

## COMPARATIVE ANALYSIS OF LONG-ACTING INSULINS: INSULIN DEGLUDEC VS. NPH INSULIN IN TYPE 1 DIABETES

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### Article Info

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### Abstract

Type 1 diabetes mellitus (T1DM) is a condition characterized by the autoimmune or idiopathic destruction of pancreatic  $\beta$ -cells, resulting in insulin deficiency. To manage T1DM, patients require insulin replacement therapy, with basal-bolus insulin injection being the mainstay of treatment. While Neutral Protamine Hagedorn (NPH) insulin is an intermediate-acting insulin commonly used as basal insulin, insulin glargine, a long-acting insulin analog, has proven to be more effective as a basal insulin compared to NPH insulin. As a result, long-acting insulin is now the preferred choice for basal insulin in basal-bolus insulin therapy. However, even with the use of long-acting insulin, blood glucose control remains suboptimal.

This study aims to address the issue of suboptimal blood glucose control in T1DM patients by exploring alternative insulin therapies. The effectiveness of long-acting insulin analogs will be evaluated in comparison to NPH insulin, and their impact on blood glucose management will be assessed.

### 1. Introduction

Type 1 diabetes mellitus (T1DM) is characterized by insulin deficiency because of autoimmune or idiopathic destruction of pancreatic  $\beta$ cells (Ikegami et al., 2011). Patients with T1DM are dependent on insulin replacement therapy, and the mainstay of insulin therapy is basal-bolus insulin injection (American Diabetes Association, 2013). Neutral protamine Hagedorn (NPH) insulin is intermediate-acting insulin mainly used as basal insulin. Insulin glargine, a long-acting insulin analog, was developed later and is more useful basal insulin compared with NPH insulin (Ratner RE et al., 2000; Albright ES et al., 2004). Now, long-acting insulin is usually used as basal

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insulin in basal-bolus insulin therapy. However even basal-bolus insulin therapy with long-acting insulin cannot control blood glucose perfectly.

Insulin degludec is newly developed ultra-long-acting (basal) insulin analog and has a longer duration of action than insulin glargine (Wang F et al., 2012). Some clinical reports show that insulin degludec has non-inferior blood glucose lowering effect and reduces frequency of hypoglycemia compared with insulin glargine in patients with T1DM (Birkeland KI et al., 2011; Bode BW et al., 2013; Vora J et al., 2014). Recently, severe hypoglycemia is known to be associated with coronary artery calcification and repeated episodes of hypoglycemia are an aggravating factor for preclinical atherosclerosis in T1DM patients (Fährmann ER et al., 2015; Giménez M et al., 2011). Insulin degludec is expected to improve coronary atherosclerosis not only by controlling blood glucose but also by reducing the frequency of hypoglycemia, but the efficacy of insulin degludec on the artery is unknown.

Prevention of macrovascular complications is very important for the treatment of diabetes. Arterial stiffness is a useful surrogate marker of atherosclerosis. Brachial-ankle PWV (baPWV) has been used to evaluate arterial stiffness or atherosclerosis in diabetic patients. An arterial stiffness parameter called cardio-ankle vascular index (CAVI) was developed as a marker of arteriosclerosis involving the aorta, femoral artery and tibial artery (Shirai K et al., 2006). CAVI is measured from an electrocardiogram, phonocardiogram, brachial artery waveform and ankle artery waveform, and is adjusted for blood pressure based on the stiffness parameter  $\beta$  (Takaki A et al., 2007). CAVI is independent of blood pressure and has adequate reproducibility for clinical use, whereas baPWV is dependent on blood pressure (Shirai K et al., 2006).

Although arterial stiffness can be evaluated by measuring either baPWV or CAVI, CAVI is superior to baPWV as an index of arterial stiffness in patients who have undergone coronary angiography (Takaki A et al., 2007). CAVI is also superior to intima-media thickness (IMT) for predicting coronary atherosclerosis (Nakamura K et al., 2008). Some hypoglycemic agents including insulin improve CAVI within 6 months in several clinical studies (Nagayama D et al., 2010; Ohira M et al., 2011; Ohira M et al., 2014). CAVI is a very useful marker for evaluating the effect on the artery in diabetic patients.

## **2. Aim**

In the present study, we investigated the effects of insulin degludec on blood glucose control, oxidative stress, and CAVI in T1DM patients.

