Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes

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Abstract

Aims/hypothesis Glucose fluctuations may help predict diabetic complications. We evaluated the relation between glucose variability and oxidative stress in patients with type 1 diabetes.

Methods Continuous glucose monitors were inserted subcutaneously in 25 patients. During the measurement, patients collected two 24 h urine samples, while 24 healthy controls collected one 24 h urine sample for determination of 15(*S*)-8-*iso*-prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) using HPLC tandem mass spectrometry. Mean of the daily differences (MODD), mean amplitude of glycaemic excursions (MAGE) and continuous overlapping net glycaemic action calculated with *n* hour time-intervals (CONGA-*n*) were calculated as markers for glucose variability and correlation with 15(*S*)-8-*iso*-PGF $_{2\alpha}$ excretion was calculated.

Results Median [interquartile range (IQR)] urinary 15(*S*)-8-*iso*-PGF $_{2\alpha}$ was higher in patients than healthy controls:

161 (140–217) pg/mg creatinine vs 118 (101–146) pg/mg creatinine ($p=0.001$). Median (IQR) MODD was 3.7 (3.2–5.0) mmol/l, MAGE 7.6 (6.4–9.0) mmol/l and CONGA-1 2.3 (2.1–2.8) mmol/l. Univariate regression did not reveal an association for MODD ($r^2=0.01$), MAGE (0.08) or CONGA-1 (0.07) with 15(*S*)-8-*iso*-PGF $_{2\alpha}$ excretion, nor was an association revealed when corrected for HbA $_{1c}$, age, sex and smoking. Spearman correlation coefficients (r) between 15(*S*)-8-*iso*-PGF $_{2\alpha}$ excretion and MODD, MAGE and CONGA-1 were non-significant: -0.112 , -0.381 and -0.177 . **Conclusions/interpretation** We report that there is no relationship between glucose variability and urinary 15(*S*)-8-*iso*-PGF $_{2\alpha}$. We also confirm that patients with type 1 diabetes have higher levels of urinary 15(*S*)-8-*iso*-PGF $_{2\alpha}$ than healthy controls, suggesting that in addition to glucose variability, other factors favouring oxidative stress may exist. We did not see a relation between high glucose variability and elevated levels of oxidative stress in patients with type 1 diabetes.

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Keywords Complications · Continuous glucose monitoring · Glucose variability · Oxidative stress · Type 1 diabetes · Urinary 15(*S*)-8-*iso*-PGF $_{2\alpha}$

Abbreviations

CONGA-*n* continuous overlapping net glycaemic action calculated with *n* hour time-intervals
HPLC HPLC tandem mass spectrometry
MS/MS
IQR interquartile range
MAGE mean amplitude of glycaemic excursions
MODD mean of the daily differences
PGF $_{2\alpha}$ prostaglandin $F_{2\alpha}$

Introduction

Hyperglycaemia is the main risk factor for developing microvascular complications and accelerates atherosclerosis in diabetes [1, 2]. The present paradigm postulates that mitochondrial glucose overload results in increased electron transfer to oxygen and formation of free oxygen radicals. This in turn activates four pathways along which hyperglycaemia can induce diabetic complications [3]. There is also some evidence that hypoglycaemia may contribute to platelet activation and thereby to macrovascular complications. The mechanisms through which both hyper- and hypoglycaemia contribute to diabetic complications is schematically depicted in Fig. 1 [4, 5].

Recent studies with continuous glucose sensors found a large range of glucose values in children with type 1 diabetes, even in those with excellent HbA_{1c} values, which raises the possibility that in addition to HbA_{1c}, glucose variability may have predictive value for the development of diabetic complications [6–8]. Although this has been negated for microvascular complications [9], it is an attractive hypothesis to explain the increased macrovascular burden of type 1 diabetes [10]. A recent study investigated the effect of long-term glycaemic exposure, as indicated by HbA_{1c}, and acute glucose fluctuations quantified with a continuous glucose monitor on the activation of oxidative stress in 21 type 2 diabetic patients on either oral glucose-lowering medication or a weight-controlling diet and compared activation of oxidative stress with age and sex-matched healthy control persons. This study revealed significantly more oxidative stress in patients with type 2 diabetes than in healthy control persons and demonstrated that acute glucose fluctuations in particular induce oxidative stress. These fluctuations were suggested to be valuable predictors for the risk of diabetic complications [11]. Activation of oxidative stress was estimated from the measurement of 24 h urinary excretion of 8-*iso*-prostaglandin

F_{2α}(PGF_{2α}). Because isoprostanes are stable products from free oxygen radical-mediated oxidation of arachidonic acid and have been demonstrated to increase with oxidant injury, they are considered good indicators of oxidative stress [12, 13]. The daily glucose fluctuations were quantified with the mean amplitude of glucose excursions (MAGE), calculated from the continuous sensor readings [11].

