Int J Clin Exp Med 2017;10(3):5406-5413
www.ijcem.com /ISSN:1940-5901/IJCEM0034423

Original Article

Relationships between glucose fluctuations and oxidative stress in short duration type 2 diabetes treated with sensor-augmented insulin pump

Chun-Hong Shi, Yan-Zhen Ye, Jian-Ling Du, Xue-Yang Zhang, Yong-Bo Wang, Ying Ba, Yu Yang

Department of Endocrinology, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, China

Received June 25, 2016; Accepted November 25, 2016; Epub March 15, 2017; Published March 30, 2017

Abstract: This study aims to explore the relationships between oxidative stress and glucose fluctuations in patients with short duration type 2 diabetes treated with sensor-augmented insulin pump (SAP). Sixty type 2 diabetes patients with duration < 1 year and HbA1c ≥ 9% were enrolled and randomly divided into the SAP group or the multiple daily injection (MDI) group, and all of them were performed 6-day enhanced treatments. Furthermore, the patients were also assigned to the severe oxidative group (group SO) or the weak oxidative group (group WO) according to median level of 8-iso-prostaglandin F2a (8-iso-PGF2a), an oxidative stress parameter. Compared with the baseline, the following parameters which indicate glucose fluctuations: the mean blood glucose (MBG), mean amplitude of glycemic excursion (MAGE), absolute mean of daily differences (MODD) and incremental area under the curve (IAUC) in group SAP were significantly decreased after 6-day therapy ($P < 0.05$), while no difference was found on terms of serum 8-iso-PGF2a levels ($P > 0.05$). After SAP therapy, the levels of MBG, MAGE, MODD and IAUC in group SAP were significantly lower than group MDI ($P < 0.05$). There was no difference in serum 8-iso-PGF2a levels and insulin daily dosage (IDD) between the groups ($P > 0.05$). The values of IDD and waistline in group SO were significantly higher than group WO ($P < 0.05$). The multiple regression analysis showed IDD (t=2.146, P=0.035) and waistline (t=4.502, P=0.011) were independently correlated with 8-iso-PGF2a. No relationship was found between oxidative stress and glucose fluctuations in short duration type 2 diabetes patients under the condition of short-term intensive SAP therapy, IDD and waistline were independent risk factors toward the activation of oxidative stress.

Keywords: Insulin infusion system, glucose fluctuation, oxidative stress, type 2 diabetes

Introduction

The global prevalence of diabetes has been rapidly increased, and until 2010, the prevalence rate of diabetes in Chinese adults has reached 11.6%, among which type 2 diabetes accounted for more than 90% [1]; the medical expenses necessary for the prevention and treatment of diabetes related macrovascular complications accounted for the main part of the related medical costs. Under high glucose conditions, because the oxidation of glucose would be increased, such mechanisms as the non-enzymatic glycosylation of proteins and the increased activities of aldose reductases could cause the production of reactive oxygen species in vivo to be increased, so the oxidative stress level would be increased. Besides the direct damages toward cells and tissues, the reactive oxygen species could also act a second messenger to activate multiple signaling pathways, such as the activation of transcription factor nuclear factor kappa B [2], activation of protein kinase C pathway [3], followed by causing the apoptosis of endothelial cells and generating a large number of inflammatory factors; the smooth muscle cells would then proliferate and migrate, and the monocytes in the circulation system would then adhere and migrate into vascular walls, thus causing the remodeling and thickening of vascular tunica media, diastolic function reduction, and gradual formation of atherosclerotic plaques. Serum 8-iso-prostaglandin F2a (8-iso-PGF2a) is a stable final product produced when free radicals injured the lipids on cell membranes and caused it to occur peroxidation, normally exists in plasma freely or with the form of esterified

phospholipid, and could be used as a specific oxidative stress marker to evaluate the peroxidation of lipids [4].

