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ORIGINAL

Comparison of effects of insulin aspart three times a day *versus* insulin detemir once a day on oxidative stress in patients with type 2 diabetes

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Abstract. The main purpose of this study was to investigate whether treatment with long-acting insulin once a day or short-acting insulin three times before each meal daily has a stronger antioxidative effect in patients with type 2 diabetes. These patients had not been treated previously with insulin and were hospitalized for initiation of glycemic control by insulin injection. The patients (n =43) were assigned consecutively and alternately to a group treated with insulin aspart injection three times daily just before each meal and a group treated with insulin detemir injection once daily before bedtime. The results showed that insulin aspart three times a day produced a greater improvement in plasma glucose, and particularly in mean postprandial plasma glucose, compared with insulin detemir once a day (p =0.0006 for comparison of changes between the two insulin treatments). The amount of insulin needed to approach the target levels of plasma glucose was larger in the insulin aspart group (26.0 ± 10.7 U/day *vs*. 13.7 ± 4.9 U/day; p <0.0001). However, only insulin detemir significantly decreased oxidative stress evaluated based on the level of urinary 8-iso-prostaglandin F2α (p =0.0079), although the mechanisms are not fully evident.

Key words: Insulin, Oxidative stress, Type 2 diabetes

RECENT evidence suggests that postprandial plasma glucose (PPG) is more strongly associated with cardiovascular disease than fasting plasma glucose (FPG) in non-diabetic subjects and in patients with type 2 diabetes [1-8]. The reason for this observation is not completely clear, but increased oxidative stress due to glucose fluctuations [9] may at least partially explain the association because oxidative stress has a critical role in the progression of diabetic complications [10-11]. An experimental study also showed that variability in glycemic control due to alternant repetition of high and low glucose concentrations is more deleterious to endothelial cells than a constant high glucose concentration [12]. These facts support the hypothesis that treatment that inhibits postprandial hyperglycemia using short-acting antidiabetic drugs is superior to that which decreases the overall glucose concentration using long-acting antidiabetic drugs because of the expected decrease of oxidative stress.

However, in the recently reported the Hyperglycemia and its Effect After Acute Myocardial Infarction on Cardiovascular Outcomes in Patients With Type 2 Diabetes Mellitus (HEART2D) trial, the risk of cardiovascular disease was similar between the prandial strategy of targeted control of postprandial glycemia by administration of three daily doses of short-acting insulin at meal times and the basal strategy of targeting fasting/interprandial glycemia by administration of long-acting insulin once or twice daily in patients with type 2 diabetes after acute myocardial infarction [13]. Insulin itself also has antiinflammatory and antioxidative effects [14-18]. Therefore, if insulin is used as an antidiabetic drug, the antioxidative effect of long-acting insulin once daily may at least not be clinically inferior to that of short-acting insulin three times daily, even though long-acting insulin cannot fully inhibit postprandial glycemic excursions. This may in part explain the results of the HEART2D trial.

As far as we are aware, the relative antioxidative effects of long-acting insulin once daily and short-act-

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ing insulin three times daily before each meal have not been compared in patients with type 2 diabetes. Therefore, in the current study, we investigated this issue using urinary 8-iso-prostaglandin F2α (8-iso-PGF2α) as a surrogate marker of oxidative stress [19] in patients with type 2 diabetes who were hospitalized to achieve better glycemic control with newly initiated insulin therapy.

Patients and Methods

Patients

A total of 43 consecutive type 2 diabetic patients who had not been previously treated with insulin and were admitted to our hospital for initiation of glycemic control by insulin injection were recruited prospectively from September 2009 to July 2011. Induction of insulin injection therapy was considered for patients who could not achieve HbA1c <8% despite treatment with oral antihyperglycemic agents (OHAs) as monotherapy or in combination with other therapy and for patients with HbA1c <8% who chose insulin injection therapy (n =2 in the insulin aspart group, n =1 in the insulin detemir group). HbA1c ≥8% is defined as "poor control" by the Japan Diabetes Society [20]. Clinical and laboratory tests showed no evidence of moderate or severe liver dysfunction, infectious disease or autoimmune disease in any patients at the time of hospitalization. Patients with serum creatinine levels >1.5 mg/dL were not included in the study. The characteristics and laboratory data at admission are shown in Table 1.

