to prevent cardiovascular disease[8,25]. In fact, increasing the dose of metformin from 750 to 1,500 mg/day resulted in improvement in HOMA-IR, which was specifically observed in the metformin group (Table 3). However, although HbA1c improved in both the metformin and linagliptin add-on groups, and HOMA-IR was significantly improved in the metformin group, improvement in FMD was observed only in the linagliptin add-on group. This shows not only improvement in glycemic control and insulin resistance, but that other factors also contribute to the improvement in FMD in the linagliptin add-on group. In this regard, linagliptin add-on therapy had a beneficial effect in patients with a baseline HbA1c of <7.0%.

To elucidate the factors associated with ΔFMD, simple and multiple linear regression analyses were carried out. The results of multiple linear regression analysis showed Δapolipoprotein B, FMD at baseline and sex as significant factors associated with ΔFMD (Table 4). Of these three factors, only apolipoprotein B changed significantly in the linagliptin add-on group, but not in the other two groups (Table S2). There were no differences in the other two factors among the groups. These findings suggest that linagliptin add-on therapy improves vascular endothelial function by reducing apolipoprotein B.

Apolipoprotein B is known to interact with very low-, intermediate- and low-density lipoproteins as well as chylomicrons[26,27], and is also known as a predictor of cardiovascular disease[28]. A previous clinical trial of fenofibrate in type 2 diabetes mellitus patients showed that Δapolipoprotein B48 was associated with ΔFMD[29]. Glucagon-like peptide-1 is known to repress intestinal apolipoprotein B production and triglyceride in rats[30], thus DPP-4 inhibitors are also expected to reduce apolipoprotein B and triglyceride through glucagon-like peptide-1 activation. In fact, it has been reported that sitagliptin decreased postprandial triglyceride and apolipoprotein B48 levels[31], and that vildagliptin also reduced apolipoprotein B and lipids in postprandial plasma, but not in a fasting situation[32]. As the blood tests were carried out in the fasting state in the present study, it is possible that no reduction in triglyceride was detected in linagliptin add-on therapy despite the reduction in apolipoprotein B under fasting conditions.

To date, other beneficial effects of DPP-4 inhibitors on atherosclerosis have been reported, together with their potential mechanisms. DPP-4 inhibitors lower postprandial glucose. This is important, as postprandial hyperglycemia is an independent risk factor of atherosclerosis[3,33]. In the present study, it is possible that the reduction of postprandial glucose might have played a role in the observed improvement in FMD. Treatment with DPP-4 inhibitors is also known to reduce inflammatory markers, such as hs-CRP and tumor necrosis factor-α, and increase anti-inflammatory markers, such as cytokine interleukin-10 and adiponectin[34–36]. In the present study, no changes were observed in hs-CRP and adiponectin (Table S3). However, one cannot exclude changes in other inflammatory cytokines that were not measured in the present study, such as tumor necrosis factor-α, intercellular adhesion molecules 1 and endothelial factors, such as vascular cell adhesion protein 1, with subsequent effects on endothelial function. Thus, further investigation is required to elucidate the mechanisms underlying the improvement in endothelial function after treatment with DPP-4 inhibitors.

Although the present study showed the positive effects of linagliptin on endothelial function, it had several limitations. First, this was an open-label design study with a relatively small number of Japanese patients, so additional long-term trials of larger sample size, preferably of patients of different ethnicities, are required to address the cardiovascular benefits of linagliptin. Second, total apolipoprotein B was measured rather than apolipoprotein B48 and B100 separately. Accordingly, we cannot discuss separately or conclude which type plays a role in the observed changes. Third, detailed lipid profiling was not carried out. Accordingly, we could not estimate the effect of linagliptin on various lipids, such as very low-density lipoprotein and chylomicrons. Fourth, all measurements were carried out using fasting plasma samples. Previous studies showed clear effects for DPP-4 inhibitor on the postprandial state[32].

In conclusion, the present study showed the potential effectiveness of metformin plus linagliptin treatment in type 2 diabetes mellitus patients with moderate hyperglycemia, by inducing both better glycemic control and improvement of endothelial function.

ACKNOWLEDGMENTS

This study was financially supported by Nippon Boehringer Ingelheim Co., Ltd. and Eli Lilly and Company, the manufacturer of linagliptin. The authors were fully responsible for all content and editorial decisions, were involved at all stages of manuscript development, and have approved the final version. The two pharmaceutical companies did not dictate or modify the results of the study. We thank Koki Shin (Shin Clinic), Makiko Sasamoto (Sasamoto Medical Clinic), Yasuyo Ando, Shuki Usui, Kayoko Ikehara, Tomoko Yagi, Kenzaburo Oda, Ken Kanazawa, Mai Hijikata, Naoko Ninomiya, Kimiko Katou, Chie Fujita, Kaori Awano, Hirokazu Yamada, Tomokazu Shibamiya and Hiroki Takayama for their excellent technical assistance. Parts of this study were presented as a poster presentation at the 76th Scientific Sessions of the American Diabetes Association, New Orleans, LA, USA, on 10–14 June 2016.