The 10-year follow-up study of U.K. Prospective Diabetes Study confirmed that the risk of myocardial infarction in the intensive reducing-glucose group was significantly reduced by 15% [5], and the strict blood glucose control caused glucose fluctuations or even hypoglycemia in type 2 diabetes patients already accompanied with macrovascular diseases not only showed no cardiovascular benefits but also increased the all-cause mortality [6, 7]; abnormal glucose fluctuations was one of the important features of glucose dysmetabolism. The sensor-augmented insulin pump (SAP) integrates the insulin pump and real-time dynamic glucose monitoring, which could not only reduce HbA1c [8]. Furthermore, it could also significantly reduce the standard deviation and the coefficient of variation of blood sugar, thus reducing the blood sugar fluctuations effectively [9]. In vitro experiments [10] showed that glucose fluctuations induced intracellular oxidative stress, caused vascular endothelial damages, and accelerated the progression of atherosclerosis. Studies targeting diabetes patients still had controversies [11, 12], and it's still not clear whether reducing glucose fluctuations could reduce oxidative stress and thus delay the progression of diabetic complications.

In this study, patients with short-duration type 2 diabetes (sd-T2DM) were applied intensive reducing-glucose treatment using SAP, and the glucose fluctuations were performed real-time continuous glucose monitoring (RT-CGM); using 8-iso-PGF2a as an oxidative stress index, the relationships between oxidative stress and glucose fluctuations in short-duration type 2 diabetes patients after intensive treatments were analyzed, aiming to provide basis for selecting the optimized treatment strategies for type 2 diabetes patients.

Materials and methods

Study design and participants

The T2DM patients admitted into the department of endocrinology in the first affiliated hospital of the Dalian medical university from August 2013 to October 2014 were selected. Inclusion criteria: 1) aged 18-70 years; 2) type 2 diabetes and duration < 1 year; 3) was not applied antidiabetic drugs or applied oral hypoglycemic agents or insulin a month ago, but the duration < 1 month; 4) HbA1c ≥ 9.0%. Exclusion criteria: 1) accompanied with such acute diabetic complications as diabetic ketoacidosis; 2) with severe stress, heart failure, hepatic dysfunction (Alanine transaminase > 100U/L), renal dysfunction (glomerular filtration rate ≤ 60 $ml^{-1}min^{-1}$), infections, or occurred acute myocardial infarction, stroke, or had surgery within 6 months; 3) with the medication history of Statins, vitamin C or E, other antioxidant drugs, or non-steroidal anti-inflammatory drugs within a month.

This study was conducted in accordance with the declaration of Helsinki and was approved by the Ethics Committee of Dalian Medical University. Written informed consent was obtained from all participants before study entry.

Randomization and masking

A computer-generated random number sequence was divided into two blocks with the ratio as 1:1, and the patients met the inclusion criteria were grouped using this number sequence before the study, among who the patients numbered from 1 to 30 were set as group SAP, and those numbered from 31 to 60 were set as group multiple daily injection (MDI) for daily multiple subcutaneous injection of insulin. The study was not masked because of the difference of the insulin delivery device and glucose monitoring device.

Procedures

Patients in Group SAP was treated with the 722 insulin pump (Medtronic, US) and insulin Aspart (Novo Nordisk, Denmark) with the initial dose as 0.5 $U{\cdot}kg^{-1}{\cdot}d^{-1}$, as well as the basic and supplementary dose as 50%, respectively, which was evenly distributed 5 min before each meal, and RT-CGM was simultaneously performed. The alarm for hypoglycemia was set as 3.0 mmol/L, and when the alarm occurred, the patient should be asked to take proper diet and pay close attention to the changes of blood sugar. The alarm for hyperglycemia was set as 17 mmol/L, and when the alarm occurred, such measures as setting the temporary baseline

Table 1. Comparison of baseline features between group SAP and MDI ($\bar{x}$±SE) (median, interquartile range)

	Age (years)	Duration (days)	Waistline (cm)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	FPG (mmol/L)	HbA1c (%)
MDI (n=30)	48.3±3.7	55 (253)	96±5	26.6±2.1	125±9	81±7	10.4±2.6	10.6±1.2
SAP (n=30)	47.4±4.2	75 (330)	95±6	26.4±1.9	131±10	81±5	10.5±1.7	10.3±0.9
	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	MAGE (mmol/L)	MODD (mmol/L)	IAUC (mmol/L)	8-iso-PGF2a (pg/ml)
MDI (n=30)	5.18±0.34	1.89 (1.15)	1.27±0.13	3.13±0.35	4.19±0.98	2.35±0.26	1.52±0.19	38.18 (55.59)
SAP (n=30)	4.99±0.52	1.96 (1.55)	1.23±0.08	2.89±0.22	4.23±1.02	2.56±0.41	1.39±0.23	37.49 (43.23)