Methods

The 43 patients were assigned consecutively and alternately to a group treated with insulin aspart (Novorapid®: short-acting insulin) injection three times daily just before each meal and a group treated with insulin detemir (Levemir®: long-acting insulin) injection once daily before bedtime. Most patients had received OHAs including sulfonylureas (SUs) before insulin therapy. Insulin-secretory drugs [SUs, glinides and dipeptidyl peptidase (DPP)-4 inhibitors] were discontinued after initiation of insulin therapy, but other OHAs (metformin, thiazolidinediones; TZD, α-glucosidase inhibitors; αGI) were continued. All patients remained hospitalized during the observation period and there were no changes in drug administration except for antidiabetic drugs in this period.

Insulin therapy was started after hospitalization. In both groups, the initial total daily dose of insulin was approximately 0.2 U × body weight. This dose was subsequently adjusted. The target levels of glucose with insulin therapy were a mean postprandial plasma glucose (PPG) (2 hours after breakfast, lunch, and dinner) level of 180 mg/dL in the insulin aspart group, and a fasting plasma glucose (FPG) level of 130 mg/dL in the insulin detemir group, because FPG <130 mg/dL and 2-hour PPG <180 mg/dL are defined as "good control" by the Japan Diabetes Society [20]. The mean observation period after initiation of insulin therapy was approximately 10 days: 9.6 ± 2.5 days in the insulin aspart group and 10.4 ± 1.9 days in the insulin detemir group, with no significant difference between these periods.

Blood and urine tests were performed at the beginning of and after the observation period. Collection of blood (except for blood sampling for measurement of plasma glucose) and urine, and measurement of blood pressure (BP) and body weight in underwear were performed from 6:00 to 7:00 a.m. after overnight fasting for at least 10 hours. Plasma glucose was measured 7 times a day (before and 2 hours after each meal, and at bedtime). After blood was collected, it was immediately placed into specific test tubes for different assays and then rapidly centrifuged at 1,500 rpm for 5 minutes to separate the serum or plasma from the clot-containing blood cells.

Measurement of plasma glucose, hemoglobin (Hb) A1c, glycoalbumin (GA) and serum lipids

Plasma glucose (PG) was evaluated immediately after blood collection using the automated glucose oxidase method (Glucose Auto Stat GA1160®; Arkray, Kyoto, Japan). For measurement of HbA1c, blood was collected in a test tube containing EDTA-2K. HbA1c was measured immediately after blood collection using high-performance liquid chromatography (HPLC; Hi-Auto A1c®, HA8150®; Arkray). Only HbA1c is detected with this method and the normal range is 4.3% to 5.8% [Japan Diabetes Society (JDS) values]. GA was measured by HPLC using a kit (Lucica GA-L®, Asahi Kasei Co., Tokyo, Japan). Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and serum triglyceride (TG) were measured using enzymatic assays. Determiner L TC II and Determiner L TG reagents (Kyowa Medics, Tokyo, Japan) were used in measurements of TC and TG, respectively. HDL-C

Table 1 Clinical characteristics of the patients treated with insulin aspart and insulin detemir

