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Assessment of glucose variability in subjects with prediabetes

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ABSTRACT

The **aim** of the study was to assess glucose variability in subjects with prediabetes by means of CGM. **Material and methods:** 32 subjects with prediabetes – mean age 56.6 ± 9.6 years, mean BMI 30.3 ± 5.3 kg/m^2 and 18 subjects with normal glucose tolerance (NGT) – mean age 54.4 ± 9.9 years, mean BMI 24.8 ± 6.9 kg/m^2, were enrolled. Glucose tolerance was studied during OGTT. HbA1c was measured by NGSP certified method. CGM was performed with FreeStyle Libre Pro sensor. **Results:** The following indices of glucose variability were significantly higher in the prediabetes group – CV ($p < 0.041$), J-index ($p < 0.014$), CONGA ($p < 0.047$) and GRADE ($p < 0.036$). A significant increase in HbA1c ($p < 0.036$), mean interstitial glucose ($p < 0.025$), time above range ($p < 0.018$) and a significant decrease in time in range ($p < 0.014$) was found in prediabetes compared to NGT. Significant correlations between HbA1c and LBGI ($r = -0.33$, $p = 0.02$), HBGI ($r = 0.31$, $p = 0.03$), CONGA ($r = 0.36$, $p = 0.01$), J-index ($r = 0.37$, $p = 0.01$) and M–value ($r = -0.34$, $p = 0.02$) were established. **Conclusion:** Glucose variability is significantly increased in prediabetes and is an additional parameter in the assessment of glucose homeostasis even at these early stages of glucose dysregulation.

1. Introduction

Glucose variability, in particular its role in the development of both acute and chronic complications of diabetes, is currently among the most discussed issues in diabetology [1–8]. The development of technology and the implementation of continuous glucose monitoring (CGM) in routine clinical practice provide new opportunities for glycemic control assessment [8–10]. There is growing evidence on the importance of this new criterion for glycemic control as it provides different information from the classical and "gold standard" criterion which has been established and used for many years in clinical practice – glycated hemoglobin (HbA1c). The significance of glucose variability in the complex evaluation of glycemic control in diabetes, as well as the best mathematical models for its assessment, are yet to be determined [8–13].

Glucose variability in prediabetes and in normal glucose tolerance (NGT), as defined by the current diagnostic criteria for glucose tolerance based on plasma glucose (fasting and 2-hour during OGTT) and HbA1c, is a subject even less investigated. The analysis of glucose variability in prediabetes would answer the question about its potential role in the development of chronic complications as it is well known that some are present in a substantial part of subjects with predi-

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abetes. It would also address the topic of its importance for their development as compared to mean glycaemia assessed by HbA1c [14–17]. In addition, the assessment of glucose variability in prediabetes and normoglycaemia, as they are defined by the currently established diagnostic criteria, could probably play an important role in the diagnosis of glucose tolerance independently of the current diagnostic criteria. Finally, it could possibly provide additional and more precise ways for the classification of glucose tolerance and for the stratification of the risk for diabetes, cardiovascular disease and chronic complications in prediabetes and even in some subjects with normal glucose tolerance [18–25].

The published until now data on CGM in prediabetes and NGT provide a different view on glucose homeostasis and question the accuracy and sufficiency of the currently established diagnostic tools – OGTT and HbA1c. CGM data reveal glucose concentrations exceeding the diagnostic thresholds for these categories; moreover, they show glucose values spreading in the diabetes range even in some subjects with NGT [22–26]. Studies which have explored directly the role of glucose variability in the assessment of glucose tolerance have demonstrated that the indices of glucose variability have a very high sensitivity in differentiating between prediabetes and NGT and a lower one in distinguishing between diabetes and prediabetes [19]. Another unresolved issue is what comes first in the course of glucose regulation impairment – the changes in glucose variability or in mean glycaemia as assessed by HbA1c [27].

In general, the published up to now data indicate that glucose variability could play a promising role in the diagnostic, clinical and prognostic assessment of glucose homeostasis in subjects without diabetes.

2. Aim

The **aim** of the present study was to assess glucose variability in subjects with prediabetes by means of continuous glucose monitoring.