To date, the role of acute glucose fluctuations in activation of oxidative stress has not been investigated in patients with type 1 diabetes. Insulin therapy, higher insulin sensitivity and affected counter-regulation to hypoglycaemia will contribute to more pronounced glucose fluctuations in patients with type 1 diabetes than in those with type 2 diabetes. The aim of our study was to evaluate the role of glucose variability, assessed using a continuous glucose monitor, in the activation of oxidative stress in patients with type 1 diabetes and to determine the amount of oxidative stress in these patients as compared with healthy control persons.

Methods

Patients

The study was approved by the local ethics committee and participants gave written informed consent. Twenty-five patients with type 1 diabetes and 24 age- and sex-matched healthy controls were enrolled in the study. The main clinical characteristics are listed in Table 1. Hypertension was defined as receiving antihypertensive treatment. Diabetic retinopathy, nephropathy and neuropathy were defined as having had laser coagulation, microalbuminuria requiring therapy with ACE inhibition and altered or diminished sensibility in hands and/or feet, respectively. Macrovascular complications were defined as coronary artery diseases, e.g. angina pectoris or acute myocardial infarction, and/or cerebrovascular events in the medical history. Seven patients were using an insulin pump, all with a rapid-acting analogue as pump insulin, and 18 patients were being treated with multiple daily injections. Of the latter, 13 were on a combination of long and rapid-acting insulin analogues (glargine, detemir and lispro, aspart), one on human insulin only, three on a combination of rapid-acting human insulin and basal insulin analogues and one on rapid-acting insulin analogues and basal human insulin.

Exclusion criteria were a BMI >30 kg/m², a GFR of less than 60 ml min⁻¹ 1.73 m⁻² according to the Cockcroft–Gault formula [14], heparin, oral anticoagulants or corticosteroid use, skin conditions prohibiting sensor needle insertion and any febrile illnesses during the 3 month period prior to the experiment.

Fig. 1 Overview of the mechanism of hyper- and hypoglycaemia-induced complications. GADPH, glyceraldehyde-3 phosphate dehydrogenase; PARP, poly (ADP ribose) polymerase; PKC, protein kinase C

Table 1 Clinical characteristics

Characteristics	Patients	Controls	<i>p</i> value
Men/women (<i>n</i>)	11/14	11/13	0.897 ^c
Age (years) ^a	39.0 (28.5–49.5)	35.0 (29.0–44.0)	0.881 ^b
Cigarette smoking [<i>n</i> (%)]	8 (32)	4 (16.7)	0.212 ^c
BMI (kg/m ²)	23.3±2.1	22.7±3.2	0.414 ^d
Diabetes duration (years)	21.1±11.4		
Diabetes treatment [<i>n</i> (%)]			
Insulin pump	7 (28)		
Multiple daily injections	18 (72)		
Total daily insulin dose (IU/24 h)	48.7 (16.3)		
Complications [<i>n</i> (%)]			
Retinopathy	6 (24)		
Nephropathy	4 (16)		
Neuropathy	3 (12)		
Macroangiopathy	2 (8)		
Systolic blood pressure (mmHg)	122±16		
Diastolic blood pressure (mmHg)	73±11		
Hypertension [<i>n</i> (%)]	4 (16)		
HbA _{1c} (%)	8.1±0.9		
HDL-cholesterol (mmol/l)	1.74±0.34		
Total cholesterol (mmol/l)	4.28±0.68		
Triacylglycerol (mmol/l)	0.69±0.31		

Unless otherwise stated, data are means±SD or ^a median (IQR)