	Insulin aspart	Insulin detemir	*p*
No. (male/female)	21 (11/10)	21 (7/14)	—
Age (year)	63.3 ± 6.6	65.1 ± 13.7	0.5804
Duration of diabetes (year)	9.3 ± 6.3	10.5 ± 8.9	0.6265
Treatment duration (day)	9.6 ± 2.5	10.4 ± 1.9	0.5339
BMI (kg/m^2)	23.8 ± 3.1	23.7 ± 4.7	0.9258
FPG (mg/dL)	189.6 ± 64.5	186.4 ± 68.3	0.8662
Mean PPG (mg/dL)	297.4 ± 76.8	288.9 ± 63.0	0.6984
Mean PG (mg/dL)	261.9 ± 66.4	258.5 ± 62.9	0.8630
PG-SD (mg/dL)	56.8 ± 18.4	53.3 ± 15.6	0.5080
HbA1c (%)	9.6 ± 1.5	10.1 ± 2.0	0.4464
GA (%)	29.9 ± 8.1	33.0 ± 10.2	0.2817
TG (mg/dL)	143.2 ± 70.8	142.1 ± 66.2	0.9590
HDL-C (mg/dL)	45.6 ± 12.4	48.5 ± 16.1	0.5223
LDL-C (mg/dL)	117.4 ± 39.0	109.9 ± 34.5	0.4670
SBP (mmHg)	123.2 ± 17.7	121.8 ± 17.0	0.7840
DBP (mmHg)	73.8 ± 10.3	71.8 ± 14.8	0.6231
Urinary 8-iso-PGF2α (pg/g.Cr)	194.8 ± 80.0	224.2 ± 67.3	0.2041
FMD (%)	10.0 ± 6.0	7.2 ± 4.6	0.1462
Diabetic therapy			
SU	19	20	—
(SU1/SU2/SU3/SU4/SU5)	7/3/4/4/1	5/5/5/5/0	—
Glynides	1	0	—
Metformin, TZD, DPP-4 inhibitors	0	1	—
Diet alone	1	0	—
Antihypertensive drugs			
(A/B/C/AC/ABC/AB/ACT/none)	3/0/0/0/0/1/1/15	1/0/1/2/1/1/0/13	—

Data are expressed as mean ± standard deviation (SD), Comparison in variables between two groups were made by use of an unpaired *t* test. *p*: *p* value, $p < 0.05$ are defined as statistical significance. BMI: body mass index, FPG: fasting plasma glucose, Mean PG: mean of PG of 7 times (before and 2hour-after each meal, and bedtime) a day, Mean PPG: mean of postprandial glucose (PPG)(2hour-after each meal), PG-SD: SD of 7 times PG, HbA1c: hemoglobin A1c; GA: glycoalbumin, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure, BW: body weight, 8-iso-PGF2α: 8-iso-prostaglandin F2α, FMD: flow mediated vasodilation, Diabetic therapy: the number of patients who received diabetic therapy; SU: sulfonylurea; Glinides: only nateglinide was used; TZD: thiazolidinediones: only pioglitazone was used; DPP-4 inhibitors: dipeptidyl depeptidase 4 inhibitors: only sitagliptin was used; α-GI: α glucosidase inhibitor; SU1:SU alone; SU2: SU and metformin; SU3: SU and α-GI; SU4: SU, α-GI and metformin and/or TZD; SU5: SU and DPP-4 inhibitors; Antihypertensive drugs: the number of patients treated with antihypertensive drugs; A: angiotensin-II receptor blocker (ARB); B: beta-blocker; C: calcium channel blocker (Ca); T: thiazide

was measured directly by a method based on selective solubilization of different lipoproteins by proprietary detergents using Cholestest N HDL-C (Daiichi Pure Chemicals, Tokyo, Japan). LDL-C was also measured directly by a homogenous method using Cholestest LDL (Daiichi Pure Chemicals), rather than indirectly using the Friedwald equation.

Urinary 8-iso-PGF2α assay

Urinary 8-iso-PGF2α was measured using first morning urine with an enzyme immunoassay (EIA) kit (ACE® EIA; Cayman Chemical Company, Ann Arbor, MI) with intra- and interassay CVs of less than 10%, based on actual values. This assay has been reported to show no differences between measurements of morning urine samples and urine samples stored for 24 hours [21]. Data were adjusted by urine creatinine concentrations. It is reported that urinary 8-iso-PGF2α levels can change by short-term treatment such as 1-2 hours [22]. We also measured urinary 8-iso-PGF2α in 8 non-diabetic healthy men (mean age 39.7 ± 9.7 years old) as a control. The mean urinary 8-iso-PGF2α level in the controls was 137.8 ± 59.2 mg/g.Cr.

Measurement of flow-mediated vasodilation (FMD)

FMD was measured as previously described [23] in the morning after fasting on the same day that the blood and urine tests were performed. Measurement of FMD

was performed in 15 patients in the insulin aspart group and in 17 patients in the insulin detemir group.