## **3. Subjects and methods**

### **3.1. Subjects**

The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of Sakura Hospital Toho University Medical Center (No. 2012-113). Before participation, the purpose of the study was explained to each subject, and consent was obtained for both participation in the study and for release of the study data.

A randomized open-label study was conducted at Sakura Hospital Toho University Medical Center. We enrolled 28 patients with T1DM, whose glycosylated hemoglobin (HbA1c) was over 7.0% and steady for 3 months. At baseline, all subjects were treated with ultra-rapid-acting insulin before each meal and once daily insulin glargine. We divided the patients into 2 groups by simple randomization using the envelope method. One group was switched from insulin glargine to the same dosage of insulin degludec (degludec group, n = 14), and the other group continued insulin glargine (glargine group, n = 14). Table 1 shows the clinical characteristics of the subjects at baseline.

The subjects were observed for 6 months after registration in this study, and the following parameters were measured before the study and after 6 months: body weight (BW), body mass index (BMI), fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), serum total cholesterol level (TC), serum triglycerides level (TG), serum high-density lipoprotein-cholesterol level (HDL-C), serum low-density lipoprotein-cholesterol level (LDL-C), lipoprotein lipase mass levels in preheparin serum (preheparin LPL mass), serum diacron-reactive oxygen metabolites (d-ROMs) and CAVI. Serum C-peptide response (CPR) was measured only once at baseline. During this study, all patients maintained the same diet and exercise therapies and did not change medication except basal insulin. Basal insulin dose was adjusted based on frequency of hypoglycemia. When hypoglycemic episodes were recognized more than three times per week, the basal insulin dose was decreased by 20%. All subjects received nutritional guidance from a dietitian every month. The dietitian analyzed the meal contents and suggested changes if necessary.

### 3.2. Body weight measurement and blood sampling

Body weight was measured and blood samples were collected in the morning after 12 hours of fasting. Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA), and plasma was separated within 1 h for measurements of HbA1c and lipids. Blood was also collected in plain test tube, and serum was separated for measurement of preheparin LPL mass.

### 3.3. Measurement of HbA1c and plasma lipid concentrations

The stable and unstable fractions of HbA1c were measured by high-pressure liquid chromatography method using the Hi-Auto A1c kit (Kyoto Daiichi Kagaku, Kyoto, Japan). Data of the stable form were used in the present analysis. The data of HbA1c was expressed according to the National Glycohemoglobin Standardization Program (NGSP) (Hoelzel et al., 2004).

Plasma TC and TG levels were measured enzymatically using kits from Nippon Shoji Co., Ltd. (Osaka, Japan) and a Hitachi 7150 analyzer (Hitachi, Ltd., Tokyo, Japan). HDL-C level was measured using a selective inhibition assay (Daiichi Pure Chemicals Co., Ltd, Tokyo) (Shirai K et al., 1992). Plasma LDL-C level was calculated using the Friedewald formula.

### 3.4. Assay of preheparin LPL mass

Serum preheparin LPL mass was measured with a sandwich enzyme-linked immunosorbent assay (ELISA) using a specific monoclonal antibody against LPL (Daiichi Pure Chemicals, Japan), as described previously (Kobayashi J et al., 1993). The linearity and the coefficient variation of this method were described in our previous report (Ohira M et al., 2011).

### 3.5. Measurement of d-ROMs

Measurement of d-ROMs was performed using the Free Radical Analytical System 4 (FRAS 4; Wismerll Co. Ltd. Tokyo, Japan). The normal range is between 250 and 300 U.CARR (Carratelli Units), where 1 U.CARR corresponds to 0.8 mg/L H<sub>2</sub>O<sub>2</sub>. Values higher than 300 U.CARR suggest increased oxidative stress. The d-ROMs test was conducted according to the methods described previously (Wakabayashi T et al., 2014).

### 3.6. Measurement of CAVI

CAVI is obtained by measuring blood pressures and pulse wave velocity (PWV) according to the following formula:  $CAVI = a\{(2p/\Delta P) \times \ln(Ps/Pd)PWV^2\} + b$ , where Ps is systolic blood pressure; Pd is diastolic blood pressure; PWV is pulse wave velocity;  $\Delta P$  is Ps - Pd;  $\rho$  is blood density, and a and b are constants. The details of CAVI and the measurement are described in our previous reports (Shirai K et al., 2006; Ohira M et al., 2011).