3. Material and methods

3.1. *Material*

50 subjects (12 males, 38 females) were enrolled in the study. According to glucose tolerance they were divided into two groups – 18 with normal glucose tolerance (NGT) and 32 with prediabetes, including 24 subjects with impaired fasting glucose (IFG) and 8 subjects with impaired glucose tolerance (IGT). The characteristics of the subjects in the two groups are presented in Table 1. Subjects taking medications that could affect glucose metabolism such as anti-diabetic and anti-obesity drugs, corticosteroids and antipsychotics, subjects with serious comorbidities and malignancies as well as pregnant women were not included in the study.

The participants were recruited at the Department of Diabetology of the University Hospital of Endocrinology, Sofia, among subjects participating in an ongoing program for screening and prevention of type 2 diabetes. These are subjects with at least one risk factor for type 2 diabetes – first degree relative with diabetes, overweight/obesity, hypertension, dislipidemia, history of previously detected prediabetes, women with PCOS, history of gestational diabetes or delivery of a baby greater than 4 kg, as well as subjects with other components of the metabolic syndrome such as insulin resistance, hyperuricemia and non-alcoholic fatty liver disease, referred to the department for evaluation.

3.2. *Methods*

All participants received detailed information about the nature and aim of the study and signed informed consent. The study was approved by the Ethics Committee of the Medical University of Sofia. A full medical history was taken from and a physical examination was performed on each participant. Continuous glucose monitoring was then performed with professional blind sensor for a 14-day period in routine everyday setting. Glucose tolerance was assessed during a standard OGTT with 75 g glucose and by glycated hemoglobin (HbA1c). An electronic database was created and analyzed with a special software.

3.2.1. *Continuous glucose monitoring*

In the present study professional sensors for continuous glucose monitoring FreeStyle Libre Pro (Abbot GmbH & KG) were used. The sensor measures the glucose concentration in the interstitial fluid by a glucose oxidase method every 15 min for a period of 14 days (336 h), with an overall number of 1344 measurements for the defined period [28]. The sensor is blind as it has been developed for professional use only. This means that the participant does not get information on the glucose levels during the study period and thus the continuous glucose monitoring is retrospective, and not a real-time one. The study is completely blind as the sensor used is factory-calibrated and does not require additional calibration with a glucose meter. As the study is a non-interventional one, the participants were not instructed to follow a certain dietary pattern or physical activity regimen. On the contrary, they were advised to stick to their usual lifestyle regardless of the sensor in order to obtain a real picture of the glucose levels and excursions under everyday conditions. The sensors were inserted on the lateral side of the arm according to its instructions for use, and removed by the researchers at the Department of Diabetology. The mean period of sensor use was 13.6 ± 2.3 days. The shorter than 14 days period in some of the subjects was due to technical issues – detachment of the sensor or discontinuation after mechanical strike. The sensor data were downloaded, processed, visualized and archived with a licensed software. A glucose concentration of 3 mmol/l was fixed as the lower limit and of 7.8 mmol/l as the upper limit of the target range. Data on time in range, time below range and time above range in percentage as well as on mean glucose concentration for the studied period in mmol/l were obtained. An interstitial glucose concentration ≥ 11.1 mmol/l in a minimum of two consecutive measurements, i.e. lasting for ≥15 min, was defined as hyperglycaemia in the diabetes range. The currently adopted in the consensus on the use of CGM indices of glucose variability – the standard deviation (SD) and the coefficient of variation (CV), were calculated by means of

Table 1 – Characteristics of the study groups.