Ethical considerations

The study was performed according to the guidelines of the Declaration of Helsinki and all subjects gave written informed consent to their inclusion in the study. The study was approved by the ethical committee of Dokkyo Medical University Koshigaya Hospital.

Statistical methods

All data are presented as means ± standard deviation (SD). The two time points for each parameter for an individual were compared using a paired *t* test, Comparisons between the two groups were made using an unpaired *t* test for normally distributed data (confirmed by a χ^2 test); a Student *t* test or a Welch *t* test was chosen based on the homogeneity of variance calculated by an F test. Changes in each parameter between the insulin aspart and insulin detemir groups were compared by Student *t* test.

A *p* value of less than 0.05 was accepted as indicating statistical significance.

Results

The study was completed by 42 patients. One patient treated with insulin detemir was dropped from the study because it was discovered after hospitalization that this patient had chronic hepatitis C. The final insulin doses were 26.0 ± 10.7 (6 to 48) U/day in the insulin aspart group and 13.7 ± 4.9 (4 to 22) U/day in the insulin detemir group ($p < 0.0001$).

In the insulin aspart group, there was a significant decrease of mean PPG (mean of 2-hour PPG after breakfast, lunch, and dinner) and a significant decrease of FPG and mean PG (mean of PG at 7 time points: before and 2 hours after each meal, and at bedtime). In the insulin detemir group, there was a significant decrease of FPG. Insulin detemir also significantly decreased the mean PPG and mean PG, but to a lesser extent than insulin aspart. Data for PG in the two groups are shown in Fig. 1A. There was a tendency for a decrease in PG-SD (SD of PG at 7 times) in the insulin aspart group, whereas insulin detemir did not change PG-SD. Mean amplitude of glycemic excursions (MAGE) at 7 times points a day was defined by the following formula; $\Sigma \lambda/x$ [λ: glycemic extent of fluctuation exceeding 1 SD (=absolute value of the difference between variables and 1 SD when variables exceed 1 SD), x: the number of variables showing glycemic extent of fluctuation exceeding 1 SD]. There was a slight tendency for a decrease in MAGE in the insulin aspart group, while no significant change in MAGE was observed in the insulin detemir group (Fig. 1B).

GA, fasting TG, and LDL-C levels were significantly reduced in both groups, and there was a weak but significant decrease of diastolic BP in the insulin aspart group. There was no significant change in body weight during the observation period in either group. There was no change in FMD in the insulin aspart and insulin detemir groups. Urinary 8-iso-PGF2α levels at baseline (before insulin treatment) in both groups were significantly higher than those in healthy subjects (both $p < 0.05$). There was a significant decrease of urinary 8-iso-PGF2α in the insulin detemir group, but no change in the insulin aspart group over the study period (Fig. 2). In a subgroup of insulin aspart group treated with final total daily dose of insulin ≤22 U/day (n =10), which was maximum dose in insulin detemir group, urinary 8-iso-PGF2α levels were respectively 200.5 ± 95.4 and 232.8 ± 109.1 pg/g.Cr before and after insulin therapy ($p = 0.0541$). There was no significant difference in final total daily dose of insulin between the entire insulin detemir group and this subgroup treated with insulin aspart (13.7 ± 4.9 *vs*. 16.5 ± 4.7 U/day, $p = 0.1442$). There were significant differences in mean PPG, mean PG, and urinary 8-iso-PGF2α between the insulin aspart and insulin detemir groups. These results are summarized in Table 2.

For FMD and urinary 8-iso-PGF2α, we also performed repeated measures analysis of variance (ANOVA) between the insulin aspart and insulin detemir groups. There was no difference in FMD between the two groups ($p = 0.5117$) and no interaction between the two types of insulin and FMD values ($p = 0.3523$). For urinary 8-iso-PGF2α, this analysis could not be performed adequately because of an interaction between the two types of insulin and 8-iso-PGF2α values ($p = 0.0030$).

There was no significant correlation between urinary 8-iso-PGF2α and FPG before and after insulin therapy in the insulin aspart group (r =0.2874, $p = 0.2064$; r =0.0080, $p = 0.9723$), the insulin detemir group (r =0.3873, $p = 0.0827$; r =0.2961, $p = 0.1923$).

