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ORIGINAL ARTICLE

Clinical evaluation of thrice-daily lispro 50/50 versus twice-daily aspart 70/30 on blood glucose fluctuation and postprandial hyperglycemia in patients with type 2 diabetes mellitus

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Abstract
Aim Our goal was to compare the effects of thrice-daily lispro 50/50 and twice-daily aspart 70/30 on blood glucose fluctuation and postprandial hyperglycemia in type 2 diabetes mellitus patients.
Methods Thirty-nine type 2 diabetes patients hospitalized at our hospital for poorly controlled disease (26 men, 13 women; mean age 62.5 years, mean BMI 24.1) received either thrice-daily lispro 50/50 (50/50 group, $n = 19$) or twice-daily aspart 70/30 (70/30 group, $n = 20$) after 1 week of multiple-daily injection insulin regimen. Efficacy measurements included HbA1c, diurnal variation in blood glucose levels and total daily insulin doses. Despite a small number of subjects, we also explored the potent effect in prevention of progression of atherosclerosis. Ultrasound examination of bilateral carotid arteries ($n = 22$) and the cardio-ankle vascular index (CAVI) ($n = 12$) were performed before and after 48 weeks of treatment.
Results Mean HbA1c levels improved significantly in each treatment group, and HbA1c at 48 weeks was significantly lower in the 50/50 group than the 70/30 group (7.0 ± 1.0 vs. 7.3 ± 1.1 %, $P = 0.03$), whereas mean fasting blood glucose levels did not differ significantly between the two groups at 48 weeks (127.4 ± 30.9 vs. 132.6 ± 20.6 mg/dL, $P = 0.47$). A significantly greater percentage of subjects in the 50/50 group achieved the target HbA1c value of ≤ 6.9 %compared to the 70/30 group [57.9 % (11/19 patients) vs. 25.0 % (5/20 patients), $P = 0.01$). The 50/50 group tended to have less daily plasma glucose fluctuation than the 70/30 group. Although there was a significant difference in the degrees of change in CAVI between the subsets of each group, there were no significant differences in the maximum thickness of the intima-media layers of the carotid arteries (IMT) and plaque scores.
Conclusions Thrice-daily lispro 50/50 injection reduces the postprandial blood glucose level and stabilizes the diurnal fluctuations of blood glucose levels more efficiently than twice-daily aspart 70/30 in type 2 diabetes mellitus patients.

Keywords Type 2 diabetes mellitus · Lispro 50/50 · Aspart 70/30 · Cardio-ankle vascular index (CAVI) · Thickness of the intima-media layers of carotid arteries (IMT)

Introduction

The goal for treatment of type 2 diabetes mellitus is prevention of the onset and progression of microvascular and macrovascular complications. The Diabetes Control Complications Trial (DCCT) and the Kumamoto Study demonstrated that intensive insulin therapy provides significantly greater improvement in HbA1c values and reduces the onset and progression of diabetic microangiopathy more effectively than conventional insulin therapy, but failed to show significant effects on cardiovascular disease [1, 2]. It has recently been reported that postprandial hyperglycemia is a risk factor for cardiovascular events in patients with type 2 diabetes mellitus [3]. Therefore, it is essential to suppress postprandial blood glucose elevation, and to stabilize the diurnal variation of

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blood glucose levels for prevention of atherosclerotic disorders, including cardiovascular disease [4]. Type 2 diabetes mellitus is a progressive and complex disorder that is difficult to treat effectively in the long-term. Most type 2 diabetes patients eventually require insulin therapy [5]. Basal-bolus insulin therapy, combining rapid-acting and long-acting human insulin analogs, is useful for controlling both pre-prandial and postprandial blood glucose levels and for lowering blood HbA1c levels. However, diabetic patients, especially elderly patients, often prefer to receive fewer injections. Previously, we often used twice-daily injections of rapid-acting insulin aspart 70/30 comprised of biphasic insulin aspart 70/30 [Novo Nordisk Pharma, Tokyo, Japan], although this twice-daily insulin regimen often did not achieve the target for lowering postprandial hyperglycemia. Insulin lispro mix 50/50 [Eli Lilly, Tokyo, Japan] contains a higher percentage of rapid-acting insulin than aspart 70/30. This thrice-daily lispro mix 50/50 injection treatment with each meal mimics basal-bolus insulin therapy, and may prevent the onset and progression of cardiovascular events by controlling daily glucose fluctuations. Here, we compared the effects of thrice-daily lispro 50/50 with twice-daily aspart 70/30 on postprandial blood glucose levels and diurnal variation of blood glucose levels, and also explored the potent effect in prevention of progression of atherosclerosis, despite the small number of subjects.