	NGT	Prediabetes	P
Number	18	32	–
Male/female	4/14	8/24	0.460
Age [years]	54.4 ± 9.9	56.8 ± 9.6	0.065
BMI [kg/m^2]	24.8 ± 6.9	30.3 ± 5.3	**0.004**
Waist circumference [cm]	85.7 ± 13.2	102.4 ± 12.9	**0.0001**
Glucose 0 min [mmol/l]	5.6 ± 0.3	6.4 ± 0.3	**0.0001**
Glucose 120 min [mmol/l]	5.1 ± 1.4	6.5 ± 1.9	**0.013**
HbA1c [%]	5.48 ± 0.4	5.71 ± 0.3	**0.036**
Mean interstitial glucose [mmol/l]	5.2 ± 0.5	5.6 ± 0.6	**0.025**

The bold values represented the results with p value < 0.05.

standard software (Excel, Microsoft Office) on the basis of the downloaded raw sensor data on overall measured glucose concentrations for the 14-day period [29]. Some other currently used indices of glucose variability were also calculated by means of a special software – Easy GV version 9.0.R2: CONGA (continuous overall net glycemic action), MAGE (mean amplitude of glycemic excursions), MAG (mean absolute glucose), M-value, HBGI (high blood glucose index), LBGI (low blood glucose index), J-index, L-index (lability index), GRADE (glycemic risk assessment in diabetes equation). SD is the amount of variation or dispersion of a data set. It is calculated as the square root of the variance of the data set and is most accurate if values are normally distributed around the mean, which is often not the case with blood glucose. CV is based on the standard deviation with correction for the mean glucose. It is less influenced when data sets with widely different mean glucose values are compared. J-index is also calculated on the basis of SD and mean blood glucose and is a measure of both the mean level and variability of glycemia. The M-value is another hybrid measure of both variability and mean glycemia. In the original formula the M-value is calculated as the mean of the logarithmic transformation of the deviation from a reference value of six blood sugar measurements taken over a 24-h period plus an amplitude correction factor. MAGE is the average of all blood glucose excursions that are greater than 1 SD of all measures for a given glucose profile. CONGA is the standard deviation of the differences of glucose readings for a defined period of hours. MAG is calculated as the summed differences between sequential 7-point profiles per 24 h divided by the time in hours between the first and last measurement. L-index is based on the change in glucose levels over time. LBGI, HBGI and GRADE are estimates of the risk associated with a glucose profile and neither of them measures glucose fluctuations directly. LBGI and HBGI are estimates of the risk for hypo- and hyperglycemia. They are generated with a correction of the skewness of glycemia by a symmetrization process expanding the hypoglycemic and reducing the hyperglycemic range. Larger values of LBGI and HBGI indicate higher risk while a high GRADE score may be a result of either hyper- or hypoglycemia [2,6,7].

3.2.2. *Physical examinations*

Weight and height were measured and BMI was calculated for each subject using the formula BMI = weight [kg]: height [m]^2.

3.2.3. *Functional tests*

Glucose tolerance was studied during a standard OGTT with 75 g glucose with measurement of serum glucose at 0 min and 120 min applying 2006 WHO criteria for defining glucose tolerance categories.

3.2.4. *Laboratory measurements*

Biochemical parameters (glucose and HbA1c) were assessed by means of a biochemical analyzer Cobas Integra 400. Serum glucose was measured by a hexokinase enzyme method (Roche Diagnostics), HbA1c was measured in whole blood by immunoturbidimetric NGSP-certified method. (Roche Diagnostics).

3.2.5. *Statistical methods*

Statistical analysis of data was performed with SPSS vs. 21.0. The type of distribution of the studied variables was determined by using histograms and curves of normal distribution. The variables without normal distribution were analyzed after logarithmic transformation. Descriptive analysis, one-way ANOVA, analysis of covariances (ANCOVA) linear regression enter method and correlations with parametric (Pearson) coefficient were used for comparisons between the groups with different glucose tolerance. Data are presented as means with standard deviation for the variables with normal distribution and as medians with interquartile ranges for the variables without normal distribution. P-value of <0.05 (2-sided) was considered statistically significant.

4. Results

Subjects with prediabetes had significantly higher anthropometric indices, fasting and 2-h glucose values, HbA1c level and mean interstitial glucose concentration as compared to the subjects with NGT. The demographic, anthropometric and glycaemic characteristics of the studied groups are presented in Table 1.

