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Insulin Degludec improves Blood Glucose but Does Not Alter Arterial Stiffness in Type 1 Diabetes Patients

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Abstract

Background: Type 1 diabetes mellitus (T1DM) is characterized by insulin deficiency.Insulin degludec is newly developed ultra-long-acting insulin analog. However, the effect of insulin degludec on the artery is unclear. **Aim:**To study the efficacy of insulin degludec on artery. **Methods:** 28T1DM patients already onbasal-bolus insulin therapy were enrolled.Patients were divided into a group that switched from insulin glargine to insulin degludec (degludec group, n=14) and a group that continued to inject insulin glargine (glargine group, n=14).We observed the change in cardio-ankle vascular index (CAVI) that reflects arterial stiffness. **Results:** After 6 months,degludecsignificantly improved fasting blood glucose (FBG; -95.57 ± 93.06 mg/dl, $P < 0.005$) and hemoglobin A1c (HbA1c: -0.73 ± 0.53 %, $P < 0.0005$).The decreasesinFBG and HbA1c in degludec groupwere significantly differentfrom the changesin glarginegroup (FBG: -95.57 ± 93.06 mg/dl vs+23.21 ± 70.44 mg/dl, $P < 0.01$; HbA1c: -0.73 ± 0.53 % vs -0.03 ± 0.75 %, $P < 0.01$).The changeinCAVIwas not significantly different between two groups. **Conclusion:**These results suggest that switching from insulin glargine to insulin degludec improves blood glucose, but does not ameliorate arterial stiffness.

Keywords: Type 1 diabetes, insulin degludec, insulin glargine, hypoglycemia, arterialstiffness

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1. Introduction

Type 1 diabetesmellitus (T1DM) is characterized by insulin deficiency because of autoimmune or idiopathic destruction of pancreatic ßcells (Ikegami et al., 2011). Patients with T1DM are dependent on insulin replacement therapy, and the mainstayof 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 developedlater and is moreuseful 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 insulin in basal-bolus insulin therapy.Howevereven 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 degludechas non-inferior blood glucose lowering effect and reduces frequencyof hypoglycemia compared with insulin glarginein patients with T1DM (Birkeland KI et al., 2011; Bode BW et al., 2013; Vora J et al., 2014). Recently, severe hypoglycemia isknown 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 atherosclerosisnot only by controlling blood glucose but also by reducing the frequencyof hypoglycemia, but theefficacy 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 ß (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 effectson the arteryin 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 conductedat Sakura Hospital Toho University Medical Center. We enrolled 28 patients with T1DM, whose glycosylated hemoglobin (HbA1c) wasover 7.0% and steady for 3 months. At baseline, all subjects were treatedwith 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 inthis 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 medicationsexceptbasal insulin.Basal insulin dose was adjusted based onfrequency 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; WismerII 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_2O_2. 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: $CAVI = a\{(2\rho/\Delta P)\times \ln(Ps/Pd)PWV^2\}+b$, where Ps is systolic blood pressure; Pd is diastolic blood pressure; PWV is pulse wave velocity;ΔP 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 areexpressed as mean ± 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 apparentlyhigher in the degludecgroup than in theglargine group, but the differences were not significant. Fasting serum CPR was low in both groups. Dosages of ultra-rapid insulin and insulin glargineat 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±15.01	50.71±16.68	0.4055
duration of type 1 diabetes (years)	14.71±10.41	12.79±7.13	0.5723
BW (kg)	58.16±10.30	60.14±10.12	0.6112
BMI (kg/m^2)	23.07±3.64	22.99±3.83	0.9561
FBG (mg/dl)	235.00±95.89	199.21±63.33	0.2545
HbA1c (%)	9.61±1.21	8.84±1.19	0.1036
serum CPR (ng/ml)	0.02±0.06	0.05±0.108	0.3282
TC (mg/dl)	214.93±33.09	201.00±27.99	0.2401
TG (mg/dl)	118.43±107.83	123.07±88.33	0.5971
HDL-C (mg/dl)	67.21±16.07	66.07±20.08	0.8692
LDL-C (mg/dl)	119.14±19.52	108.36±21.90	0.1806
preheparin LPL mass (ng/ml)	76.10±25.51	77.31±27.58	0.9052
d-ROM (U.CARR)	367.86±71.36	361.86±60.19	0.8118
Dosage of ultra-rapid-acting insulin (U)	19.21±6.54	21.93±6.32	0.2743
Dosage of insulin glargine (U)	12.57±4.29	13.86±4.75	0.4593

Data are presented as mean±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 inclinical parameters after 6 months in two groups