Materials and methods

We performed a retrospective observational study to analyze clinical data for adult type 2 diabetic patients at the Department of Endocrinology and Metabolism, Tonami General Hospital. We recruited 39 patients with type 2 diabetes mellitus who were admitted to Tonami General Hospital for improvement of poor glycemic control, and were treated with thrice-daily lispro 50/50 before each meal (50/50 group) or twice-daily aspart 70/30 before breakfast and dinner (70/30 group). All participants were enrolled sequentially between June 2007 and May 2009. Clinical and laboratory tests showed no evidence of severe kidney disease (plasma creatinine 1.50 mg/dL), severe liver dysfunction, infectious disease, or autoimmune disease in any of the patients at the time of hospitalization. Patients were excluded from the study if they had type 1 diabetes mellitus, were positive for anti-glutamic acid decarboxylase antibody, or had urinary C-peptide excretion $\leq$ 20.0 μg/day. All patients provided informed consent and confirmed their willingness to inject insulin and carry out glucose self-monitoring. The study protocol was approved by the ethics review committee of Tonami General Hospital, Toyama, Japan. The study was carried out in accordance with the principles of the Declaration of Helsinki.

The characteristics and laboratory data on admission are shown in Table 1. Most patients had received oral antihyperglycemic agents (OHAs), including sulfonylurea or insulin therapy. After hospitalization, these medications were discontinued, and intensive insulin therapy was started with insulin aspart (Novo Nordisk A/S, Bagsvaerd, Denmark) or insulin lispro (Eli Lilly Nederland B.V.,

Table 1 Baseline characteristics of patients in the 50/50 or 70/30 group

	50/50 group	70/30 group	*P* value
Number of patients, *n*	19	20	
Gender (male/female), *n*	12/7	14/6	0.76
Age (years)	63.2 ± 11.1	61.2 ± 12.6	0.51
BMI (kg/m^2)	23.7 ± 5.0	24.5 ± 4.3	0.13
Current smoking, *n* (%)	3 (15.8)	3 (15.0)	0.65
Current alcohol use, *n* (%)	5 (26.3)	4 (20.0)	0.43
Exercise habits, *n* (%)	2 (10.5)	3 (15.0)	0.77
Duration of diabetes (years)	10.5 ± 8.8	11.9 ± 9.8	0.27
HbA1c (NGSP) (%)	9.9 ± 2.0	10.1 ± 1.8	0.32
Urinary C-peptide (μg/day)	66.4 ± 38.0	60.2 ± 31.6	0.42
Diabetic complications			
Retinopathy, *n* (%)	7 (36.8)	7 (35.0)	0.73
Nephropathy, *n* (%)	7 (36.8)	6 (30.0)	0.90
Neuropathy, *n* (%)	4 (21.1)	1 (5.0)	0.13
Prior therapy			
Diet/Exercise only, *n* (%)	7 (36.8)	8 (40.0)	0.88
Oral antidiabetic agents, *n* (%)	3 (15.8)	4 (20.0)	0.79
Insulin, *n* (%)	9 (47.4)	8 (40.0)	0.72
Arterial hypertension, *n* (%)	11 (57.9)	11 (55.0)	0.59
Dyslipidemia, *n* (%)	11 (57.9)	13 (65.0)	0.38
Ischemic heart disease, *n* (%)	3 (15.8)	3 (15.0)	0.69
Cerebral infarction, *n* (%)	2 (10.5)	1 (5.0)	0.27
Lipid-lowering agents			
Statins	11 (57.9)	13 (65.0)	0.77
Fibrates	0 (0.0)	1 (5.0)	0.41
Antihypertensive agents			
RAS inhibitors	11 (57.9)	11 (55.0)	0.83
CCB	7 (36.8)	6 (30.0)	0.33
Diuretics	2 (10.5)	1 (5.0)	0.21
Antiplatelet agents	3 (15.8)	4 (20.0)	0.40
Max IMT (mm)	1.23 ± 0.56	1.15 ± 0.52	0.58
Plaque score	8.09 ± 5.02	7.05 ± 5.50	0.65

Data are represented as mean ± standard deviation or number (percentage). *P* values are for differences between the two groups using the Mann–Whitney's *U* test

RAS renin-angiotensin system, *CCB* calcium channel blockers, *IMT* thickness of the intima-media layers of the carotid arteries

Houten, The Netherlands) before each meal and glargine (Lantus, Aventis Pharma, Frankfurt, Germany) at bedtime. Basal insulin doses were titrated to target fasting plasma glucose levels between 90 and 130 mg/dL. Premeal insulin doses were adjusted according to 2-h postprandial plasma glucose levels to achieve the target of $\leq$ 180 mg/dL. After 1 week of intensive insulin therapy with a multiple-daily injection insulin regimen, treatment with lispro 50/50 or aspart 70/30 was started. The target levels of glucose with each insulin therapy were a mean postprandial plasma glucose (PPG) (2 h after each meal) value of 180 mg/dL and a fasting plasma glucose (FPG) level of 130 mg/dL, based on the definition of "good control" by the Japan Diabetes Society (JDS). The mean observation periods after initiation of each type of insulin therapy were 11.6 ± 3.2 months in the 50/50 group and 10.5 ± 5.1 months in the 70/30 group; there was no significant difference between these two periods. Body weight and height were measured, and body mass index (BMI) was calculated as weight in kilograms, divided by the square of height in meters as an index of obesity. Systolic blood pressure (sBP) and diastolic blood pressure (dBP) were measured at the brachial artery. Blood was taken in the morning after breakfast with insulin injection or intake of medication. Hemoglobin A1c (HbA1c), serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglyceride (TG) were measured every 4 weeks throughout the observation period. The HbA1c values in the present study are shown as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%), calculated with the following formula: HbA1c (NGSP) (%) = HbA1c (JDS) (%) + 0.4 (%) [6].