4.1. *Comparison of the CGM derived parameters of glycemic control between the groups with normal glucose tolerance and prediabetes*

4.1.1. *Time in ranges*

Time in target was significantly decreased and time above target was significantly increased in the group with prediabetes

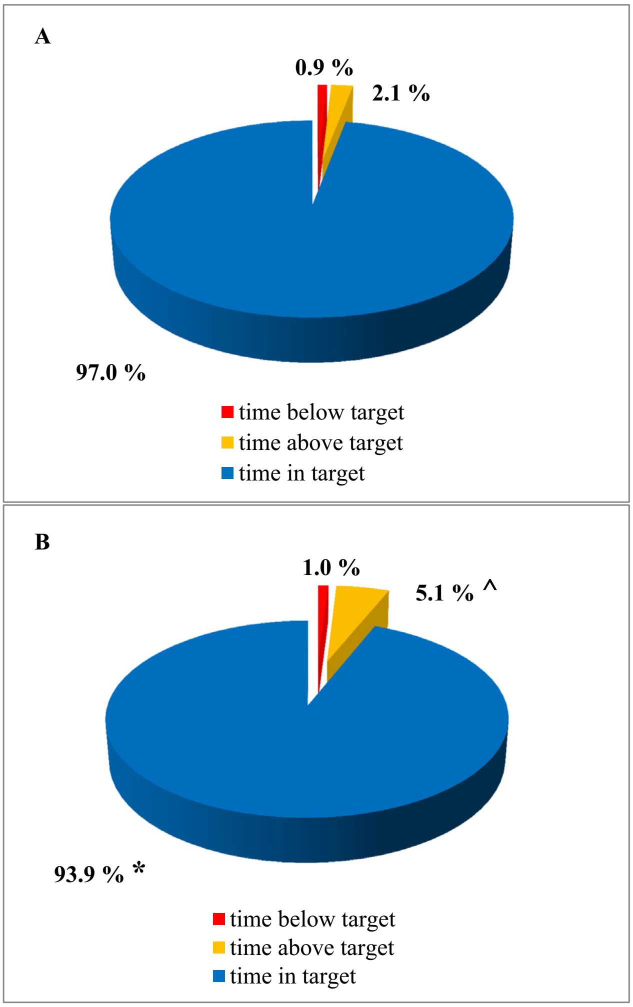

Fig. 1 – Time in ranges in the groups with normal glucose tolerance (NGT) (A) and prediabetes (B). *p = 0.014 vs. NGT; ^p = 0 018 vs. NGT.

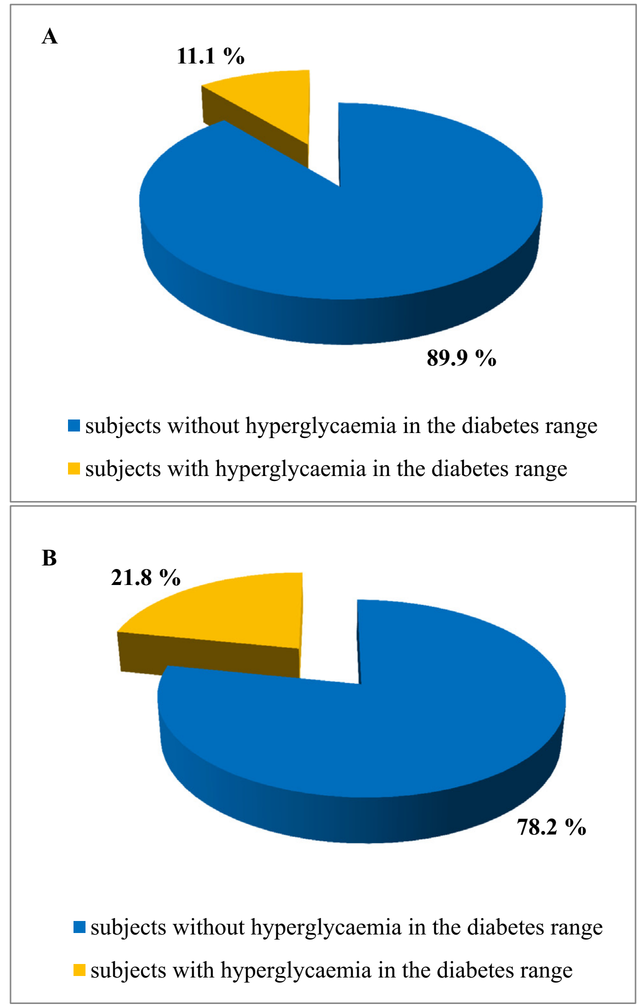

Fig. 2 – Percentage of subjects with hyperglycaemia in the diabetes range in the groups with normal glucose tolerance (NGT) (A) and prediabetes (B).

compared to the group with NGT. No difference was found in the time below target between the two studied groups (Fig. 1).