In the present study, CAVI was measured using a VaSera CAVI instrument (Fukuda Denshi Co., Ltd., Tokyo, Japan) as described previously (Shirai K et al., 2006). Systolic and diastolic blood pressures were measured at the time of CAVI measurement.

### 3.7. Statistical analysis

Data are expressed as mean  $\pm$  S.D. Normal distribution was tested using the Shapiro-Wilk test. Some data were not normally distributed, and normality was obtained by logarithmic transformation.

Statistical analysis was performed using Student's t-test and ANOVA. All analyses were performed using JMP computer software version 9.0 (SAS, Cary, NC, USA). P values  $< 0.05$  were considered significant.

## 4. Results

### 4.1. Baseline characteristics in degludec and glargine groups

The mean age, FBG and HbA1c were apparently higher in the degludec group than in the glargine group, but the differences were not significant. Fasting serum CPR was low in both groups. Dosages of ultra-rapid insulin and insulin glargine at baseline were almost the same in these two groups. Other parameters were almost identical in the two groups (Table 1).

**Table 1. Comparison of baseline characteristics in the two groups**

|                                          | Degludec group      | Glargine group     | P value |
|------------------------------------------|---------------------|--------------------|---------|
| No. of subjects (male/female)            | 14(4/10)            | 14(7/7)            | 0.2620  |
| Age (years)                              | 55.69 $\pm$ 15.01   | 50.71 $\pm$ 16.68  | 0.4055  |
| duration of type 1 diabetes (years)      | 14.71 $\pm$ 10.41   | 12.79 $\pm$ 7.13   | 0.5723  |
| BW (kg)                                  | 58.16 $\pm$ 10.30   | 60.14 $\pm$ 10.12  | 0.6112  |
| BMI (kg/m <sup>2</sup> )                 | 23.07 $\pm$ 3.64    | 22.99 $\pm$ 3.83   | 0.9561  |
| FBG (mg/dl)                              | 235.00 $\pm$ 95.89  | 199.21 $\pm$ 63.33 | 0.2545  |
| HbA1c (%)                                | 9.61 $\pm$ 1.21     | 8.84 $\pm$ 1.19    | 0.1036  |
| serum CPR (ng/ml)                        | 0.02 $\pm$ 0.06     | 0.05 $\pm$ 0.108   | 0.3282  |
| TC (mg/dl)                               | 214.93 $\pm$ 33.09  | 201.00 $\pm$ 27.99 | 0.2401  |
| TG (mg/dl)                               | 118.43 $\pm$ 107.83 | 123.07 $\pm$ 88.33 | 0.5971  |
| HDL-C (mg/dl)                            | 67.21 $\pm$ 16.07   | 66.07 $\pm$ 20.08  | 0.8692  |
| LDL-C (mg/dl)                            | 119.14 $\pm$ 19.52  | 108.36 $\pm$ 21.90 | 0.1806  |
| preheparin LPL mass (ng/ml)              | 76.10 $\pm$ 25.51   | 77.31 $\pm$ 27.58  | 0.9052  |
| d-ROM (U.CARR)                           | 367.86 $\pm$ 71.36  | 361.86 $\pm$ 60.19 | 0.8118  |
| Dosage of ultra-rapid-acting insulin (U) | 19.21 $\pm$ 6.54    | 21.93 $\pm$ 6.32   | 0.2743  |
| Dosage of insulin glargine (U)           | 12.57 $\pm$ 4.29    | 13.86 $\pm$ 4.75   | 0.4593  |

Data are presented as mean  $\pm$  S.D. BW; body weight, BMI; body mass index, FBG fasting blood glucose, HbA1c; glycosylated hemoglobin, CPR; C-peptide response, TC; total cholesterol, TG; triglycerides, HDL-C; high density lipoprotein cholesterol, LDL-C; LDL-cholesterol, preheparin LPL mass; lipoprotein lipase mass levels in preheparin serum, d-ROMs; diacron-reactive oxygen metabolites.