Cardio-ankle vascular index (CAVI) was measured non-invasively using a Vasera VS-1000 system (Fukuda Denshi, Tokyo, Japan), and CAVI values were automatically calculated by the Vasera VS-1000. CAVI values were obtained by substituting the stiffness parameter β in the following equation for detecting vascular elasticity and PWV: stiffness parameter $\beta = 2\rho \times 1/(P_s - P_d) \times \mathrm{In}\,(P_s/P_d) \times \mathrm{PWV}^2$ (ρ is blood density, P_s and P_d are SBP and DBP in mmHg, and PWV was measured between the aortic valve and the ankle), as described in detail by Yambe et al. and Shirai et al. [7, 8]. Therefore, CAVI was unaffected by BP. CAVI has good reproducibility. The average coefficient of variation of CAVI is less than 5 %, and is small enough for clinical use [9]. Measurement of CAVI was performed in six patients in the 50/50 group and in six patients in the 70/30 group, before and after treatment.

The thickness of the intima-media layers of the carotid arteries (IMT) was measured by high-resolution B-mode ultrasonography (SSA-340A; Toshiba, Tokyo, Japan) with an electronic linear transducer (mid-frequency of 8 MHz). When using 8.0 MHz, the detection limit of this echo system was 0.1 mm. The IMT value was determined as originally described by Pignoli et al. [10]. The IMT was measured as the distance between two parallel echogenic lines corresponding to the blood-intima and media-adventitia interfaces on the posterior wall of the artery. Three determinations of IMT were performed at the site of the thickest point: the maximum IMT and the IMTs two adjacent points (1 cm upstream and 1 cm downstream from this site). These three determinations were averaged (mean IMT). The maximum IMT was determined as the average of the maximal wall thickness of these six segments: near and far walls of the left and right common carotid artery, carotid bulb, and internal carotid artery. As previously reported, localized thicknesses of more than 2.0 mm were considered as plaques, and excluded from the analysis [11]. Plaque score (PS) was defined as the sum of IMT at the site of the thickest point greater than 1.1 mm in each area, on scanning of the extracranial common carotid artery (S1), the carotid bulb (S2), the proximal portion (S3), and the distal portion of the internal carotid artery (S4) in the neck performed bilaterally, as described previously [12, 13]. To avoid intersonographer variability, the prescribed study examination was examined by the same sonographer with the same equipment. The intra-observer variability for repeat measurements, indicated by the mean difference and the standard deviation in IMT between these two determinations, was 0.04 and 0.05 mm, respectively. This variability demonstrates good reproducibility.

All data are presented as the mean $\pm$ standard deviation, unless otherwise specified. To compare the two points for each parameter in each group, Wilcoxon signed-rank test was carried out. The significance of differences between two groups was tested using Mann–Whitney's U test. The χ^2 test was used to compare categorical variables. Correlations between two groups were tested using Pearson's correlation coefficient. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

After hospitalization, a total of 39 patients with poor glycemic control began insulin therapy with a multiple-daily injection regimen. After 1 week of this therapy, the patients were divided by turns into one of two groups receiving a thrice-daily lispro 50/50 regimen before each meal (50/50 group; $n = 19$) or a twice-daily aspart 70/30 regimen before breakfast and dinner (70/30 group; $n = 20$). The characteristics of subjects in the 50/50 group and the 70/30 group at baseline are presented in Table 1. Patients in both groups were similar with respect to age, BMI, duration of diabetes, and HbA1c. With regard to

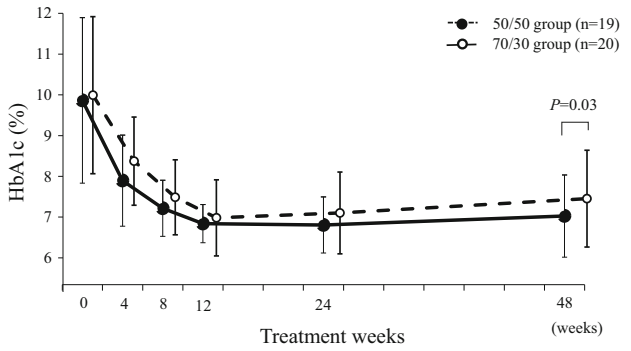

Fig. 1 Changes in mean HbA1c of the 50/50 (*filled circle*, $n = 19$) and 70/30 (*open circle*, $n = 20$) groups. Data are mean ± SD. *P* values are for differences between the two treatment groups at 48 weeks using the Mann–Whitney's *U* test