Note: Group MDI: performed multiple daily subcutaneous injection of insulin; Group SAP: treated using sensor-augmented insulin pump; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; TC : total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MAGE: mean amplitude of glycemic excursion; MODD: absolute mean of daily differences; IAUC: incremental area under the curve; 8-iso-PGF2a: serum 8-iso-prostaglandin F2a.

rate, applying large dose of insulin, or adjusting the baseline rate to restore the blood sugar into the ideal range as soon as possible. Meanwhile, the patient should also be told to observe the trend symbols on the screen, and inform doctors promptly to avoid excessive glucose fluctuations. Group MDI was subcutaneously injected insulin Aspart (Novo Nordisk, Denmark) and insulin Detemir (Novo Nordisk, Denmark), four times a day, with the initial dose of the total insulin as 0.5 U·kg^{-1}·d^{-1} and the initial dose of insulin Detemir as 0.2 U·kg^{-1} together with retrospective continuous glucose monitor (CGM) (MiniMed, Medtronic, USA). The finger glucose levels in both groups were monitored before meals and bedtime to calibrate CGM, with probe replacement once every 3 days and probe removal 6 days later.

Detection of different indexes

The age, disease duration, waist circumference, body weight, height, blood pressure were recorded, and then body mass index (BMI) was calculated [BMI=body weight (kg)/height^2 (m^2)]. Automatic biochemical analyzer (Hitachi 7600-210, Japan) was used to measure the fasting plasma glucose (FPG), biochemical liver functions, renal functions, and blood lipids. HbA1c was determined using the ion exchange high performance liquid chromatography method (Variant II, BIO-RAD, USA). Serum 8-iso-PGF2a was measured with the dual-antibody sandwich avidin biotin complex-ELISA (8-iso-PGF2a ELISA Kit, USCN, US) method, the anti-human 8-iso-PGF2a monoclonal antibody was coated onto the microtiter plates; after the 8-iso-PGF2a molecules in the standards and samples combined with the monoclonal antibody, the biotinylated anti-human 8-iso-PGF2a was added inside so as to form the immune complex; the horseradish peroxidase-labeled Streptvidin then bound with the biotin, and appeared blue after added the working solution; after added the termination solution, the OD values were measured at 450 nm. The 8-iso-PGF2a concentration was positively proportional to the OD value, so the 8-iso-PGF2a concentration in each sample could be obtained from the standard curve. The minimum detectable concentration was < 16 pg/ml, and the inner- and inter-plate variation coefficients were both less than 9.6%.

Assessment parameters of SAP

1) Mean amplitude of glycemic excursion (MAGE): glucose fluctuation greater than one standard deviation of blood glucose within 24 h were selected; the glucose fluctuation was then performed statistics according to the first valid fluctuation direction, and MAGE was the average amplitude of all the glucose fluctuations; 2) Absolute mean of daily differences (MODD): the absolute difference corresponding to the measurement value of CGM within two consecutive 24 h detection periods was MODD; 3) Incremental area under the curve (IAUC): the increased area of the postprandial curve which was higher than the preprandial blood glucose level.

Statistical analysis

SPSS19.0 was used for the statistical analysis. The normally distributed measurement data were expressed as mean ± standard error ($\bar{x}$ ±SE), and the non-normally distributed measurement data were expressed as (median,

Table 2. Comparison of serum indexes in group SAP and MDI before and after the treatment ($\bar{x}$±SE) (median, interquartile range)

		MAGE (mmol/L)	MODD (mmol/L)	IAUC (mmol/L×h)	MBG (mmol/L)	8-iso-PGF2a (pg/ml)	IDD (U·kg^{-1}·d^{-1})
MDI (n=30)	Before	4.19±0.98	2.35±0.26	1.52±0.19	10.19±1.45	27.18 (55.59)	-
	After	4.75±0.77	2.17±0.37	1.21±0.12	9.25±1.28$^{\#}$	30.57 (43.91)	0.60±0.02
	P	0.326	0.259	0.297	0.029	0.586	
SAP (n=30)	Before	4.23±1.02	2.56±0.41	1.39±0.23	10.23±1.36	28.49 (43.23)	-
	After	2.65±0.56$^{*,\#}$	1.49±0.25$^{*,\#}$	0.48±0.08$^{*,\#}$	7.35±1.04$^{*,\#}$	33.13 (36.32)	0.69±0.06
	P	0.018	0.024	0.007	0.006	0.632	
P		0.012	0.031	0.014	0.017	0.825	0.451