Mean body weight and BMI changed slightly after 6 months in both groups, butthere 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 ± 93.06 mg/dl, P < 0.005, HbA1c: -0.73 ± 0.53 %, P < 0.0005 in degludec group). FBG and HbA1c were significantly reduced in degludec group compared with glarginegroup (FBG: -95.57 ± 93.06 mg/dl vs+23.21 ± 70.44 mg/dl, P < 0.01; HbA1c: -0.73 ± 0.53 % vs -0.03 ± 0.75 %, P < 0.01). Mean preheparinLPL mass changedslightly after 6 monthsin degludec group, butthe changes inpreheparin LPL massfrom baseline werenotsignificantly 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. ExceptFBG and HbA1c in degludec group,all other clinical parameters were not significantly different at 6 months compared to baseline in bothgroups. The changes in theseclinical 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^2)	+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 patientsin 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
Final dosage of basal insulin (mean±S.D.; U)	**12.14±4.52**	**13.86±4.75**	**0.3372**
Status of hypoglycemic episodes (number of patients)			
increased	**1**	**1**	
unchanged	**10**	**13**	
decreased	**3**	**0**	

4.4. Change in CAVIin degludec groupand glargine group

In degludecgroup, 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 glarginegroup than in the degludecgroup, but the difference was not significant(+0.04± 0.59 vs +0.25± 0.73, P = 0.4172).

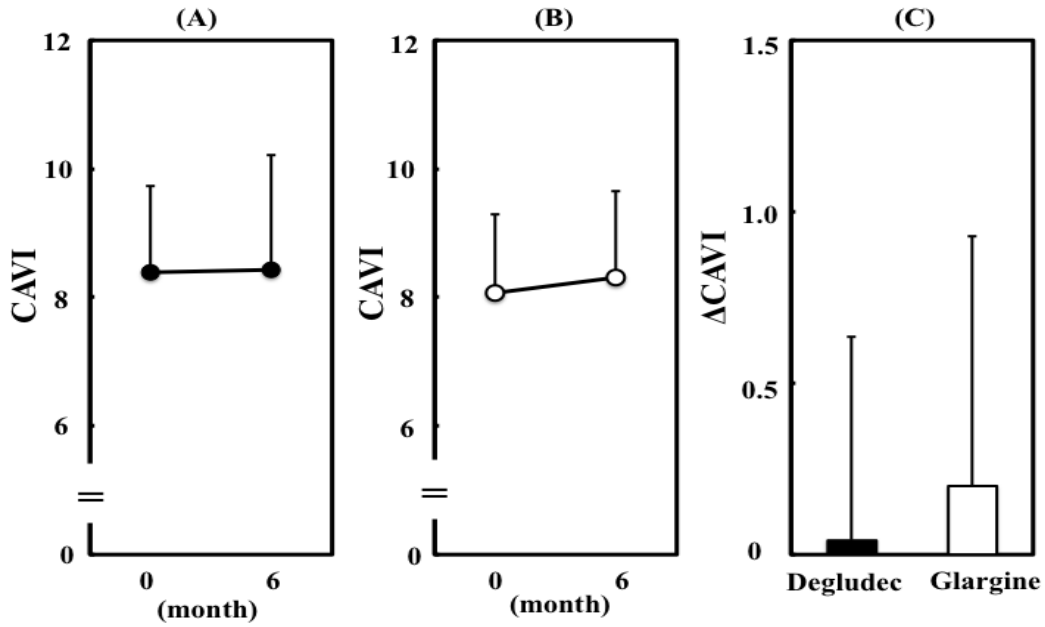

Figure 1: Comparison of cardio-ankle vascular index (CAVI) at baseline and after 6 months.

(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 ± S.D. ∆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 mainstayof 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 isa moreuseful 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 degludecin HbA1c control isequivalent to that of insulin glargine (Vora J et al., 2014). Although the two basal insulin analogs showedalmost the same decrease in HbA1c in previous studies, insulin glarginerequired 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 degludec group, and the mean basal insulin dosage at the end of this study was almost the same in thetwo groups.Thus, the two previous reports and our studyindicate that the glucose-lowering effect of insulin degludec is superior tothat 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 hypoglycemiais lower with insulin degludec than with insulin glarginein 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, butCAVI was unchanged. A previous report shows that change inCAVI is independent of HbA1c (Nagayama D et al., 2010). Glibenclamide, a sulfonylurea agent, significantly reducedboth FBG and HbA1c, butdid 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 andimprovingpostprandial hyperglycemia (Nagayama D et al., 2010; Ohira M et al., 2011; Ohira M et al., 2014).Insulin degludecmay not be able to improve insulin resistance or postprandial hyperglycemia, because it is a basal insulin analog.We speculate that this is one of thereasons why insulin degludec could not improve CAVI.