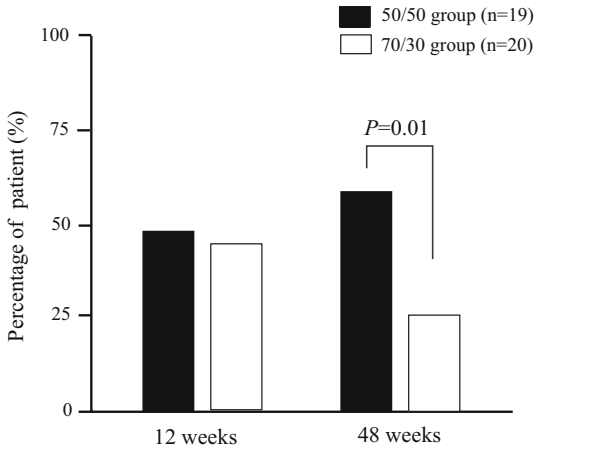

Fig. 2 Percentage of patients reaching HbA1c target of <6.9 % according to treatment: 50/50 group ($n = 19$) and 70/30 group ($n = 20$). Data are mean ± SD. *P* values are for differences between the two treatment groups using the Mann–Whitney's *U* test

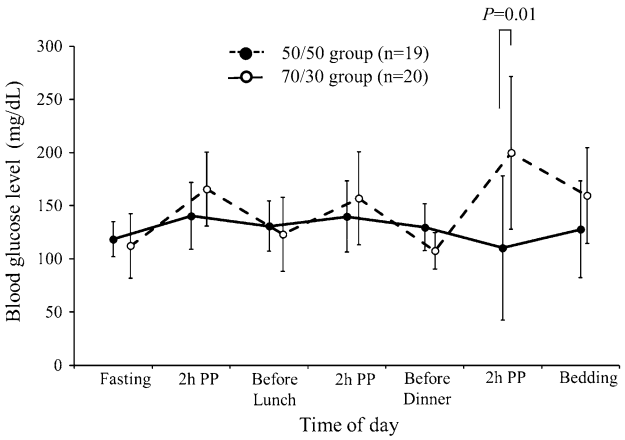

Fig. 3 Mean seven-point blood glucose profiles according to treatment: 50/50 group (*filled circle*, $n = 19$) and 70/30 group (*open circle*, $n = 20$). Data are mean ± SD. *PP* postprandial. *P* values are for differences between the two treatment groups using the Mann–Whitney's *U* test

treatments prior to the switch to lispro 50/50, seven of the 19 patients were on diet and exercise therapy, three patients were being treated with oral anti-diabetic drugs, and the remaining nine patients were treated with insulin therapy. On the other hand, for the treatments prior to the switch to aspart 70/30, eight of the 20 patients were on diet and exercise therapy, four patients were being treated with oral antidiabetic drugs, and the remaining eight patients were treated with insulin therapy. Three patients in the 50/50 group and four patients in the 70/30 group were prescribed α-glucosidase inhibitors. Prior treatments did not differ significantly between the two groups. The proportion of habitual smokers, alcohol users, the patients in the habit of exercise, and the patients taking lipid-lowering agents or anti-hypertensive agents did not differ between the two groups. The maximum IMT of common carotid arteries and the plaque score at baseline also did not differ between the two groups.

The mean baseline HbA1c values were 9.9 ± 2.0 and 10.1 ± 1.8 % in the 50/50 group and the 70/30 group, respectively. During the follow-up period, the mean HbA1c value decreased significantly in both groups (6.9 ± 0.5 and 7.0 ± 0.9 %, respectively, at 12 weeks, and 7.0 ± 1.0 and 7.3 ± 1.1 %, respectively, at 48 weeks). After 48 weeks, the reduction in mean HbA1c was significantly greater in the 50/50 group than in the 70/30 group (Fig. 1). The mean HbA1c decreased to a lesser extent in the 70/30 group than in the 50/50 group. Fasting blood glucose levels were 119.4 ± 22.0 mg/dL at baseline and 127.4 ± 30.9 mg/dL at 48 weeks in the 50/50 group. In the 70/30 group, fasting blood glucose levels were 122.6 ± 18.3 mg/dL at baseline and 132.6 ± 20.6 mg/dL at 48 weeks. Thus, fasting blood glucose levels did not differ significantly between the two groups at baseline and at 48 weeks. At 48 weeks, the proportions of patients who reached HbA1c targets of < 6.9 % were significantly greater in the 50/50 group than the 70/30 group, as shown in Fig. 2 (60.0 vs. 25.0 %, respectively; $P = 0.01$). Figure 3 shows the mean seven-point self-monitored blood glucose (SMBG) profiles after 1 week of treatment with thrice-daily lispro 50/50 or twice-daily aspart 70/30. The seven-point SMBG profile levels were different between the two groups. Two-hour postprandial blood glucose levels were lower in the 50/50 group than the 70/30 group, including 2-h post-breakfast (147.6 ± 45.9 vs. 170.0 ± 34.9 mg/dL, respectively; $P = 0.34$), post-lunch (146.9 ± 48.3 vs. 161.0 ± 43.9 mg/dL, respectively; $P = 0.51$), and post-supper (116.0 ± 77.7 vs. 204.0 ± 72.2 mg/dL, respectively; $P = 0.01$). The plasma glucose levels after breakfast and lunch were lower in the 50/50 group compared with the 70/30 group, but the differences did not reach statistical