DISCLOSURE

N Kumashiro received research funds from Eli Lilly Japan K.K. and lecture fees from Novo Nordisk Inc. T Hirose received research funds from AstraZeneca; Boehringer Ingelheim Pharmaceuticals, Inc.; Astellas Pharma Inc.; Ono Pharmaceutical Co., Ltd.; Novo Nordisk Inc.; Sanofi-Aventis Deutschland GmbH; Daiichi-Sankyo Co., Ltd.; Eli Lilly Japan K.K.; Takeda Pharmaceutical Company Limited; Mitsubishi Tanabe Pharma Corporation; Dainippon Sumitomo Pharma Co., Ltd.; Kissei Pharmaceutical Co., Ltd.; and Johnson & Johnson; and received lecture fees from Sanofi-Aventis Deutschland GmbH; Eli Lilly Japan K.K.; Novo Nordisk Inc.; Takeda Pharmaceutical Company Limited; Daiichi-Sankyo Co., Ltd.; Mitsubishi Tanabe Pharma Corporation; Merck & Co., Inc.; Dainippon Sumitomo Pharma Co., Ltd.; Novartis Pharma K.K.; Kissei Pharmaceutical Co., Ltd.; Boehringer Ingelheim Pharmaceuticals, Inc.; Ono Pharmaceutical Co., Ltd.; and AstraZeneca. The funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The other authors declare no conflict of interest.

REFERENCES

1. Kim JA, Montagnani M, Koh KK, *et al.* Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006; 113: 1888–1904.
2. Fox CS, Coady S, Sorlie PD, *et al.* Trends in cardiovascular complications of diabetes. *JAMA* 2004; 292: 2495–2499.
3. Node K, Inoue T. Postprandial hyperglycemia as an etiological factor in vascular failure. *Cardiovasc Diabetol* 2009; 8: 23.