^b Patients vs controls, Mann–Whitney *U* test

^c Patients vs controls, Pearson's χ^2 test

^d Patients vs controls, independent samples *t* test

Study protocol

The experiment consisted of two short visits to the clinical trial room. During the first visit the continuous glucose sensor (CGMSgold; MedtronicMiniMed, Sylmar, CA, USA) was inserted subcutaneously in the abdominal periumbilical area of the patients and, if present, the insulin pump on the other side. Patients injecting insulin were asked not to inject on the same side as the sensor insertion side in the week prior to and during the period of measurement. Home blood glucose meters (Accu-Check Compact Plus; Roche Diagnostics, Quebec, Canada) were provided to perform the sensor calibration procedure four times daily. Information about diabetes duration, complications and current mode and dose of insulin therapy was recorded in the case record form. A venous blood sample was collected for HbA_{1c} determination. Patients were asked to collect urine during two consecutive 24 h periods. They were instructed to preserve the urine-containing jars in the refrigerator when at home and in special cooling bags when outdoors, in order to avoid artificial formation of isoprostanes. For optimal prevention of artificial isoprostane formation, patients noted the total urine volume after 24 h, mixed the urine thoroughly, and divided 14 ml urine equally into two tubes, which were placed in a freezer until the second visit. The same procedure was done with the second 24 h urine collection. In addition, patients received a study diary to record their meals and symptomatic hypo- or hyperglycaemic events. Three days later, patients returned to hospital to have the sensor removed and data

downloaded, and to hand in the frozen urine tubes together with the study diary. Healthy controls were asked to do one 24 h urine collection, as described above for the patients with type 1 diabetes. For both the patients and healthy controls, age, concomitant medication, smoking, height and weight and the resulting BMI were recorded. As the association with lower oxidative stress levels is still controversial [15–18], participants were asked not to take vitamin E tablets during the experiment. Finally, the urine-containing tubes were stored at –80°C until analyses for 15(*S*)-8-*iso*-PGF_{2α} and creatinine in one run.

Laboratory measurements

HbA_{1c} was measured using an HPLC assay (Variant II; Bio-Rad Laboratories, Montreal, QC, Canada). Activation of oxidative stress was estimated by determining the urinary isoprostane excretion (15(*S*)-8-*iso*-PGF_{2α}), using HPLC tandem mass spectrometry (HPLC-MS/MS). Urine samples were collected and stored without additives as 7 ml aliquots at –80°C. Initial creatinine concentrations were established by colorimetric Jaffé assay. Two millilitres of sample was mixed with ²H₄-labelled 15(*S*)-8-*iso*-PGF_{2α} as internal standard and applied to an 8-*iso*-PGF_{2α} immunoaffinity column (CaymanChemical Company, Ann Arbor, MI, USA). After washing, the extract was eluted, evaporated to dryness (60°C, N₂) and reconstituted in 200 µl 0.05 mol/l formic acid–ethanol (75:25, vol./vol.); 50 µl was injected on the HPLC-MS/MS. Chromatographic separation was achieved on a modular HPLC system (Surveyor; Thermo Finnigan,

San Jose, CA, USA) consisting of a cooled autosampler ($T=12^{\circ}\text{C}$), a low-flow quaternary MS pump and analytical HPLC column: 2.1×150 mm, $5\text{ }\mu\text{m}$ Alltech C8 (Alltech Lexington, KT, USA). Samples were eluted with a flow rate of $200\text{ }\mu\text{l/min}$ and a programmed gradient between A ($\text{HCOOH-H}_2\text{O}$; 0.01% , vol./vol.) and B (CH_3CN). MS/MS analyses were performed on a TSQ Quantum AM (Thermo Finnigan) operated in the positive ion electrospray ionisation mode. The surface-induced dissociation was set at 15 V ; spray voltage was $3,000\text{ V}$ and the capillary temperature was 300°C . In the MS/MS experiments argon was used as collision gas at a pressure of 0.2 Pa ; collision energy was 20 eV for the optimised transitions: $m/z\ 353.24\rightarrow m/z\ 193$ and $m/z\ 357.24\rightarrow m/z\ 197.10$. The inter-assay ($n=5$) and intra-assay (average of 5 days, $n=3$) variability allowed for determination at physiological concentrations with a CV $<7\%$.

Assessment of glycaemic variability

Inter-day glycaemic variability The day-to-day variation of the glucose pattern was calculated with the mean of the daily differences (MODD), which is defined as the mean of the absolute differences between glucose values on day 2 and the corresponding values on day 1, at the same time [19, 20].