### 4.2. Comparisons of changes in clinical parameters after 6 months in two groups

Mean body weight and BMI changed slightly after 6 months in both groups, but there were no significant differences between two groups. Switching from insulin glargine to insulin degludec significantly reduced FBG and HbA1c after 6 months (FBG:  $-95.57 \pm 93.06$  mg/dl,  $P < 0.005$ , HbA1c:  $-0.73 \pm 0.53$  %,  $P < 0.0005$  in degludec group). FBG and HbA1c were significantly reduced in degludec group compared with glargine group (FBG:  $-95.57 \pm 93.06$  mg/dl vs  $+23.21 \pm 70.44$  mg/dl,  $P < 0.01$ ; HbA1c:  $-0.73 \pm 0.53$  % vs  $-0.03 \pm 0.75$  %,  $P < 0.01$ ). Mean preheparin LPL mass changed slightly after 6 months in degludec group, but the changes in preheparin LPL mass from baseline were not significantly different between two groups. Diacron ROMs changed slightly both in

degludec and glargine groups, but the changes from baseline were almost the same in the two groups. Except FBG and HbA1c in degludec group, all other clinical parameters were not significantly different at 6 months compared to baseline in both groups. The changes in these clinical parameters from baseline were not significantly different between two groups (Table 2).

Hypoglycemia occurred in some patients. Other drug-related adverse effects were not observed in any of the patients.

**Table 2. Comparisons of the changes in clinical parameters after 6 months in the two groups**

|                              | Change from baseline |                | P value |
|------------------------------|----------------------|----------------|---------|
|                              | Degludec group       | Glargine group |         |
| ΔBW (kg)                     | +0.90±3.68           | +0.96±1.75     | 0.9586  |
| ΔBMI (kg/m <sup>2</sup> )    | +0.32±1.42           | +0.41±0.76     | 0.8521  |
| ΔFBG (mg/dl)                 | -95.57±93.06*        | +23.21±127.81  | 0.0093  |
| ΔHbA1c (%)                   | -0.73±0.53**         | -0.03±0.75     | 0.0084  |
| ΔTC (mg/dl)                  | -5.86±31.23          | +4.86±16.92    | 0.2693  |
| ΔTG (mg/dl)                  | -2.71±60.98          | -20.71±68.05   | 0.4994  |
| ΔHDL-C (mg/dl)               | -1.36±7.43           | +1.43±4.50     | 0.2409  |
| ΔLDL-C (mg/dl)               | -0.07±24.85          | +7.71±15.58    | 0.3298  |
| Δpreheparin LPL mass (ng/ml) | +6.84±23.74          | -4.34±15.07    | 0.1487  |
| Δd-ROM (U.CARR)              | +14.71±26.47         | +6.86±46.47    | 0.5872  |

Data are presented as mean±S.D. Δ denotes the difference between the value at baseline and that after 6 months.

\*P < 0.005, \*\*P < 0.0005 for within group treatment effect. Abbreviations are as in Table 1.

#### 4.3. Comparisons of hypoglycemic episodes and dosage of basal insulin after 6 months in two groups

The dosage of basal insulin at the end of this study was slightly reduced in degludec group, and was unchanged in glargine group. The dosage of basal insulin at end of this study was not significantly different between two groups (Table 3). Hypoglycemia frequency over 3 times per week was observed in none of the patients in glargine group and in one patient in degludec group. In that patient, the dose of degludec was reduced by 5 U, with no recurrence of hypoglycemia frequency >3 per week.

Hypoglycemic episodes increased in one patient each in both groups. However, hypoglycemic episodes decreased in three patients in degludec group, but in none of the patients in glargine group (Table 3).