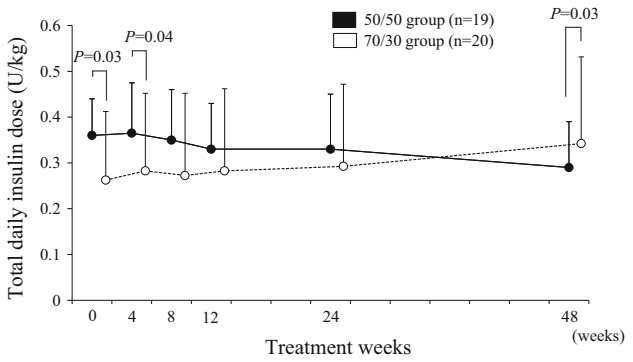

Fig. 4 Changes in mean total daily insulin dose (U/kg) according to treatment over the course of the study: 50/50 group ($n = 19$) and 70/30 group ($n = 20$). Data are mean ± SD. P values are for differences between the two treatment groups at 48 weeks using the Mann–Whitney's U test

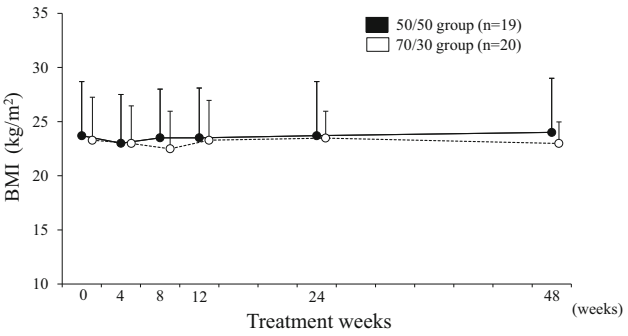

Fig. 5 Changes in mean body mass index according to treatment over the course of the study: 50/50 group ($n = 19$) and 70/30 group ($n = 20$). Data are mean ± SD

significance. The plasma glucose values after dinner were significantly lower in the 50/50 group than in the 70/30 group. Fasting blood glucose were similar between the two groups. Therefore, postprandial blood glucose excursions after each of the three meals and the mean daily blood glucose excursions were lower in the 50/50 group. The M value, an expression of mean glycemia and the effects of glucose swings, was calculated from the SMBG profile [14]. The M value was significantly lower in the 50/50 group than in the 70/30 group (14.8 ± 11.3 vs. 19.4 ± 17.4, respectively; $P = 0.04$).

The changes in mean total daily insulin dose according to treatment over the course of the study are shown in Fig. 4. At the start of the treatment in each group, the daily of insulin doses for the 50/50 group (0.36 ± 0.10 U/kg per day, distributed to 0.13 ± 0.05 : 0.09 ± 0.03 : 0.14 ± 0.05 U/kg at breakfast : lunch : dinner) were significantly larger than those for the 70/30 group (0.26 ± 0.15 U/kg per day, distributed to 0.14 ± 0.10 : 0.12 ± 0.10 U/kg at breakfast : dinner). The daily insulin doses in the 50/50 group decreased from 0.36 ± 0.10 to 0.27 ± 0.11 U/kg per day after 48 weeks, whereas those in the 70/30 group increased from 0.26 ± 0.15 to 0.33 ± 0.19 U/kg per day after 48 weeks. The between-group difference after 48 weeks was significant.

There were no significant changes in BMI in either group during the observation period (Fig. 5). No significant changes in blood pressure, LDL-C, or TG levels were observed in either group (Table 2).

Although the cases for which the follow-up measurements of CAVI, IMT and plaque score before and after treatment were performed were limited, the atherosclerotic changes were detected based on IMT of the carotid artery and CAVI. CAVI values at baseline were similar between the two groups. After 48 weeks, there was a significant increase in CAVI in the 70/30 group ($n = 6$) (8.49 ± 0.81 at baseline to 9.13 ± 0.55 at 48 weeks; $P = 0.01$), but no change in the 50/50 group ($n = 6$) (8.51 ± 0.80 at baseline to 8.83 ± 1.50 at 48 weeks; $P = 0.68$) (Fig. 6). The mean changes from baseline to week 48 in the max IMT of common carotid arteries and plaque scores are shown in Fig. 7. There were no significant differences in the degrees of change in the max IMT between the 50/50 group ($n = 8$) and the 70/30 group ($n = 14$) (+0.18 ± 0.52 vs. +0.02 ± 0.11, respectively; $P = 0.72$). There were also no significant differences in the increase in plaque score from baseline between the two groups (−0.06 ± 2.90 vs. +0.39 ± 1.28, respectively; $P = 0.81$). The plaque score decreased in 50.0 % of subjects in the 50/50 group and in 37.5 % of subjects in the 70/30 group, although the difference was not significant. To further explore the effects of the treatment of thrice-daily lispro 50/50 on progression of IMT, we examined the clinical characteristics divided into two subgroups: "improvers," which showed negative changes in plaque score; and "non-improvers," which showed positive or no changes (Table 3). The mean HbA1c value at baseline was significantly higher in the improver group than in the non-improver group, whereas the mean HbA1c value at 48 weeks was significantly lower in the improver group than in the non-improver group. The daily insulin dose in the non-improver group was significantly larger than that in the improver group at 48 weeks. There was a tendency for longer duration of diabetes and older age in the non-improver group, although the differences between the two groups were not significant.

Neither the improvement in HbA1c (ΔHbA1c = baseline HbA1c – HbA1c at 48 weeks) nor the mean HbA1c value at 48 weeks had a significant correlation with the improvement in plaque score (Δ plaque score = baseline plaque score – plaque score at 48 weeks).