Intra-day glycaemic variability Because there is no consensus on how to optimally assess glucose variability and to prevent the results from being affected by the method chosen, we performed multiple calculations to estimate the glucose variability. The easiest measure is the CV defined as the SD divided by the mean of the glucose values. Another marker of glucose variability is the MAGE, designed by Service et al. [21] in 1970. The MAGE over

24 h is the mean of the absolute differences between peak and nadir values over 24 h, with peaks (nadirs) defined as glucose values preceded and followed by an increase (decrease) and decrease (increase), respectively, in excess of at least 1 SD of the mean glucose. If a decrease of more than 1 SD was the first excursion, only peak-to-nadir excursions (>1 SD) were included in the calculation of the MAGE and vice versa. We calculated the MAGE over both 24 h urine collection periods separately and over the two periods taken together.

Rapid glucose variability We also calculated the recently proposed continuous overlapping net glycaemic action calculated with n hour time-intervals (CONGA- n), to gain insight into the rapid glucose variability. With this method, the difference between each glucose reading and the glucose reading n hours previously (as determined by the investigator) is calculated. The CONGA- n is the standard deviation of these differences. We used CONGA-1, indicating intra-day variability based on 1 h time differences, to allow for comparison of our results with those reported in other articles [19, 22]. In normal non-diabetic participants, CONGA values vary between 0.4 and 1.2: values above 1.5 indicate glycaemic lability [19]. Furthermore, we calculated the within-hour glucose SD and range, as markers of rapid glucose variability.

Statistical analysis

Mean urinary excretion of $15(S)\text{-}8\text{-iso-PGF}_{2\alpha}$, inter- and intra-day and rapid glucose variability were assessed using standard statistics. The intra-individual day-to-day variation of the above-mentioned variables was calculated using a paired samples t test or Wilcoxon's signed rank test, depending on the distribution of the data. Oxidative stress was

Table 2 Glucose variability and glycaemic excursions

	CGM, urine collection 1	CGM, urine collection 2	CGM, collections 1+2	p value
Mean glucose (mmol/l)	9.0 (± 2.2)	9.3 (± 1.8)	9.1 (± 1.8)	0.465
SD (mmol/l)	3.29 (± 1.07)	3.45 (± 1.28)	3.61 (± 1.05)	0.654
Inter-day variation				
MODD (mmol/l)	NA	NA	3.7 (3.2–5.0)	NA
Intra-day variation				
CV	0.37 (0.29–0.44)	0.37 (0.30–0.44)	0.42 (0.31–0.47)	1.0
MAGE (mmol/l)	6.9 (6.2–8.7)	7.8 (5.2–9.7)	7.6 (6.4–9.0)	0.977
Rapid glucose variability				
CONGA-1	2.5 (2.0–2.7)	2.5 (1.9–3.1)	2.3 (2.1–2.8)	0.195
Within-hour range (mmol/l)	2.0 (1.7–2.2)	2.1 (1.6–2.8)	2.0 (1.7–2.5)	0.057
Within-hour SD (mmol/l)	0.69 (0.58–0.74)	0.70 (0.55–0.95)	0.66 (0.58–0.84)	0.076

Variability and excursions were indicated by several markers, derived from continuous glucose monitoring data during the first, second and total 48 h of urine collections, respectively

Data are means ($\pm$ SD) or medians (25th–75th percentile)

CGM, continuous glucose monitoring

Fig. 2 Urinary excretion of 15(*S*)-8-*iso*-PGF_{2α} for all 25 patients with type 1 diabetes during the first (black bars) and second (grey bars) 24 h urine collection

compared between the patients with type 1 diabetes and the age and sex-matched healthy controls, using an independent samples *t* test or Mann–Whitney *U* test, again depending on the distribution of the data. Correlation was calculated and univariate regression analysis was performed to evaluate the relation between the excretion rate of urinary 15(*S*)-8-*iso*-PGF_{2α} and the markers of glycaemic variability, results being corrected in multivariate regression analysis for long-term glycaemic control as indicated by the HbA_{1c} level [15, 23], sex [24], age [25] and smoking [17, 18, 26], all variables that have been reported to be involved in the activation of oxidative stress. For lipid values and blood pressure, we also evaluated the association with urinary excretion of 15(*S*)-8-*iso*-PGF_{2α} in a univariate model. In the event of a significant relation in the univariate analysis, variables were included in a multivariate model to evaluate the determinants for oxidative stress. *p* values of <0.05 were considered significant.