4.1.2. *Diabetic hyperglycaemia*

Diabeteic hyperglycaemia defined as at least two consecutive measurements ≥ 11.1 mmo/l was detected in 2 subjects (11%) of the group with NGT and in 7 subjects (21.8%) of the group with prediabetes (Fig. 2).

4.1.3. *Glucose variability indices*

When analyzing the indices of glucose variability, significantly higher CV, CONGA, J-index and GRADE were found in the group with prediabetes as compared to the group with NGT. After adjustment for BMI, SD, J-index, L-index and MAGE appeared to be significantly increased in the group with prediabetes as compared to the group with NGT (Table 2).

When dividing prediabetes into IFG and IGT, a significant difference was found in J-index being higher in IFG as compared to NGT (Table 3).

4.2. Association between glycated hemoglobin and indices of glucose variability

Significant correlation was established between HbA1c and some of the indices of glucose variability – CONGA, J-index, LBGI, HBGI and M-value. Fasting plasma glucose correlates significantly with CONGA, J-index, LBGI and M-value. No correlation was found for the 2-h postload glucose (Table 4).

5. Discussion

The present study evaluates glucose variability based on data from CGM at the early stages of glucose dysregulation. The group with prediabetes has the typical anthropometric and metabolic characteristics of this condition with significantly increased BMI, waist circumference, fasting and 2-h glucose as compared to the group with NGT. The comparison between the two study groups shows significant differences in both mean glycaemia, presented as glycated hemoglobin and

Table 2 – Indices of glucose variability in the groups with normal glucose tolerance (NGT) and prediabetes – unadjusted and adjusted for BMI analyses. Data are presented as means ± standard deviations for the variables with normal distribution and as medians with interquartile ranges for the variables without normal distribution.

	NGT (n = 18)	Prediabetes (n = 32)	P (unadjusted)	P (adjusted for BMI)
SD	0.99 (0.85–1.1)	1.07 (0.87–1.27)	0.093	**0.015**
% CV	19 ± 2	20 ± 4	**0.041**	0.059
CONGA	4.64 ± 0.43	4 95 ± 0.52	**0.047**	0.183
L-index	1.11 (0.72–1.37)	1.23 (0.93–1.94)	0.056	**0.016**
J-index	12.65 ± 2.38	14.86 ± 2.95	**0.014**	**0.029**
LBGI	2.91 (1.74–3.30)	2.24 (1.18–3.32)	0.170	0.568
HBGI	0.90 (0.55–1.18)	0.92 (0.71–1.44)	0.156	0.065
GRADE	0.37 (0.27–0.62)	0.62 (0.40–1.02)	**0.036**	0.094
MAGE	2.58 (2.25–2.86)	2.73 (2.35–3.51)	0.051	**0.005**
M-value	4.30 ± 3.10	3.28 ± 2.47	0.231	0.505
MAG	1.21 ± 0.19	1.29 ± 0.21	0.203	0.091

Table 3 – Indices of glucose variability in the groups with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and normal glucose tolerance (NGT). Data are presented as means ± standard deviations for the variables with normal distribution and as medians with interquartile ranges for the variables without normal distribution.