Note: Group MDI: performed multiple daily subcutaneous injection of insulin; Group SAP: treated using sensor-augmented insulin pump; MAGE: Mean amplitude of glycemic excursion; MODD: absolute mean of daily differences; IAUC: incremental area under the curve; 8-iso-PGF2a: serum 8-iso-prostaglandin F2a. Comparison between group SAP and MDI, $^{*}P < 0.05$; comparison between the data before and after the treatment, $^{\#}P < 0.05$.

Table 3. Comparison between the patients with different 8-iso-PGF2a levels ($\bar{x}$±SE) (median, interquartile range)

	Age (years)	Duration (days)	Waistline (cm)	BMI (kg/m^2)	FCP (nmol/L)	
WO (n=30)	46.8±3.2	85 (139)	92±5	25.9±1.9	1.28±0.32	
SO (n=30)	48.7±4.1	49 (122)	98±4*	26.8±1.5	1.46±0.26	
P	0.748	0.699	0.045	0.221	0.472	
	IDD (U·kg^{-1}·d^{-1})	MBG (mmol/L)	HbA1c (%)	MAGE (mmol/L)	MODD (mmol/L)	IAUC (mmol/L×h)
WO (n=30)	0.49±0.03	10.55±1.22	10.2±0.8	4.17±0.85	2.31±0.32	1.24±0.15
SO (n=30)	0.63±0.04*	10.59±1.50	10.4±1.0	4.22±0.91	2.25±0.40	1.29±0.17
P	0.035	0.931	0.898	0.879	0.713	0.776

According to the median of 8-iso-PGF2a (32.70 pg/ml), the patients were divided into group severe oxidative (SO) and group weak oxidative (WO). Note: BMI: body mass index; FCP: fasting C-peptide; MBG: mean blood glucose; HbA1c: glycated hemoglobin; MAGE: mean amplitude of glycemic excursion; MODD: absolute mean of daily differences; IAUC: incremental area under the curve. Compared with group WO, $^{*}P < 0.05$.

interquartile range). The intragroup comparison, which had normally distributed data, before and after the treatment used the paired-sample *t* test, and the intergroup comparison used the independent-sample *t* test; the intergroup comparison which had non-normally distributed data used the rank sum test. The factors that affected 8-iso-PGF2a were analyzed using the multiple linear regression analysis, with $P < 0.05$ considered as statistically significant.

Results

Baseline features

Seventy five type 2 diabetes were screened, and 15 were excluded due to not meeting the inclusion criteria. A total of 60 patients were enrolled and divided into group SAP (n=30) and group MDI (n=30), with the mean age as (47.9±4.3) years old, disease duration as 63 (237) days. The intergroup comparison of baseline features showed no statistically significant difference (**Table 1**).

Comparison of serum indexes

Compared with the data before the treatment, mean blood glucose (MBG) (*P*=0.006), MAGE (*P*=0.018), MODD (*P*=0.024), and IAUC (*P*=0.007) in group SAP after the treatment were reduced significantly, and MBG (*P*=0.029) in group MDI was decreased significantly; While 8-iso-PGF2a showed no statistically significant difference in both groups. After the treatment, MBG (*P*=0.017), MAGE (*P*=0.012), MODD (*P*=0.031), and IAUC (*P*=0.014) in SAP were significantly lower than group MDI, but the serum 8-iso-PGF2a and insulin daily dose (IDD) showed no statistical significance (**Table 2**).

Table 4. Factors that might affect 8-iso-PGF2a

Factor	Partial regression coefficient	SD	Standard regression coefficient	95% CI	*t*	*P*
Waistline	1.349	0.376	0.756	0.264-1.248	4.502	0.011
BMI	0.610	0.845	0.337	-0.024-0.698	1.985	0.062
MBG	0.503	0.639	0.310	-0.043-0.663	0.698	0.164
MAGE	0.389	0.128	0.131	-0.120-0.382	0.537	0.212
MODD	0.214	0.136	0.086	-0.355-0.527	0.280	0.487
IAUC	0.337	0.430	0.174	-0.139-0.487	0.524	0.266
IDD	0.861	0.804	0.454	0.076-0.832	2.146	0.035

Multiple linear regression analysis of 8-iso-PGF2a. Note: BMI: body mass index; MBG: mean blood glucose; MAGE: mean amplitude of glycemic excursion; MODD: absolute mean of daily differences; IAUC: incremental area under the curve. IDD: insulin daily dose.