**Table 3. Comparison of basal insulin dosage and hypoglycemic episodes after 6 months in the two groups**

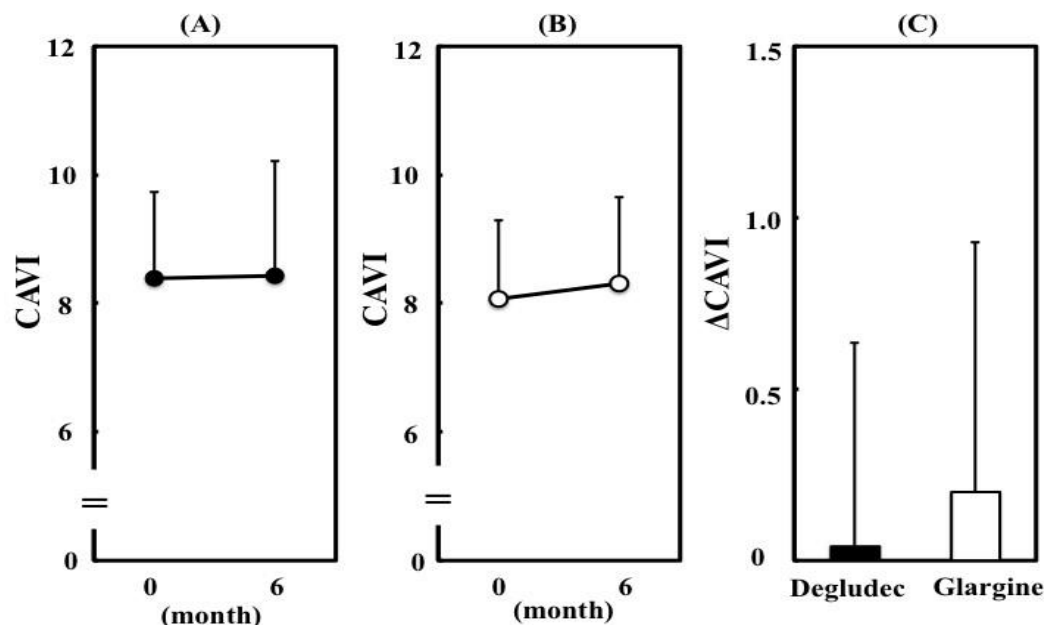
|                                                             | Degludec group    | Glargine group    | P value       |
|-------------------------------------------------------------|-------------------|-------------------|---------------|
| <b>Final dosage of basal insulin (mean±S.D.; U)</b>         | <b>12.14±4.52</b> | <b>13.86±4.75</b> | <b>0.3372</b> |
| <b>Status of hypoglycemic episodes (number of patients)</b> |                   |                   |               |
| <b>increased</b>                                            | <b>1</b>          | <b>1</b>          |               |
| <b>decreased</b>                                            | <b>10</b>         | <b>13</b>         |               |
|                                                             | <b>3</b>          | <b>0</b>          |               |

#### 4.4. Change in CAVI in degludec group and glargine group

In degludec group, CAVI was almost unchanged from baseline (8.39± 1.35) to 6 months of study (8.43± 1.78) (P = 0.7911; Figure 1A). In glargine group, CAVI changed from 8.06±1.23 at baseline to 8.31± 1.35 at 6 months,

but the change was not significant ( $P = 0.2220$ ; Figure 1B). The changes in CAVI during this study are shown in Figure 1C.

The mean change in CAVI appeared slightly greater in the glargine group than in the degludec group, but the difference was not significant ( $+0.04 \pm 0.59$  vs  $+0.25 \pm 0.73$ ,  $P = 0.4172$ ).



**Figure 1: Comparison of cardio-ankle vascular index (CAVI) at baseline and after 6 months.**

(A) Change in CAVI in patients who switched to insulin degludec. (B) Change in CAVI in patients who continued to inject insulin glargine. (C) Changes in CAVI after 6 months in two groups. Data are presented as mean  $\pm$  S.D.  $\Delta$  denotes the difference between the value at baseline and that after 6 months.

## 5. Discussion

In the present study, switching basal insulin from glargine to degludec significantly improved FBG and HbA1c in T1DM patients. However, other clinical parameters including preheparin LPL mass, d-ROMs and CAVI were not different in the two groups. T1DM is characterized by deficiency of insulin secretion, and the mainstay of insulin replacement therapy is basal-bolus insulin therapy (American Diabetes Association. 2013).

Long-acting insulin plays an important role for maintaining basal insulin levels in patients with T1DM. Insulin glargine is a long-acting insulin analog and is a more useful basal insulin compared with NPH insulin, which is an intermediate-acting insulin (Ranter RE et al., 2000; Albright ES et al., 2004). Insulin degludec is a newly developed basal insulin analog and the efficacy of insulin degludec in HbA1c control is equivalent to that of insulin glargine (Vora J et al., 2014). Although the two basal insulin analogs showed almost the same decrease in HbA1c in previous studies, insulin glargine required higher insulin dosage than insulin degludec (Birkeland KI et al., 2011; Bode BW et al., 2013). In the present study, FBG and HbA1c decreased significantly only in the degludec group, and the mean basal insulin dosage at the end of this study was almost the same in the two groups. Thus, the two previous reports and our study indicate that the glucose-lowering effect of insulin degludec is superior to that of insulin glargine.