	NGT (n = 18)	IFG (n = 8)	IGT (n = 24)	p
SD	0.99 (0.85–1.1)	1.03 (0.87–1.27)	1.16 (0.86–1.43)	IFG vs NGT, p = 0.301 IGT vs NGT, p = 0.436 IGT vs IFG, p = 0.934
% CV	19 ± 2	19 ± 4	21 ± 5	IFG vs NGT, p = 0.936 IGT vs NGT, p = 0.642 IGT vs IFG, p = 0.801
CONGA	4.64 ± 0.43	4.98 ± 0.54	4.86 ± 0.49	IFG vs NGT, p = 0.096 IGT vs NGT, p = 0.650 IGT vs IFG, p = 0.918
L-index	1.11 (0.72–1.37)	1.23 (1.01–1.94)	1.17 (0.86–2.24)	IFG vs NGT, p = 0.137 IGT vs NGT, p = 0.668 IGT vs IFG, p = 0.995
J-index	12.65 ± 2.38	14.94 ± 3.06	14.64 ± 2.77	**IFG vs NGT, p = 0.039** IGT vs NGT, p = 0.295 IGT vs IFG, p = 0.992
LBGI	2.91 (1.74–3.30)	2.0 (1.11–3.18)	2.53 (1.63–4.19)	IFG vs NGT, p = 0.235 IGT vs NGT, p = 0.988 IGT vs IFG, p = 0.792
HBGI	0.90 (0.55–1.18)	0.92 (0.72–1.44)	1.02 (0.51–1.95)	IFG vs NGT, p = 0.399 IGT vs NGT, p = 0.873 IGT vs IFG, p = 0.997
GRADE	0.37 (0.27–0.62)	0.62 (0.37–1.08)	0.59 (0.55–0.92)	IFG vs NGT, p = 0.188 IGT vs NGT, p = 0.084 IGT vs IFG, p = 0.971
MAGE	2.58 (2.25–2.86)	2.73 (2.35–3.51)	2.89 (2.29–3.71)	IFG vs NGT, p = 0.111 IGT vs NGT, p = 0.548 IGT vs IFG, p = 1.000
M-value	4.30 ± 3.10	2.87 ± 1.98	4.53 ± 3.44	IFG vs NGT, p = 0.224 IGT vs NGT, p = 0.996 IGT vs IFG, p = 0.772
MAG	1.21 ± 0.19	1.28 ± 0.19	1.32 ± 0.26	IFG vs NGT, p = 0.655 IGT vs NGT, p = 0.692 IGT vs IFG, p = 0.973

mean interstitial glucose, and in glucose variability assessed by some but not all of the studied indices – CV, CONGA, J-index, GRADE.

Significant differences were also found in some other parameters of glycemic control derived from CGM – the time spent in the different ranges of glucose concentration. Thus

Table 4 – Correlation between glycated hemoglobin (HbA1c) and its corresponding estimated average glucose (eAG), fasting and 2-h plasma glucose and indices of glucose variability.

	HbA1c	eAG	Fasting glucose	2-h glucose
Ln SD	r = 0.019 p = 0.21	r = 0.16 p = 0.30	r = 0.18 p = 0.22	r = 0.19 p = 0.19
% CV	r = 0.01 p = 0.93	r = −0.02 p = 0.86	r = 0.02 p = 0.91	r = 0.14 p = 0.34
CONGA	r = 0.36 **p = 0.01**	r = 0.37 **p = 0.01**	r = 0.36 **p = 0.01**	r = 0.17 p = 0.26
Ln L-index	r = 0.23 p = 0.12	r = 0.19 p = 0.20	r = 0.27 p = 0.06	r = 0.07 p = 0.65
J-index	r = 0.37 **p = 0.01**	r = 0.36 **p = 0.01**	r = 0.39 **p = 0.006**	r = 0.22 p = 0.14
Ln LBGI	r = −0.33 **p = 0.02**	r = −0.33 **p = 0.02**	r = −0.29 **p = 0.04**	r = −0.04 p = 0.78
Ln HBGI	r = 0.31 **p = 0.03**	r = 0.25 p = 0.09	r = 0.27 p = 0.06	r = 0.09 p = 0.53
Ln GRADE	r = 0.09 p = 0.56	r = 0.09 p = 0.54	r = 0.27 p = 0.058	r = 0.20 p = 0.18
Ln MAGE	r = 0.14 p = 0.37	r = 0.10 p = 0.50	r = 0.24 p = 0.09	r = 0.16 p = 0.29
Ln M-value	r = −0.34 **p = 0.02**	r = −0.33 **p = 0.02**	r = −0.30 **p = 0.04**	r = −0.03 p = 0.83
MAG	r = 0.16 p = 0.27	r = 0.16 p = 0.29	r = 0.11 p = 0.47	r = 0.08 p = 0.58

time in target (from 3.0 to 7.8 mmol/l) is significantly lower in the group with prediabetes, while time above target is significantly higher as compared to the group with NGT.