Results

One patient discontinued the sensor measurement after approximately 24 h, because of a putative allergic reaction against the inserted sensor needle. Patients wore the continuous glucose monitor for a period ranging from 50 to 72 h. In total, data of continuous glucose measurements during 49 24-h periods, together with the concomitant urinary 15(*S*)-8-*iso*-PGF_{2α} excretion levels, were obtained in 25 patients with type 1 diabetes. The 15(*S*)-8-*iso*-PGF_{2α} excretion levels during a single 24-h period were obtained in 24 age- and sex-matched healthy controls. There were no major differences in age, BMI and smoking between the two groups (see Table 1).

Data on inter-day, intra-day and rapid glycaemic variability are summarised in Table 2. The median (interquartile range [IQR]) urinary excretion of 15(*S*)-8-*iso*-PGF_{2α}, calculated over two urine collection periods, in the patient group was 161 (140–217) pg/mg creatinine (Fig. 2). There was no significant difference in 8-*iso*-PGF_{2α} excretion between the first and the second urine collection (172 [147–231] pg/mg creatinine vs 157 [139–218] pg/mg creatinine, *p*=0.162, Wilcoxon signed rank test), while the between-patient variability significantly exceeded the within-patient variability (*p*=0.009, Kruskal–Wallis test). The median (IQR) urinary excretion of 15(*S*)-8-*iso*-PGF_{2α} was significantly lower in healthy control persons than in diabetes patients: 118 (101–146) pg/mg creatinine (*p*=0.001, Mann–Whitney *U* test). Data on MAGE and oxidative stress, assessed in the patients with type 1 diabetes, are plotted in Fig. 3, clearly illustrating the absence of any relationship between these two variables. Univariate regression analysis revealed that none of the markers of glucose variability were associated with the urinary excretion rate of 15(*S*)-8-*iso*-PGF_{2α} (an-

Fig. 3 Scatter plot showing MAGE vs oxidative stress. A regression line could not be drawn

Table 3 Spearman correlation coefficients between urinary excretion rates of 15(*S*)-8-*iso*-PGF_{2α} and markers of glycaemic variability, glycaemic control and some variables that were earlier reported to be involved in the activation of oxidative stress

	MODD	HbA _{1c}	Sex	Age	Smoking	CV	MAGE	CONGA-1	Within-hour range	Within-hour SD
HbA _{1c}	0.261									
Sex ^a	−0.024	0.028								
Age	0.062	−0.124	0.073							
Smoking ^b	−0.305	0.074	0.943 ^c	−0.327						
CV	0.739 ^c	0.023	−0.110	−0.015	−0.146					
MAGE	0.749 ^c	0.371	−0.073	0.123	−0.166	0.613 ^d				
CONGA-1	0.617 ^d	0.202	−0.073	−0.026	−0.074	0.391	0.665 ^c			
Within-hour range	0.459 ^d	0.169	−0.135	0.007	−0.106	0.151	0.539 ^d	0.924 ^c		
Within-hour SD	0.482 ^d	0.164	−0.141	0.047	−0.133	0.189	0.530 ^d	0.934	0.991 ^c	
8- <i>iso</i> -PGF	−0.112	0.028	0.281	−0.289	0.102	0.048	−0.381	−0.177	−0.26	−0.285

^a Labelled as 1=male, 2=female^b Labelled as 0=non-smoking, 1=smoking^c Pearson χ^2 ^d $p < 0.05$; ^e $p < 0.0001$ 8-*iso*-PGF, urinary excretion of 15(*S*)-8-*iso*-PGF_{2α}

analysed as dependent variable, with maximum r^2 values of 0.01, 0.08 and 0.07 for the inter-day, intra-day and rapid glucose variability, respectively). In addition, no significant relationship was found between blood pressure, hypertension and lipid values (HDL-cholesterol, total cholesterol, triacylglycerol) on the one hand, and the amount, on the other hand, of oxidative stress, as can be seen from poor r^2 values ranging from 0.002 ($p=0.85$) for triacylglycerol values to 0.056 ($p=0.364$) for systolic blood pressure. Correction for HbA_{1c}, sex, age and smoking did not reveal a significant association for any of the markers. Regression analysis, with and without correction for HbA_{1c}, age and smoking, revealed that sex had the strongest, though very poor, association with oxidative stress ($r^2=0.1$, $p=0.085$). Regression analysis over the total study group, including patients and control persons, revealed that smoking, but not sex and age, had a significant though weak association ($r^2=0.14$, $p=0.036$) with oxidative stress.