We hypothesized that decrease of hypoglycemiarateimproves arterial stiffness, becauserepeated 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 inonly 20% of the patients.Therefore, decrease of hypoglycemic episodes hadno 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 massis low in type 2 diabetes patients and is increased by insulin therapy (Miyashita Y et al., 2002).Preheparin LPL mass is considered to reflectinsulin 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 inpreheparin 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; VarvarovskáJ et al., 2004). Some clinical markers such as isoprostanes or 8-hydroxy-deoxyguanosineare 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 increasedslightly in both groups, but the changeind-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 whyd-ROM didnot 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 toadjust 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 HbA1csignificantly. However, this switch did not change CAVI ord-ROMs, an oxidative stress marker.

6. Disclosure

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

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References

Albright ES, Desmond R, Bell DS. (2004). Efficacy of conversion from bedtime NPH insulin injection to once- or twice-daily injections of insulin glargine in type 1 diabetic patients using basal/bolus therapy. Diabetes Care. 27 (2), 632-633.

American Diabetes Association. (2013). Standards of medical care in diabetes—2013. Diabetes Care. 36 Suppl 1, S11-66.

Bensadoun A. Lipoprotein lipase.(1991). Annu Rev Nutr.11, 217-237.

Birkeland KI, Home PD, Wendisch U, Ratner RE, Johansen T, Endahl LA, Lyby K, Jendle JH, Roberts AP, DeVries JH, Meneghini LF. (2011). Insulin degludec in type 1 diabetes: a randomized controlled trial of a new-generation ultra-long-acting insulin compared with insulin glardine. Diabetes Care. 34 (3), 661-665.

Bode BW, Buse JB, Fisher M, Garg SK, Marre M, Merker L, Renard E, Russell-Jones DL, Hansen CT, Rana A, Heller SR; BEGIN® Basal-Bolus Type 1 trial investigators. (2013). Insulin degludec improves glycaemic control with lower nocturnal hypoglycaemia risk than insulin glargine in basal-bolus treatment with mealtime insulin aspart in Type 1 diabetes (BEGIN(®) Basal-Bolus Type 1): 2-year results of a randomized clinical trial. Diabet Med. 30 (11), 1293-1297.

Fährmann ER, Adkins L, Loader CJ, Han H, Rice KM,Denvir J, Driscoll HK. (2015). Severe hypoglycemia and coronary artery calcification during the diabetes control and complications trial/epidemiology of diabetes interventions and complications (DCCT/EDIC) study.DiabetesRes ClinPract.107 (2), 280-289.

Giménez M, Gilabert R, Monteagudo J, Alonso A, Casamitjana R, Paré C, Conget I. (2011). Repeated episodes of hypoglycemia as a potential aggravating factor for preclinical atherosclerosis in subjects with type 1 diabetes. Diabetes Care.34 (1), 198-203.

Heise T, Hermanski L, Nosek L, Feldman A, Rasmussen S, Haahr H. (2012). Insulin degludec: four times lower pharmacodynamic variability than insulin glargine under steady-state conditions in type 1 diabetes. Diabetes ObesMetab.14 (9), 859-864.

Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM; IFCC Working Group on HbA1c Standardization. (2004). IFC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem. 50 (1): 166-174.

Ikegami H, Noso S, Babaya N, Kawabata Y. (2011). Genetics and pathogenesis of type 1 diabetes: prospects for prevention and intervention. J Diabetes Investing.2 (6), 415-420.

Kobayashi J, Hashimoto H, Fukamachi I, Tashiro J, Shirai K, Saito Y, Yoshida S. (1993). Lipoprotein lipase mass and activity in severe hypertriglyceridemia.ClinChimActa.216 (1-2), 113-123.

Miyashita Y, Shirai K, Sasaki H, Totsuka M, Murano T, Watanabe H. (2002). Low lipoprotein lipase mass in preheparin serum of type 2 diabetes mellitus patients and its recovery with insulin therapy. Diabetes Res ClinPract.56 (3), 181-187.

Miyashita Y, Ebisuno M, Ohhira M, Endoh K, Saiki A, Koide N, Ohtsuka M, Oyama T, Shirai K. (2006). Enhancement of serum lipoprotein lipase mass levels by intensive insulin therapy. Diabetes Res ClinPract.72 (1), 61-67.

Nagayama D, Saiki A, Endo K, Yamaguchi T, Ban N, Kawana H, Ohira M,Oyama T, Miyashita Y, Shirai K.(2010). Improvement of cardio-ankle vascular index by glimepiride in type 2 diabetic patients. Int J ClinPract. 64 (13), 1796-1801.