These results show that the changes in glucose homeostasis in prediabetes include both higher level of mean glycaemia and increased glucose variability.

Mean glycaemia appears to be higher in prediabetes as assessed by all the studied parameters while glucose variability is found to be higher when evaluated by only some of the indices. The latter, however, are among the main and best established parameters of glucose variability. As the best indices of glucose variability are yet to be determined, the established differences in the present study may be regarded as an indicator of the higher sensitivity and diagnostic value of the respective indices as they succeeded in finding significant differences at these early stages of glucose intolerance. The established significant difference in the major parameter of glucose variability (according to the consensus for use of CGM) – the coefficient of variation (CV) is probably of fundamental importance and provides greatest input in support of the higher glucose variability in prediabetes [29]. CV is an improved parameter based on SD divided by mean glucose so that the effect of dispersion and the single outlying values in the two poles of glucose concentrations be eliminated. This result is supported by logic by the data on another similar index, namely the J-index. It is also based on SD and mean glucose and found to be significantly elevated in the group with prediabetes. CONGA and MAGE are the other two widely used indices of glucose variability which are not currently included in the consensus and in the standard report on CGM [2,7]. A significant difference between the two groups (with prediabetes and with NGT) was found only in CONGA, but the borderline significance of MAGE also needs to be pointed out. The indices LBGI and HBGI estimate the risk of hypo- and hyperglycaemia, respectively, and have clinical implications mainly in patients with diabetes. As there are no extreme glucose concentrations at the stages of normoglycaemia and prediabetes, the lack of significant difference in these indices between the two groups is not unexpected. Probably LBGI and HBGI are not of clinical importance in such a population. The results on the third index for the assessment of the risk associated with glucose variability – GRADE, contrary to the results for LBGI and HBGI, show significant difference and higher risk in prediabetes which may be regarded as additional support for the concept of higher glucose variability in prediabetes.

After adjustment for BMI the results undergo slight change but still support the concept of the higher glucose variability in prediabetes – SD, J-index, L-index and MAGE are significantly increased in prediabetes compared to NGT. The borderline result for the CV should also be pointed out. The results of this analysis also support the role of BMI as an independent factor for glucose variability as shown in other studies [22]. It should be noted that in both analyses – adjusted and unadjusted – the results for the J-index remain significant. This consistency of results has to be taken into consideration in qualification of the different indices of glucose variability. Furthermore in a subanalysis dividing prediabetes into its separate categories – IFG and IGT, the only significant difference appeared to be the increased J-index in IFG as compared to NGT. Taken together the different analyses performed in the present study probably give some advantage to and focus the attention on the J-index which needs further investigation and confirmation in larger studies.

The assessment of glucose variability in the separate prediabetes categories – IFG and IGT, is a topic of particular interest. As IFG and IGT are distinct entities in regard to the underlying pathophysiology, the risk for diabetes and probably the associated cardiovascular risk, it can be presumed that glucose variability in these two categories may be different. A subanalysis dividing prediabetes into IFG and IGT was performed in the present study and the results showed a significant difference between the groups with IFG and NGT in just one of the indices – the J-index; there was no difference between the groups with IGT and NGT and also between IFG and IGT. As the number of subjects with prediabetes in the study is rather small and comprises predominantly of cases with IFG (24 subjects with IFG, 8 – with IGT), the analysis is not powered to address the issue of glucose variability in IFG and IGT and does not allow any other conclusions despite those regarding the potential role of the J-index. The topic of glucose variability in IFG and IGT certainly needs further investigations in larger groups.

The available data on glucose variability in prediabetes is rather limited. The published studies report on small groups – up to 50 subjects with prediabetes without differentiation of the categories of prediabetes, and CGM has been performed for a rather short period of time – 72 h, with real-time CGM requiring calibration with finger prick testing [18,19,23–27,30–34].