Correlations between 15(*S*)-8-*iso*-PGF_{2α} and the markers of glucose variability are given in Table 3. Again, none of the markers of glucose variability were associated with the urinary excretion rate of 15(*S*)-8-*iso*-PGF_{2α}.

Discussion

In this study, none of the measures of glucose variability was associated with oxidative stress, estimated by the urinary excretion of 15(*S*)-8-*iso*-PGF_{2α}, in patients with type 1 diabetes. In accordance with previously reported studies, our results confirm that patients with type 1 diabetes have higher levels of oxidative stress than healthy controls matched for age and sex [15, 23, 27, 28].

Because MAGE depends on the frequency of glucose measurements and the direction, either peak-to-nadir or nadir-to-peak, of the glucose excursions [21], it is open to several interpretations and is not acknowledged as the gold standard for glucose variability. So, to account for the influence of the method chosen, we calculated various different markers for glucose variability. The mean MODD and CONGA-1 for the 25 patients in our study appeared to be similar to values reported for ten patients with type 1 diabetes in an earlier study [19] (MODD, 4.0 mmol/l vs 4.3 mmol/l and CONGA-1, 2.5 mmol/l, in that and our studies). The mean MAGE in our study was higher than previously reported for 21 patients with type 2 diabetes (8.3 vs 4.2 mmol/l) [11], confirming that glucose variability in patients with type 1 diabetes is higher than in those with type 2 diabetes. Thus, based on the higher MAGE and the correlation reported earlier between glucose variability and the activation of oxidative stress, we expected a strong association between the excretion of 15(*S*)-8-*iso*-PGF_{2α} and glucose variability in our study group [11]. Our failure to find a relationship between glucose variability and oxidative stress contrasts sharply with the strong correlation ($r=0.86$) between MAGE and urinary excretion of 8-*iso*-PGF_{2α}, as previously reported for patients with type 2 diabetes [11]. With MAGE as the only reported marker of glucose variability, that study of patients with type 2 diabetes does not allow a complete comparison with respect to glucose variability measures in relation to oxidative stress.

According to our data, HbA_{1c}, sex, age and smoking were not related to oxidative stress. The role of these factors in the activation of oxidative stress is being debated, given the fact that other studies were either unable to detect an

association for HbA_{1c} [29] and age [24, 26] with oxidative stress or reported conflicting data on the effect of sex, with both male [30, 31] and female [24] sex being associated with increased activation of oxidative stress. The limited number of smokers might explain why our results on smoking are not in accordance with earlier reports on the association between smoking and elevated urinary excretion of 8-isoprostanes [17, 18, 26].

An explanation for the lack of correlation between glucose variability and oxidative stress may be that, unlike patients with type 2 diabetes, patients with type 1 diabetes are not sensitive to glucose variability as a stimulator of oxidative stress, because of different underlying pathophysiological mechanisms [28, 32–35]. The significantly higher excretion of 15(*S*)-8-*iso*-PGF_{2α} in patients with type 1 diabetes as compared with healthy controls does suggest the existence of a factor favouring oxidative stress. However, glucose variability, despite being greater than in type 2 diabetes, does not seem to be this factor.

At first glance, the excretion of 15(*S*)-8-*iso*-PGF_{2α} in patients and healthy control persons was substantially lower in our study than in several previous studies [11, 15]. Rather than an immunoassay, we used tandem mass spectrometry, a modern and precise method, which is not sensitive to components other than 8-isoprostanes. This sensitivity has been acknowledged to be a possible confounding factor when using an immunoassay [11]. The method used in our study selectively determined 15(*S*)-8-*iso*-PGF_{2α}, while less specific methods include its enantiomere ent-15(*S*)-8-*iso*-PGF_{2α} in the quantification of oxidative stress. This is why in the literature 15(*S*)-8-*iso*-PGF_{2α} levels are often reported to be higher.

In conclusion, we report that there is no relationship between urinary excretion of 15(*S*)-8-*iso*-PGF_{2α}, determined with a highly specific method, and various measures of glucose variability. Our findings confirm that the amount of oxidative stress, estimated by urinary excretion of 15(*S*)-8-*iso*-PGF_{2α}, is higher in patients with type 1 diabetes than in healthy control persons, arguing against a role of glucose variability in the activation of oxidative stress.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript. The sensors were made available free of charge by Medtronic NL (Heerlen, the Netherlands).

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