No major hypoglycemic episodes or adverse events were observed in either group. There were a few minor hypoglycemic episodes in both groups, most of which were recorded as symptoms only. This did not reach statistical significance.

Table 2 Changes in various variables before and after insulin therapy

	50/50 group ($n = 19$)			70/30 group ($n = 20$)			P^a
	Before	After	P	Before	After	P	
Systolic BP (mmHg)	125.1 ± 20.1	128.6 ± 16.6	0.42	123.8 ± 11.1	124.5 ± 9.1	0.74	0.52
Diastolic BP (mmHg)	65.3 ± 12.6	63.8 ± 9.5	0.51	71.7 ± 8.5	75.2 ± 10.9	0.20	0.30
LDL-C (mg/dL)	115.9 ± 30.8	96.9 ± 33.6	0.24	126.5 ± 27.8	108.7 ± 13.8	0.45	0.66
TG (mg/dL)	164.4 ± 82.4	131.0 ± 57.9	0.66	144.0 ± 34.4	119.3 ± 40.7	0.49	0.71
HDL-C (mg/dL)	59.5 ± 22.0	58.1 ± 24.9	0.49	54.7 ± 14.9	51.5 ± 7.3	0.18	0.26

Data are expressed as mean ± standard deviation. P values are for change before and after therapy by the Wilcoxon signed-rank test

BP blood pressure

[a] Values are for differences between the two treatment groups in the amount of change from baseline using the Mann–Whitney's U test

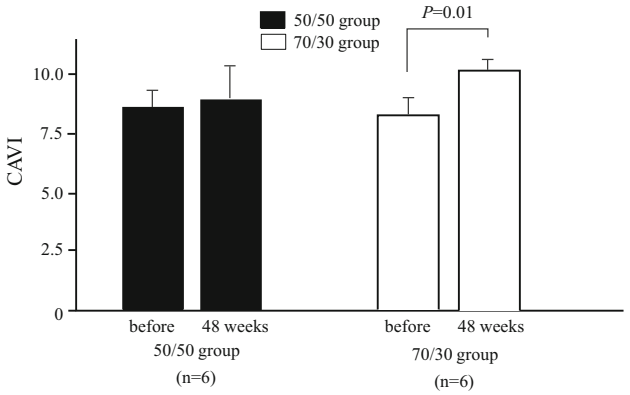

Fig. 6 Changes in CAVI according to treatment. Data are mean ± SD. P values are for differences between before and after 48 weeks in each group using the Wilcoxon signed-rank test

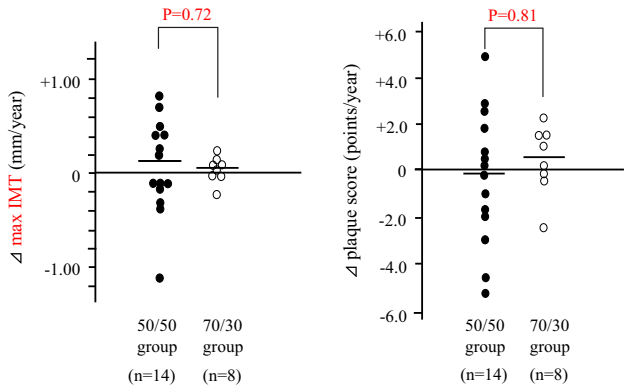

Fig. 7 Changes in the maximum IMT and plaque score according to treatment. The mean changes of the max IMT and plaque score in each group are denoted with *bars*

Discussion

In the present study, thrice-daily lispro 50/50 injection showed greater reduction in postprandial blood glucose level compared with twice-daily aspart 70/30 injection, and

Table 3 Comparison of clinical parameters between the two subgroups in the 50/50 group:"improvers" showed negative changes in plaque score and "non-improvers" showed positive or no changes

	Improver	Non-improver	P
Number of patients, n	6	8	0.70
Gender (male/female), n	4–2	6–2	0.25
Age (years)	58.0 ± 11.9	70.0 ± 8.3	0.11
Duration of diabetes (years)	7.5 ± 5.5	11.5 ± 8.8	0.44
Urinary C-peptide (μg/day)	67.7 ± 37.2	55.5 ± 18.9	0.69
BMI at baseline (kg/m^2)	26.1 ± 6.8	23.2 ± 4.3	0.20
BMI at 48 weeks (kg/m^2)	27.4 ± 7.6	22.7 ± 4.5	0.13
HbA1c at baseline (%)	11.4 ± 2.1	9.1 ± 1.2	0.02
HbA1c at 48 weeks (%)	5.8 ± 0.1	6.9 ± 1.0	0.01
Systolic blood pressure at baseline (mmHg)	125.1 ± 20.1	123.8 ± 11.1	0.53
Systolic blood pressure at 48 weeks (mmHg)	128.6 ± 16.6	124.5 ± 9.1	0.71
Diastolic blood pressure at baseline (mmHg)	65.3 ± 12.6	71.7 ± 8.5	0.41
Diastolic blood pressure at 48 weeks (mmHg)	63.8 ± 9.5	75.2 ± 10.9	0.08
LDL-C at baseline (mg/dL)	115.9 ± 30.8	126.5 ± 27.8	0.39
LDL-C 48 weeks (mg/dL)	96.9 ± 33.6	108.7 ± 13.8	0.45
TG at baseline (mg/dL)	164.4 ± 82.4	144.0 ± 34.4	0.36
TG 48 weeks (mg/dL)	131.0 ± 57.9	119.3 ± 40.7	0.52
HDL-C at baseline (mg/dL)	59.5 ± 22.0	54.7 ± 14.9	0.65
HDL-C 48 weeks (mg/dL)	58.1 ± 24.9	51.5 ± 7.3	0.50
Total daily insulin dose at baseline (U/kg)	0.41 ± 0.05	0.32 ± 0.12	0.13
Total daily insulin dose at 48 weeks (U/kg)	0.27 ± 0.06	0.37 ± 0.02	0.02
Diabetic complications			
Retinopathy, n (%)	33.3	37.5	0.67
Nephropathy, n (%)	33.3	50.0	0.22
Neuropathy, n (%)	16.7	12.5	0.45