Comparison between the patients with different 8-iso-PGF2a levels

According to the median of 8-iso-PGF2a (32.70 pg/ml), the patients were divided into group severe oxidative (SO) and group weak oxidative (WO), and the data of waistline (P=0.045) and IDD (P=0.035) in group SO were significantly higher than group WO (**Table 3**).

Factors that might affect 8-iso-PGF2a

The multiple linear regression analysis with 8-iso-PGF2a as the dependent variable and the waistline, BMI, MBG, MAGE, MODD, IAUC, and IDD as the independent variables showed that IDD (t=2.146, P=0.035) and waistline (t=4.502, P=0.011) were independently associated with 8-iso-PGF2a (**Table 4**).

Discussion

SAP is one RT-CGM-integrated insulin infusion system, in which the range of blood glucose control could be set, so the blood sugar levels and fluctuation trends could be reflected in time, and the blood glucose control could thus be improved. The glucose fluctuations could promote the expression of apoptotic genes in endothelial cells [13], increase the secretion of inflammatory cytokines [14], thus accelerating the progression of diabetic complications. This study enrolled 60 short-duration type 2 diabetes patients, and randomly divided them into group SAP or group MDI for the comparison before and after 6-day intensive treatments: MBG, MAGE, MODD, and IAUC in group SAP were significantly decreased, while only MBG in group MDI was significantly reduced after the treatment, indicating that the SAP protocol could effectively reduce the glucose fluctuations in a short term. After the treatment, MBG, MAGE, MODD, and IAUC in group SAP were significantly lower than group MDI, suggesting that the average blood glucose level and the glucose fluctuations controlled at different time points by the SAP protocol were better than MDI. 1-year study targeting 152 type 1 diabetes adolescents showed that IAUC in group SAP was significantly lower than group MDI, and the improvement of glucose fluctuations was better than group MDI [15], consistent with the results of this study.

Studies of cell culture prompted that intermittent high glucose would induce the excessive production of reactive oxygen species, thereby increasing the apoptosis of pancreatic β cells [16]. Animal experiments also confirmed that glucose fluctuations could produce more malondialdehyde than sustained hyperglycemia, thus enhancing the oxidative stress and increasing the expressions of apoptosis-related proteins [17]. In this study, the type 2 diabetes patients were treated with SAP for 6 days, and the serum 8-iso-PGF2a showed no statistical significance than that before the treatment; furthermore, the serum 8-iso-PGF2a levels between group SAP and MDI after the treatment also showed no statistical significance, indicating that though the short-term SAP protocol could effectively improve the glucose control in short-duration type 2 diabetes patients, it failed to reduce the oxidative stress level in vivo. Moreover, according to the median of 8-iso-PGF2a, the patients were divided into group SO and WO, and the results showed that the intergroup comparison of MAGE, MODD, and IAUC had no significant difference, and the

multiple linear regression analysis showed that the glucose fluctuations were irrelevant with 8-iso-PGF2a, inconsistent with the above basic experiments. Studies targeting normal populations, patients with impaired glucose regulation, and newly diagnosed type 2 diabetes revealed that when no drug intervention was performed, MAGE was positively correlated with 8-iso-PGF2a [12], and in the T2DM patients with oral hypoglycemic drugs, MAGE was also positively correlated with 8-iso-PGF2a [18], but in the DM patients with insulin treatment, there was no correlation between these two factors [19, 20], suggesting that insulin played an important role in the relationships of glucose fluctuations and oxidative stress.