There were no correlations between changes in urinary 8-iso-PGF2α and those in FPG, mean PPG, and mean PG before and after insulin therapy in the insu-

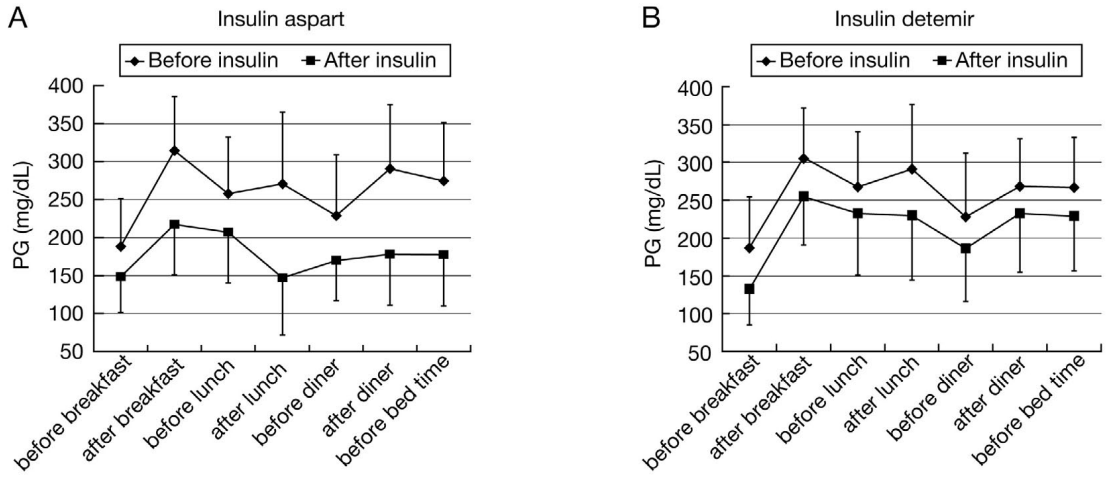

Fig.1A PG measured before and 2 hr after each meal and at bedtime before initiation of insulin therapy and after insulin therapy in insulin aspart groups (A) and in the insulin detemir groups (B)

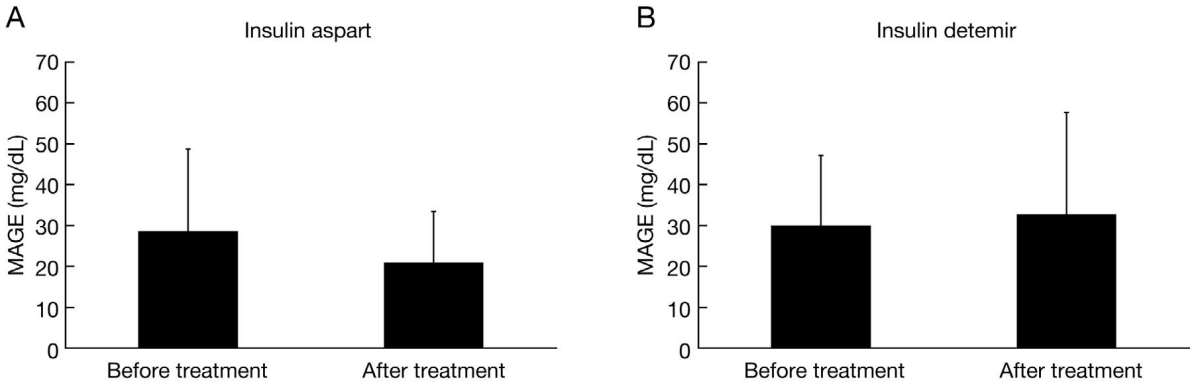

Fig.1B Mean amplitude of glycemic excursions (MAGE) before initiation of insulin therapy and after insulin therapy in insulin aspart groups (A) and in the insulin detemir groups (B)