Data are expressed mean ± standard deviation, number or percentage. P values are for differences between the two groups using the Mann–Whitney's U test

stabilized the diurnal fluctuations of blood glucose levels as estimated from the *M*-value, which reflects mean glycemia and the effect of glucose swings. These effects could be due to the different modes of administration and different blend ratios of rapid-acting and neutral protamine insulin. This suggests that rapid-acting insulin component injections three times daily are preferable to two times daily to achieve improved glycemic control.

Postprandial hyperglycemia is associated with progression of systemic arteriosclerosis, leading to macroangiopathy, such as ischemic heart disease and cerebrovascular disease. Recent studies have shown that the 2-h plasma glucose level in a 75-g oral glucose tolerance test is significantly correlated with cardiovascular complications, and is the cause of morbidity and mortality [14–16]. Experimental studies have shown that postprandial hyperglycemia increases free radical production in cells and increases monocyte adhesion to the endothelial surface in vivo [17]. These observations initiate the process of atherosclerogenesis. Drugs able to suppress postprandial plasma glucose elevation are useful for preventing the development of macrovascular complications [18]. A prospective intervention study demonstrated that reducing postprandial glucose by pharmacological intervention with an α-glucosidase inhibitor significantly reduced macrovascular events [19]. Basal-bolus insulin therapy, combining rapid-acting insulin and neutral protamine Hagedorn (NPH) insulin or insulin glargine, is effective for controlling both preprandial and postprandial blood glucose levels, and for lowering blood HbA1c levels. However, many patients often prefer to receive fewer injections.

In recent years, insulin analogs have been combined at different blend ratios (Humalog® Mix 25: 25 % lispro, 75 % protaminated lispro; Humalog® Mix 50: 50 % lispro, 50 % protaminated lispro; Novorapid® mix 30/70: 70 % aspart, 30 % protaminated aspart). Humalog® Mix 50 is an insulin analog preparation composed of a 1:1 mixture of insulin lispro and neutral protamine insulin lispro suspension. This 1:1 ratio has been often used for continuous subcutaneous insulin injection therapy [20, 21]. It has been reported that the ratio of basal and bolus insulin requirements is approximately 1:1 in healthy individuals [22]. It was previously reported that increasing the dose of insulin glargine up to half of the total insulin requirement could lead to better glycemic control in type 2 diabetes patients whose basal insulin of intensive insulin therapy was converted from bed-time NPH insulin to morning insulin glargine [23]. Therefore, thrice-daily mealtime lispro 50/50 is expected to allow reduction of both postprandial and preprandial blood glucose levels, resulting in adequate blood glucose control by conventional basal-bolus therapy. Thrice-daily lispro 50/50 injection have the potential to mimic the endogenous serum insulin profile, thereby providing a more flexible and convenient alternative to a basal-bolus insulin therapy that requires at least four daily injections.

Four weeks after the start of treatments, the mean HbA1c values improved remarkably in both groups. It cannot be denied that there might have been carry-over effect of hospitalization therapy. However, it is thought to be an indisputable fact that thrice-daily, mealtime lispro 50/50 therapy itself contributed to improvement of glycemic control, because the mean HbA1c values were further improved in both groups at 48 weeks. After 48 weeks, the reduction in the mean HbA1c value was significantly greater in the 50/50 group than in the 70/30 group. The reduction of postprandial blood glucose levels led to a significant reduction in mean HbA1c at 48 weeks in the 50/50 group, because fasting blood glucose levels did not differ significantly between the two groups at 48 weeks. The lower mean HbA1c value in the 50/50 group might have been due to the larger dose of insulin used at the start of treatment in this group compared with the 70/30 group. The difference in dose may have occurred because both preprandial and postprandial blood glucose levels were targeted in the 50/50 group, and only preprandial blood glucose levels such as fasting blood glucose and pre-supper blood glucose were targeted in the 70/30 group. After 48 weeks, the daily insulin doses in the 50/50 group decreased, whereas those in the 70/30 group increased. This daily insulin dose result is probably attributable to erasure of insulin resistance following improvement in blood glucose control, leading to reduced insulin requirements.