Nakamura K, Tomaru T, Yamamura S, Miyashita Y, Shirai K, Noike H. (2008). Cardio-ankle vascular index is a candidate predictor of coronary atherosclerosis.Circ J. 72 (4), 598-604.

Ohira M, Endo K, Oyama T, Yamaguchi T, Ban N, Kawana H, Nagayama D, Nagumo A, Saiki A, Murano T, Watanabe H, Miyashita Y, Shirai K. (2011). Improvement of postprandial hyperglycemia and arterial stiffness upon switching from premixed human insulin 30/70 to biphasic insulin aspart 30/70. Metabolism. 60 (1), 78-85.

Ohira M, Yamaguchi T, Saiki A, Ban N, Kawana H, Nagumo A, Murano T, Shirai K, Tatsuno I.(2014). Pioglitazone improves the cardio-ankle vascular index in patients with type 2 diabetes mellitus treated with metformin. Diabetes MetabSyndrObes. 7, 313-319.

Peterson J, Bihain BE, Bengtsson-Olivecrona G, Deckelbaum RJ, Carpentier YA, Olivecrona T. (1990). Fatty acid control of lipoprotein lipase: a link between energy metabolism and lipid transport. ProcNatlAcadSci U S A. 87 (3), 909-913.

Ratner RE, Hirsch IB, Neifing JL, Garg SK, Mecca TE, Wilson CA. (2000). Less hypoglycemia with insulin glargine in intensive insulin therapy for type 1 diabetes. U.S. Study Group of Insulin Glargine in Type 1 Diabetes. Diabetes Care. 23(5), 639-643.

Saxena U, Witte LD, Goldberg IJ. (1989). Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids. J Biol Chem. 264 (8), 4349-4355.

Scott JA, King GL. (2004). Oxidative stress and antioxidant treatment in diabetes.Ann N Y Acad Sci. 1031, 204-213.

Shirai K, Nema T, Hiroh Y, Itoh Y, Miyashita Y, Watanabe H. (1992). Clinical efficacy of the direct assay method using polymers for serum high density lipoprotein cholesterols. J Clin Lab Anal.11 (2), 82-86.

Shirai K, Utino J, Otsuka K, TakataM. (2006). A novel blood pressure-independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI).J Atherosclerosis Thromb.13 (2), 101-107.

Takaki A, Ogawa H, Wakayama T, Iwami T, Kimura M, Hadano Y, Matsuda S, Miyazaki Y, Matsuda T, Hiratsuka A, Matsuzaki M. (2007).Cardio-ankle vascular index is a new noninvasive parameter of arterial stiffness. Circ J. 71 (11), 1710-1714.

Tornvall P, Olivecrona G, Karpe F, Hamsten A, Olivecrona T. (1995). Lipoprotein lipase mass and activity in plasma and their increase after heparin are separate parameters with different relations to plasma lipoproteins. AtheriosclerThrombVasc Biol. 15 (8), 1086-1093.

VarvarovskáJ, Racek J, Stetina R, Sýkora J, Pomahacová R, Rusavý Z, Lacigová S, Trefil L, Siala K, Stozický F. (2004). Aspects of oxidative stress in children with type 1 diabetes mellitus. Biomed Pharmacother.58 (10), 539-545.

Vassalle C, Pratali L, Boni C, Mercuri A, Ndreu R. (2008). An oxidative stress score as a combined measure of the pro-oxidant and anti-oxidant counterparts in patients with coronary artery disease.ClinBiochem. 41 (14-15), 1162-1167.

Vîrgolici B, Mohora M, Stoian I, Lixandru D, Găman L, Paveliu F. (2005). A comparative oxidative stress study--obesity with and without diabetes mellitus.Rom J Intern Med. 43 (3-4), 261-268.

Vora J, Christensen T, Rana A, Bain SC. (2014). Insulin degludec versus insulin glargine in type 1 and type 2 diabetes mellitus: a meta-analysis of endopoints in phase 3a trials. Diabetes Ther. 5 (2), 435-446.

Wakabayashi T, Kawashima T, Matsuzawa Y. (2014). Evaluation of reactive oxygen metabolites in patients with non-small cell lung cancer after chemotherapy.MultidiscipRespir Med. 9 (1), 44. doi: 10.1186/2049-6958-9-44.

Wang F, Surh J, Kaur M. (2012). Insulin degludec as an ultralong-acting basal insulin once a day: a systematic review. Diabetes MetabSyndrObes. 5, 191-204.