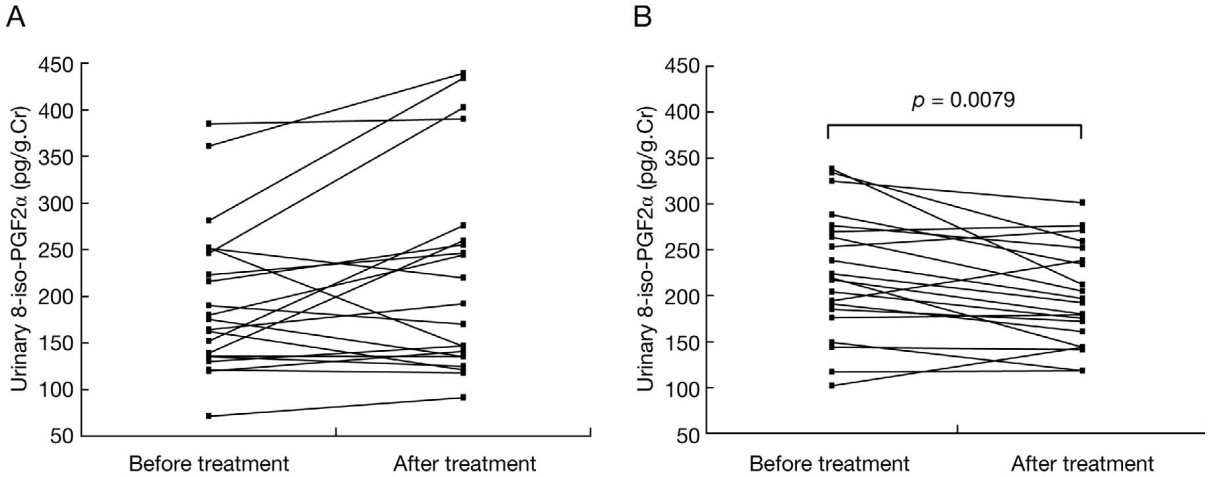

Fig. 2 Effect of insulin aspart (A) and insulin detemir (B) on urinary 8-iso-prostaglandin F2α levels before and after insulin therapy

Table 2 Changes in various variables before and after insulin therapy

	Insulin aspart × 3/day (n =21)			Insulin detemir × 1/day (n =21)			
	Before insulin	After insulin	*p*	Before insulin	After insulin	*p*	p^a
FPG (mg/dL)	189.9 ± 64.5	148.6 ± 47.0	0.0004*	186.4 ± 68.3	131.9 ± 46.6	0.0025*	0.4820
Mean PPG (mg/dL)	301.4 ± 76.5	178.7 ± 57.3	<0.0001*	288.9 ± 63.0	238.4 ± 65.1	0.0003*	0.0006*
Mean PG (mg/dL)	261.9 ± 66.4	178.1 ± 47.8	<0.0001*	258.5 ± 63.0	213.8 ± 57.3	0.0002*	0.0190*
PG-SD (mg/dL)	56.8 ± 18.4	49.1± 17.8	0.0511	53.3 ± 15.6	57.4 ± 23.6	0.3877	0.0550
MAGE (mg/dL)	28.5 ± 20.3	20.7 ± 12.7	0.1424	29.9 ± 17.3	32.6 ± 25.1	0.6138	0.1619
GA (%)	29.9 ± 8.1	26.5 ± 6.1	0.0005*	32.3 ± 10.0	28.4 ± 8.5	0.0001*	0.6409
TG (mg/dL)	145.9 ± 74.0	109.5 ± 49.9	0.0295*	148.6 ± 67.2	107.1 ± 43.6	0.0075*	0.8035
HDL-C (mg/dL)	46.7 ± 12.3	46.5 ± 13.1	0.9200	47.8 ± 17.1	48.2 ± 14.6	0.8469	0.8291
LDL-C (mg/dL)	116.2 ± 41.1	97.4 ± 33.5	0.0314*	111.8 ± 34.3	95.3 ± 23.2	0.0014*	0.8087
SBP (mm Hg)	123.2 ± 17.7	116.2 ± 14.2	0.0519	121.8 ± 16.9	116.3 ± 28.3	0.3325	0.8099
DBP (mm Hg)	73.8 ± 10.3	68.1 ± 9.4	0.0308*	71.8 ± 14.8	66.5 ± 11.4	0.0858	0.9309
BW (kg)	62.4 ± 15.4	61.7 ± 13.9	0.2807	60.1 ± 12.3	59.6 ± 12.9	0.1481	0.8880
FMD (%)	10.0 ± 6.0	11.2 ± 8.0	0.6683 (n =15)	7.2 ± 4.6	11.2 ± 9.6	0.1392 (n =17)	0.3523
8-iso-PGF2α (pg/g.Cr)	194.8 ± 79.6	223.2 ± 111.3	0.0676	224.2 ± 67.6	198.5 ± 53.4	0.0079*	0.0034*