In this study, the intensive insulin treatment protocol was performed toward these short-duration type 2 diabetes patients, and the results revealed that the intragroup comparison before and after the treatment, as well as the intergroup comparison after the treatment, showed no difference in 8-iso-PGF2a, and there was no difference in IDD between the SAP and MDI group after treatment, but IDD in group S0 was significantly higher than that in group W0, and the multiple linear regression analysis showed that IDD was independently correlated with 8-iso-PGF2a, suggesting that insulin might have the role of pro-oxidative stress. Studies had shown that insulin could effectively reduce the H_2O_2 and malondialdehyde levels in the brain tissues of rats with septic encephalopathy, thus reducing the oxidative stress [21]. A multi-center cohort study showed that compared with the protocols of basal insulin plus oral hypoglycemic agents and oral hypoglycemic agents alone, continuous subcutaneous infusion of insulin could significantly reduce the serum oxidized LDL level, as well as reduce the degree of oxidative stress in T2DM patients [22]. However, our study results contradictory to the above studies, and the possible reasons might be that Monnier [23] found that the type 2 diabetes patients with IDD less than 0.4 U/kg exhibited the oxidative stress level controlled within the normal range, while those with IDD beyond this dose exhibited significantly increased oxidative stress level, indicating that a relatively large dose of insulin might contribute to the oxidative stress. In this study, IDDs of the two groups with insulin treatment were 0.60 $U \cdot kg^{-1} \cdot d^{-1}$ and 0.69 $U \cdot kg^{-1} \cdot d^{-1}$, respectively, significantly exceeding the level used by Monnier (0.4 $U \cdot kg^{-1} \cdot d^{-1}$), and It was due to the supplementary insulin when the blood glucose exceeded the preset hyperglycosemic range, group SAP was treated with relatively larger dose; considering the pro-oxidative stress roles of such a large dose of insulin exceeded the decline of oxidative stress caused by the reduction of glucose fluctuations, although group SAP showed significant improvement of glucose fluctuations, neither of the groups ultimately exhibited one reduced oxidative stress level. In addition, this study showed that the waistline was independently associated with 8-iso-PGF2a, indicating that the imbalance of oxidative stress was also closely related with the degree of abdominal obesity, and it might be considered as the endogenous hyperinsulinism-induced oxidative stress. Studies had shown that obese children had increased in vivo oxidative stress, among who the children accompanied with metabolic syndromes exhibited more obvious oxidative stress, and their antioxidant capacities were negatively correlated with BMI [24], consistent with the results of this study.

Our limitation was that the patients enrolled in the group were relatively few, and the study time was shorter, this may not be enough for a statistically significant difference between the groups, longer follow-up and more subjects participating in the trial should be performed in the future.

In short, as for short-duration type 2 diabetes, the short-term intensive SAP protocol could improve the glucose control, reduce the glucose fluctuations, but the super-physiological dose of insulin might promote the oxidative stress, thus reducing the glucose fluctuation reduction-caused anti-oxidative stress efficiency; smaller dose insulin-induced glucose control could benefit the oxidative stress control, and then be beneficial to delay the development of chronic diabetic complications.

Acknowledgements

This research was supported by funds from Chinese Medical Association Clinical Medical Research Funds (No. 13040720457).

Disclosure of conflict of interest

None.

Address correspondence to: Jian-Ling Du, Department of Endocrinology, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, China. Tel: +86 411 83635963 2172; Fax: +86 411 83630057/87614934; E-mail: cnjianlingdu@163.com

References

[1] Xu Y, Wang L, He J, Bi Y, Li M, Wang T, Wang L, Jiang Y, Dai M, Lu J, Xu M, Li Y, Hu N, Li J, Mi S, Chen CS, Li G, Mu Y, Zhao J, Kong L, Chen J, Lai S, Wang W, Zhao W and Ning G. Prevalence and control of diabetes in Chinese adults. JAMA 2013; 310: 948-959.

[2] Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y and Ali F. Atherosclerotic cardiovascular disease: a review of initiators and protective factors. Inflammopharmacology 2016; 24: 1-10.

[3] Kizub IV, Klymenko KI and Soloviev AI. Protein kinase C in enhanced vascular tone in diabetes mellitus. Int J Cardiol 2014; 174: 230-242.

[4] Morrow JD. Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans. Arterioscler Thromb Vasc Biol 2005; 25: 279-286.

[5] Holman RR, Paul SK, Bethel MA, Matthews DR and Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008; 359: 1577-1589.

[6] ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R and Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008; 358: 2560-2572.

[7] Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH Jr, Probstfield JL, Simons-Morton DG and Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008; 358: 2545-2559.

[8] Reznik Y, Cohen O, Aronson R, Conget I, Runzis S, Castaneda J and Lee SW. Insulin pump treatment compared with multiple daily injections for treatment of type 2 diabetes (OpT2mise): a randomised open-label controlled trial. Lancet 2014; 384: 1265-1272.