4. Ter Avest E, Stalenhoef AF, de Graaf J. What is the role of non-invasive measurements of atherosclerosis in individual cardiovascular risk prediction? *Clin Sci (Lond)* 2007; 112: 507–516.
5. Shirai K, Utino J, Otsuka K, *et al.* A novel blood pressure-independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). *J Atheroscler Thromb* 2006; 13: 101–107.
6. Namekata T, Suzuki K, Ishizuka N, *et al.* Establishing baseline criteria of cardio-ankle vascular index as a new indicator of arteriosclerosis: a cross-sectional study. *BMC Cardiovasc Disord* 2011; 11: 51.
7. Nagayama D, Saiki A, Endo K, *et al.* Improvement of cardio-ankle vascular index by glimepiride in type 2 diabetic patients. *Int J Clin Pract* 2010; 64: 1796–1801.
8. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* 1998; 352: 854–865.
9. Consoli A, Gomis R, Halimi S, *et al.* Initiating oral glucose-lowering therapy with metformin in type 2 diabetic patients: an evidence-based strategy to reduce the burden of late-developing diabetes complications. *Diabetes Metab* 2004; 30: 509–516.
10. Rojas LB, Gomes MB. Metformin: an old but still the best treatment for type 2 diabetes. *Diabetol Metab Syndr* 2013; 5: 6.
11. White WB, Cannon CP, Heller SR, *et al.* Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* 2013; 369: 1327–1335.
12. Scirica BM, Bhatt DL, Braunwald E, *et al.* Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; 369: 1317–1326.
13. Green JB, Bethel MA, Armstrong PW, *et al.* Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; 373: 232–242.
14. Johansen OE, Neubacher D, von Eynatten M, *et al.* Cardiovascular safety with linagliptin in patients with type 2 diabetes mellitus: a pre-specified, prospective, and adjudicated meta-analysis of a phase 3 programme. *Cardiovasc Diabetol* 2012; 11: 3.
15. Kroller-Schon S, Knorr M, Hausding M, *et al.* Glucose-independent improvement of vascular dysfunction in experimental sepsis by dipeptidyl-peptidase 4 inhibition. *Cardiovasc Res* 2012; 96: 140–149.
16. Schurmann C, Linke A, Engelmann-Pilger K, *et al.* The dipeptidyl peptidase-4 inhibitor linagliptin attenuates inflammation and accelerates epithelialization in wounds of diabetic ob/ob mice. *J Pharmacol Exp Ther* 2012; 342: 71–80.
17. 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* 2002; 39: 257–265.
18. Takeno K, Mita T, Nakayama S, *et al.* Masked hypertension, endothelial dysfunction, and arterial stiffness in type 2 diabetes mellitus: a pilot study. *Am J Hypertens* 2012; 25: 165–170.
19. Hu H, Cui H, Han W, *et al.* A cutoff point for arterial stiffness using the cardio-ankle vascular index based on carotid arteriosclerosis. *Hypertens Res* 2013; 36: 334–341.
20. Gomez-Marcos MA, Recio-Rodriguez JI, Patino-Alonso MC, *et al.* Cardio-ankle vascular index is associated with cardiovascular target organ damage and vascular structure and function in patients with diabetes or metabolic syndrome, LOD-DIABETES study: a case series report. *Cardiovasc Diabetol* 2015; 14: 7.
21. Naka KK, Papathanassiou K, Bechlioulis A, *et al.* Effects of pioglitazone and metformin on vascular endothelial function in patients with type 2 diabetes treated with sulfonylureas. *Diab Vasc Dis Res* 2012; 9: 52–58.
22. Suzuki K, Watanabe K, Suzuki T, *et al.* Sitagliptin improves vascular endothelial function in Japanese type 2 diabetes patients without cardiovascular disease. *Journal of Diabetes Mellitus* 2012; 2: 338–345.
23. Inaba H, Takeshita K, Uchida Y, *et al.* Recovery of flow-mediated vasodilatation after repetitive measurements is involved in early vascular impairment: comparison with indices of vascular tone. *PLoS ONE* 2014; 9: e83977.
24. Hoshida S, Miki T, Nakagawa T, *et al.* Determining factors for carotid mean/max intima-media thickness and brachial flow-mediated dilation in healthy young women. *World J Cardiovascular Diseases* 2012; 2: 43–49.
25. Bailey CJ, Turner RC. Metformin. *N Engl J Med* 1996; 334: 574–579.
26. Irshad M, Dubey R. Apolipoproteins and their role in different clinical conditions: an overview. *Indian J Biochem Biophys* 2005; 42: 73–80.
27. Dominiczak MH, Caslake MJ. Apolipoproteins: metabolic role and clinical biochemistry applications. *Ann Clin Biochem* 2011; 48: 498–515.
28. Olofsson SO, Boren J. Apolipoprotein B: a clinically important apolipoprotein which assembles atherogenic lipoproteins and promotes the development of atherosclerosis. *J Intern Med* 2005; 258: 395–410.
29. Chan DC, Wong AT, Yamashita S, *et al.* Apolipoprotein B-48 as a determinant of endothelial function in obese subjects with type 2 diabetes mellitus: effect of fenofibrate treatment. *Atherosclerosis* 2012; 221: 484–489.
30. Ussher JR, Drucker DJ. Cardiovascular biology of the incretin system. *Endocr Rev* 2012; 33: 187–215.
31. Tremblay AJ, Lamarche B, Deacon CF, *et al.* Effect of sitagliptin therapy on postprandial lipoprotein levels in patients with type 2 diabetes. *Diabetes Obes Metab* 2011; 13: 366–373.
32. Matikainen N, Manttari S, Schweizer A, *et al.* Vildagliptin therapy reduces postprandial intestinal triglyceride-rich

lipoprotein particles in patients with type 2 diabetes. *Diabetologia* 2006; 49: 2049–2057.
33. Mah E, Bruno RS. Postprandial hyperglycemia on vascular endothelial function: mechanisms and consequences. *Nutr Res* 2012; 32: 727–740.
34. Tremblay AJ, Lamarche B, Deacon CF, *et al.* Effects of sitagliptin therapy on markers of low-grade inflammation and cell adhesion molecules in patients with type 2 diabetes. *Metabolism* 2014; 63: 1141–1148.
35. Satoh-Asahara N, Sasaki Y, Wada H, *et al.* A dipeptidyl peptidase-4 inhibitor, sitagliptin, exerts anti-inflammatory effects in type 2 diabetic patients. *Metabolism* 2013; 62: 347–351.
36. Hibuse T, Maeda N, Kishida K, *et al.* A pilot three-month sitagliptin treatment increases serum adiponectin level in Japanese patients with type 2 diabetes mellitus–a randomized controlled trial START-J study. *Cardiovasc Diabetol* 2014; 13: 96.

SUPPORTING INFORMATION

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

Figure S1| Changes in flow-mediated dilation during the 16-week study.
Table S1| Changes in cardio-ankle vascular index.
Table S2| Changes in parameters of lipid metabolism.
Table S3| Changes in parameters for inflammation, oxidative stress and urinary albumin.
Table S4| Results of simple linear regression analysis for change in FMD flow-mediated dilation.
Table S5| Adverse events.
Appendix S1| List of 11 medical institutions participating in the study.