Body weight gain with intensive insulin therapy is one of the main expected side effects often observed. In this study, the increase in BMI was not significantly observed during the observation period in the 50/50 group and the changes in BMI were not significantly different between the two groups, although the daily of insulin doses at the start of treatment for the 50/50 group were significantly larger than those for the 70/30 group.

In this study, the atherosclerotic changes were detected based on IMT of the carotid artery and CAVI, established as surrogate markers for cardiovascular diseases [24–26]. The proportion of habitual smokers, alcohol users, patients in the habit of exercise, and patients taking lipid-lowering agents and antihypertensive agents did not differ between the two groups, although these habits and medications might to affect the results of IMT and CAVI. The IMT represents morphological changes in the local arterial wall, such as plaque formation, stenosis, and wall thickening, while CAVI represents the functional changes in systemic arteries and is generally believed to be impaired in the early stages of atherosclerotic changes. Therefore, the interrelations between IMT and CAVI may be discrepant, although both are recognized as surrogate markers for

cerebrovascular diseases. In the present study, the prevention of increase in CAVI was significantly greater in the 50/50 group compared to the 70/30 group. However, there were no significant differences in the changes in maximum IMT and plaque scores between the two groups. There was a discrepancy in the interrelation between IMT and CAVI. This may have been because glucose intolerance can produce mild atherosclerotic changes, which are expressed as increased arterial stiffness. Multiple regression analysis identified HbA1c as a risk factor for high CAVI [27]. Huang et al. reported a positive correlation between CAVI and post-challenge hyperglycemia [28]. Moreover, it has recently been demonstrated that weight-reduction therapy significantly decreases CAVI in parallel with increasing adiponectin [29]. Thus, there is likely a close relationship between hyperglycemia and increased CAVI. Among the 12 patients for which follow-up measurements of CAVI were performed in the present study, the mean HbA1c decreased significantly in both groups (9.2 ± 1.4 and 10.2 ± 1.6 %, respectively, at baseline, and 6.2 ± 0.5 and 6.6 ± 0.3 %, respectively, at 48 weeks), although the reduction in mean HbA1c was significantly greater in the 50/50 group than the 70/30 group. But there was no significant difference in fasting blood glucose levels between before (127.3 ± 26.6 and 117.6 ± 23.5 mg/dL, respectively) and at 48 weeks (119.4 ± 27.3 vs. 124.0 ± 24.4 mg/dL, respectively). Based on the time course of HbA1c values and fasting blood glucose levels, the reduction of postprandial blood glucose levels might have contributed to the significant differences in the reduction of mean HbA1c values between the two groups. Therefore, CAVI may be useful for detecting the effects of short-term insulin therapy on arterial stiffness. To evaluate the effects on preventing the progression of carotid IMT, longitudinal observations may be necessary. It is known that hyperinsulinemia is associated with adverse cardiovascular risk. It has been reported that insulin stimulates proliferation of arterial smooth muscle cells in culture, which is considered one of the most important initial steps in atherogenesis, and the cumulative dose of regular insulin shows a positive relation with carotid IMT [30, 31]. In the present study, the daily insulin dose in the 50/50 group decreased after 48 weeks, and this may have played an important role in preventing the increase of CAVI. However, the cases in which the follow-up measurements of CAVI, IMT and plaque score were performed before and after treatment were limited in the present study. There were certain limitations of our study that need to be considered, including its retrospective design, the limited number of patients and potential study bias, so-called 'selected bias'. Therefore, at this time, we cannot adopt the finding that thrice-daily lispro 50/50 may prevent the increase of CAVI. In the future, a long-term follow-up should be conducted in a larger number of patients, in order to establish insulin regimens that prevent the progression of atherosclerosis.

The United Kingdom Prospective Diabetes Study suggested that a 1 % decrease in HbA1c was associated with a 21 % reduction in overall diabetic complications risk, including a 14 % reduction in myocardial infarction, a 37 % reduction in microvascular complications, and a 21 % decrease in death [32]. Thus, the 1.12 % HbA1c decrease observed in this study in patients treated with thrice-daily lispro 50/50, if maintained, would be anticipated to reduce the risk of future complications associated with diabetes mellitus. From a practical viewpoint, the results of the present study suggest that anti-diabetic therapy should target not only reducing HbA1c, but also flattening acute glucose fluctuations over the daily period, because glucose variations over time are associated with activation of oxidative stress leading to chronic complications [33].

Based on these results, we recommend thrice-daily mealtime lispro 50/50 as initial insulin therapy for type 2 diabetes mellitus. This insulin analog consists of 50 % insulin lispro and 50 % neutral protamine insulin lispro. This 1:1 ratio of basal to bolus may enable good glycemic control with single insulin therapy.

In this study, the efficacy of thrice-daily lispro 50/50 was comparable to twice-daily aspart 70/30 on HbA1c reduction over a 12-month study period, without increases in body weight. Nevertheless, the effects on glucose fluctuations over a day were more pronounced in the 50/50 group than in the 70/30 group. Thrice-daily lispro 50/50 appeared to be a simple and effective regimen for patients with type 2 diabetes mellitus initiating insulin therapy.

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Conflict of interest The author has no conflicts of interest to disclose.

Human rights statement and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or a substitute for it was obtained from all patients for being included in the study.

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