[9] Buse JB, Kudva YC, Battelino T, Davis SN, Shin J and Welsh JB. Effects of sensor-augmented pump therapy on glycemic variability in well-controlled type 1 diabetes in the STAR 3 study. Diabetes Technol Ther 2012; 14: 644-647.

[10] Saito S, Thuc LC, Teshima Y, Nakada C, Nishio S, Kondo H, Fukui A, Abe I, Ebata Y, Saikawa T, Moriyama M and Takahashi N. Glucose fluctuations aggravate cardiac susceptibility to ischemia/reperfusion injury by modulating MicroRNAs expression. Circ J 2016; 80: 186-195.

[11] Siegelaar SE, Barwari T, Kulik W, Hoekstra JB and DeVries JH. No relevant relationship between glucose variability and oxidative stress in well-regulated type 2 diabetes patients. J Diabetes Sci Technol 2011; 5: 86-92.

[12] Zheng F, Lu W, Jia C, Li H, Wang Z and Jia W. Relationships between glucose excursion and the activation of oxidative stress in patients with newly diagnosed type 2 diabetes or impaired glucose regulation. Endocrine 2010; 37: 201-208.

[13] Zhang W, Zhao S, Li Y, Peng G and Han P. Acute blood glucose fluctuation induces myocardial apoptosis through oxidative stress and nuclear factor-κB activation. Cardiology 2013; 124: 11-17.

[14] Zhang XG, Zhang YQ, Zhao DK, Wu JX, Zhao J, Jiao XM, Chen B and Lv XF. Relationship between blood glucose fluctuation and macrovascular endothelial dysfunction in type 2 diabetic patients with coronary heart disease. Eur Rev Med Pharmacol Sci 2014; 18: 3593-3600.

[15] Slover RH, Welsh JB, Criego A, Weinzimer SA, Willi SM, Wood MA and Tamborlane WV. Effectiveness of sensor-augmented pump therapy in children and adolescents with type 1 diabetes in the STAR 3 study. Pediatr Diabetes 2012; 13: 6-11.

[16] Zhang Z, Li J, Yang L, Chen R, Yang R, Zhang H, Cai D and Chen H. The cytotoxic role of intermittent high glucose on apoptosis and cell viability in pancreatic beta cells. J Diabetes Res 2014; 2014: 712781.

[17] Saito S, Teshima Y, Fukui A, Kondo H, Nishio S, Nakagawa M, Saikawa T and Takahashi N. Glucose fluctuations increase the incidence of atrial fibrillation in diabetic rats. Cardiovasc Res 2014; 104: 5-14.

[18] Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP and Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006; 295: 1681-1687.

[19] Monnier L, Colette C, Mas E, Michel F, Cristol JP, Boegner C and Owens DR. Regulation of oxidative stress by glycaemic control: evidence for an independent inhibitory effect of insulin therapy. Diabetologia 2010; 53: 562-571.

[20] Altıncık A, Tuğlu B, Demir K, Çatlı G, Abacı A and Böber E. Relationship between oxidative stress and blood glucose fluctuations evaluat-

ed with daily glucose monitoring in children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2016; 29: 435-439.

[21] Chen Q, Yu W, Shi J, Shen J, Gao T, Zhang J, Xi F, Li J and Li N. Insulin alleviates the inflammatory response and oxidative stress injury in cerebral tissues in septic rats. J Inflamm (Lond) 2014; 11: 18.

[22] Megson IL, Treweeke AT, Shaw A, MacRury SM, Setford S, Frias JP and Anhalt H. Continuous subcutaneous insulin infusion in patients with type 2 diabetes: a cohort study to establish the relationship between glucose control and plasma oxidized low density lipoprotein. J Diabetes Sci Technol 2015; 9: 573-580.

[23] Monnier L, Colette C, Michel F, Cristol JP and Owens DR. Insulin therapy has a complex relationship with measure of oxidative stress in type 2 diabetes: a case for further study. Diabetes Metab Res Rev 2011; 27: 348-353.

[24] Faienza MF, Francavilla R, Goffredo R, Ventura A, Marzano F, Panzarino G, Marinelli G, Cavallo L and Di Bitonto G. Oxidative stress in obesity and metabolic syndrome in children and adolescents. Horm Res Paediatr 2012; 78: 158-164.