Insulin glargine has a broad peak with a duration of action ranging from 20 to 36 hours, and this profile is more physiological than NPH insulin (Ranter RE et al., 2000). Insulin degludec has four times lower pharmacodynamic variability than insulin glargine (Heise T et al., 2012). This difference between insulin degludec and glargine is

considered to be one of the reasons why the rate of hypoglycemia is lower with insulin degludec than with insulin glargine in T1DM patients (Birkeland KI et al., 2011; Heise T et al., 2012). Indeed, hypoglycemic episodes decreased in some patients switched to degludec in this study.

CAVI is a useful surrogate marker of atherosclerosis and is improved by treatment with hypoglycemic agents including insulin (Nagayama D et al., 2010; Ohira M et al., 2011; Ohira M et al., 2014). In this study, HbA1c decreased significantly in patients switched to degludec, but CAVI was unchanged. A previous report shows that change in CAVI is independent of HbA1c (Nagayama D et al., 2010). Glibenclamide, a sulfonylurea agent, significantly reduced both FBG and HbA1c, but did not decrease CAVI significantly (Nagayama D et al., 2010). The decrease of CAVI may depend on mechanisms of glucose-lowering effect, such as reducing insulin resistance and improving postprandial hyperglycemia (Nagayama D et al., 2010; Ohira M et al., 2011; Ohira M et al., 2014). Insulin degludec may not be able to improve insulin resistance or postprandial hyperglycemia, because it is a basal insulin analog. We speculate that this is one of the reasons why insulin degludec could not improve CAVI. We hypothesized that decrease of hypoglycemia rate improves arterial stiffness, because repeated episodes of hypoglycemia are an aggravating factor for preclinical atherosclerosis in type 1 diabetes patients (Giménez M et al., 2011). However, CAVI was unchanged in degludec group. Hypoglycemic episodes decreased in the group switched to degludec, but in only 20% of the patients. Therefore, decrease of hypoglycemic episodes had no influence on CAVI in this study.

LPL is a TG hydrolase that is crucial for the catabolism of TG-rich very low density lipoprotein (VLDL) and chylomicron particles (Bensadoun A, 1991). In early clinical studies, LPL levels were analyzed using postheparin plasma (Sexena U et al., 1989; Peterson et al., 1990). However, a sensitive immunoassay using a specific monoclonal antibody against LPL has demonstrated the presence of LPL mass in preheparin serum (Kobayashi J et al., 1993; Tornvall P et al., 1995). Preheparin LPL mass is low in type 2 diabetes patients and is increased by insulin therapy (Miyashita Y et al., 2002). Preheparin LPL mass is considered to reflect insulin action in the whole body (Miyashita Y et al., 2006). These reports indicate that preheparin LPL mass is a good marker for evaluating insulin action. In this study, although the change in preheparin LPL mass was not significantly different between two groups, mean preheparin LPL mass increased in degludec group but decreased in glargine group. This result may reflect that insulin degludec has slightly stronger insulin action compared with insulin glargine.

Oxidative stress is an important factor for the development of diabetic complications and increases in children with T1DM (Scott JA et al., 2004; Varvarovská J et al., 2004). Some clinical markers such as isoprostanes or 8-hydroxydeoxyguanosine are available for detecting oxidative stress. d-ROM test is another oxidative stress marker used in patients with diabetes or coronary artery disease (Vîrgolici B et al., 2005; Vassalle C et al., 2008). In this study, d-ROM increased slightly in both groups, but the change in d-ROM was not significantly different between two groups. Although FBG and HbA1c decreased significantly in degludec group, d-ROM was not improved after 6 months. Despite the significant improvement in HbA1c, the level remained high, which may explain why d-ROM did not decrease in degludec group.

There are some limitations in the present study. First, we investigated 14 patients in each treatment arm, which was relatively small in number. Second, the dose of basal insulin is usually adjusted based on self-measured fasting blood glucose (Birkeland KI et al., 2011). In this study, self-measured fasting blood glucose was unstable in many patients. Therefore, we were not able to adjust basal insulin dose by this method. Third, the study duration was only 6 months. Long-term effects of insulin degludec are unclear. Despite these limitations, we were able to demonstrate the influences of switching to insulin degludec on blood glucose, oxidative stress and arterial stiffness in T1DM patients.

In summary, switching from insulin glargine to insulin degludec in patients with T1DM reduced FBG and HbA1c significantly. However, this switch did not change CAVI or d-ROMs, an oxidative stress marker.

## **6. Disclosure**

Potential conflicts of interest with any of the authors: None.

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