p: *p* value for change before and after therapy by paired *t* test. $p < 0.05$ are defined as statistically significant (*). Data are expressed as means ± SD. The mean observation-period was 9.6 ± 2.5 days in the insulin aspart group and 10.4 ± 1.9 days in the insulin detemir group, with no significant difference between these periods. p^a: comparison of changes between the insulin aspart and insulin detemir groups was performed by Student *t* test

lin aspart group (r =-0.0231, p =0.9208; r =0.0671, p =0.7786; r =0.0057, p =0.9803, respectively) and the insulin detemir group (r =0.2395, p =0.2956; r =-0.3833, p =0.0863 r =-0.2275, p =0.3213, respectively). There were also no correlations between changes in urinary 8-iso-PGF2α and those in FMD in the insulin aspart (r =0.2769, p =0.3175) and insulin detemir (r =-0.1528, p =0.5582) groups.

Discussion

In the current study, administration of insulin aspart was more effective than insulin detemir for improving postprandial glucose (PG), as expected. In addition, PG-SD, which reflects glucose variability, showed a tendency for improvement in the insulin aspart group, but was unaffected by insulin detemir. Interestingly, insulin aspart significantly decreased not only PPG but also FPG and fasting TG. As a result, insulin aspart decreased the mean PG more effectively than insulin detemir, which indicates better overall glycemic control with insulin aspart than with insulin detemir. However, the effect of insulin detemir on GA was not inferior to that of insulin aspart. Improvement of FPG has been suggested to be more important than that of PPG for obtaining better glycemic control in patients who initially have relatively poor glycemic control [24] and this may explain the similar effects of the two insulins on GA. Despite the induction of insulin therapy, there was no increase in body weight in either group. However, this was probably due to the relatively short observation period and the improvement of diet by hospitalization.

We found a significant decrease of urinary 8-iso-PGF2α, a reliable systemic oxidative stress marker [19], only in the insulin detemir group, even though insulin aspart gave better glycemic control, especially of postprandial glucose. Glucose variability is an important risk factor for increased systemic oxidative stress [9], but insulin aspart was unable to reduce urinary 8-iso-PGF2α. It is difficult to account for these results, but it has been suggested that insulin itself has antiinflammatory and antioxidative effects [14-18]. Also, in our previous study, we found that initiation of insulin therapy significantly reduced high sensitivity C reactive protein in patients with type 2 diabetes [25]. The blood lifetimes of insulin aspart and insulin detemir are approximately 3 to 4 hours and 24 hours, respectively, which indicates that the full effect of insulin aspart given 3 times a day is not present for more than half of each day [26-28]. This may at least partially explain the different results for the two insulins. Interestingly, Monnier *et al*. recently reported an association of glucose variability and oxidative stress evaluated by urinary 8-iso-PGF2α in patients with type 1 diabetes, type 2 diabetes treated with an OHA, and type 2 diabetes treated with an OHA and insulin [29]. Despite the similar levels of HbA1c (8-9%) in the three groups, MAGE, which

reflects glucose variability, was largest in patients with type 1 diabetes and smallest in those with type 2 diabetes treated with an OHA. However, urinary 8-iso-PGF2α in the patients with type 2 diabetes treated with an OHA was higher than in the other two groups. This suggests that the effect of insulin itself has a stronger influence on inhibition of oxidative stress than the lower glucose variability. Monnier *et al*. also found that addition of insulin, but not metformin, significantly decreased urinary 8-iso-PGF2α in patients with type 2 diabetes. Notably, all patients were treated with basal insulin (i.e., long-acting insulin) or basal bolus insulin (i.e., long-acting insulin and short-acting insulin), and the results in Monnier *et al*. are supportive of those in the current study, although the decrease in urinary 8-iso-PGF2α was smaller in our study. This might be due to differences in patient background, including ethnicity and body mass index (BMI).

The administered dose of insulin may also explain the difference between the effects of the two insulins in this study. The dose of insulin aspart (26.0 ± 10.7 U/day) was larger than that of insulin detemir (13.7 ± 4.9 U/day). Very recently, Monnier *et al*. found that the clinical antioxidative effect of insulin is attenuated at a relatively high dose (>0.4 U/kg/day)[30], although the patients' background was different compared with that in this study. This may also explain why insulin aspart did not significantly decrease urinary 8-iso-PGF2α. However, insulin aspart treatment did not decrease urinary 8-iso-PGF2α levels even in a subgroup of patients treated with final total daily dose of insulin ≤22 U/day, which corresponded to maximum dose of insulin detemir. Therefore it may be unlikely that the antioxidative effect of insulin detemir is explained only by the issue of dose of insulin.

In addition, the mean bed-time PG in the insulin detemir group was >220 mg/dL and it is likely that patients in this group might have had nocturnal urination. Urinary 8-iso-PGF2α was measured in first morning urine samples, and these samples may not have reflected whole-body production of urinary 8-iso-PGF2α at night in the insulin detemir group. In contrast, both bed-time and fasting PG were <180 mg/dL in the insulin aspart group. This difference could also explain the negative results for insulin aspart in this study. However, this is unlikely since urinary 8-iso-PGF2α did not correlate with FPG in the insulin aspart and insulin detemir groups before and after insulin therapy.

Taken together, mechanisms of antioxidative effect by insulin detemir observed in this study may be complex, and other unknown mechanisms may also be involved.

Regarding FMD, which reflects vascular endothelial function, we found no significant increase in the insulin aspart and insulin detemir groups, despite the improvement of glycemic control in both groups. However, this result was expected because the diabetic duration of the patients was relatively long and most may have developed systemic atherosclerosis to some degree.

The recent HEART2D trial showed that the risk of cardiovascular disease was similar in patients with type 2 diabetes after acute myocardial infarction who were treated with a prandial strategy targeting control of postprandial glycemia with short-acting insulin three times daily before each meal or a basal strategy targeting fasting/short interprandial glycemia with long-acting insulin given once or twice daily [13]. This suggests that a basal strategy is at least not clinically inferior to a prandial strategy in this population, even though the basal strategy cannot fully improve postprandial hyperglycemia. We speculate that the probable antioxidative effect of long-acting insulin contributed to the non-inferiority of long-acting insulin compared with short-acting insulin in the HEART2D trial.

The current study has several limitations. First, the number of patients was relatively small, especially for measurement of FMD. Second, we used a protocol in which insulin was started after discontinuation of SU, glinides, and DPP-4 inhibitors, in order to decrease the influence of exogenous insulin. However, it may also have been important to investigate the effect of insulin add-on therapy on oxidative stress. Third, it would have been interesting to explore the effect of concomitant use of insulin aspart and insulin detemir (i.e., basal bolus insulin therapy). In addition, ideally, treatment-duration should have been completely matched in all patients. Furthermore, regarding measurements of urinary 8-iso-PGF2α, we used morning urine samples. However it would have been better if the measurements were performed also in 24h-stored urine samples, although it is reported that there was no difference between measurements of morning urine samples and urine samples stored for 24 hours [21], as described in method section. Finally, the observation period in this study was relatively short, and the longer-term effects of insulin aspart and insulin detemir on oxidative stress require evaluation in a further study.

In conclusion, in the current study in patients with type 2 diabetes, insulin aspart given 3 times a day (before each meal) was more effective for improving PG, and particularly postprandial PG and glucose variability in a day, compared with insulin detemir given once a day (before bedtime). The amount of insulin needed to approach the target levels of PG was significantly larger in the insulin aspart group. However, only insulin detemir significantly decreased oxidative stress evaluated by urinary 8-iso-PGF2α, although the mechanisms are not fully evident.

Disclosure

The authors have nothing to disclose.

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