The results of the present study are much in line with the published data which report significantly increased glucose variability in prediabetes [24,27,30–34]. Hanefeld at al., found significantly increased SD and MAGE in subjects with prediabetes. Contrary to our results, they found no difference in HbA1c and mean glycaemia, respectively, and made the conclusion that the changes in glucose variability better characterize the altered glucose homeostasis in prediabetes, preceding the changes in HbA1c [27]. Significantly increased indices of glucose variability in prediabetes have also been reported by Zheng et al. [30]. Their analysis has given an intermediate position of prediabetes, with a significantly higher variability compared to NGT and significantly lower one when compared to diabetes. Similar in design and results are the studies of Wang et al. and Chen et al. which show a progressive increase in glucose variability from NGT to prediabetes and diabetes [31,32].

The results of the present study regarding the time spent in the different glucose concentration ranges support the potential role of CGM in the diagnosis of glucose tolerance. The data showing a certain percentage of time above the target range in some of the subjects with both NGT and prediabetes mean that the currently used diagnostic cut-offs for plasma glucose concentrations are actually exceeded under everyday conditions and out of the experimental environment of the diagnostic tests. Time above target is significantly higher in the group with prediabetes compared to the group with NGT. In addition, individuals with at least one episode of hyperglycaemia in the diabetes range were detected in both groups – 2 subjects (11%) in the group with NGT and 7 subjects (21.8%) in the group with prediabetes. Similar results have been reported by other researchers as well [22,23]. With regard to the time below range in the present study no difference was found between the groups but changes in this parameter have been reported by other researchers in subjects with IGT. Castaldo et al. have found significantly increased time spent in hypoglycaemia in IGT subjects compared to NGT and association of this parameter with preclinical atherosclerosis [25].

The current study has evaluated the relation between glycated hemoglobin and glucose variability, or, in other words, between the well-established parameter of glycemic control, providing information on just mean glycaemia with no notion of glucose excursions, and the indices of glucose variability. Overall, the results do not show strong correlations – a fact which supports the concept that glycated hemoglobin and glucose variability represent two different and independent aspects of glycemic control and when taken together provide an overall and complementary estimate of glycemic control. These data support the separate and independent role of glucose variability in the complex assessment of glucose homeostasis at the early stages of glucose intolerance, the prediabetic states. Among the glucose variability indices significant but relatively weak correlations with HbA1c were found for LBGI, HBGI, CONGA, J-index and M-value which can probably be explained by their dependence on mean glycaemia. The results are repeated for the fasting plasma glucose which correlates significantly with the same indices – LBGI, CONGA, J-index, M-value. The result for HBGI is of borderline significance, while the 2-hour plasma glucose does not correlate with any of the indices. As the postload glucose is an expression of glucose variability, these results are somewhat contradictory. A possible explanation could be the small number of subjects with IGT compared to those with IFG in the study and the lack of marked increase in the 2-h glucose concentrations.

5.1. *Limitations and advantages of the study*

The present study has its advantages and limitations.

A main disadvantage of the study is the small number of participants which does not allow the subdivision of prediabetes into its separate categories (IFG and IGT). It thus limits the power of the statistical analysis and the possibility for well-grounded conclusions. Regardless of this, the results outline clear tendencies and show the necessity of future research on glucose variability in subjects with prediabetes. Another limitation of the study is the significant difference in BMI between the groups which might influence the results. As the study is a non-interventional one, food amount and composition, physical activity and their timing were not prespecified which can also be considered as a limitation.

A main advantage of the study is the length of the period of CGM and the type of sensor used, namely a 14 day period and a professional blind sensor. Another advantage is the studied population (subjects with prediabetes), which is still less studied in terms of glucose variability and its role in the complex assessment of glucose homeostasis in these states as well as in the associated risk for the development of diabetes and its chronic complications. Last but not least, this is the first study in our country using CGM in subjects with prediabetes.

6. Conclusion

Glucose variability is significantly increased in prediabetes and could be an additional parameter in the assessment of glucose homeostasis even at these early stages of glucose dysregulation.

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Conflict of interest

The authors declare no conflict of interest.

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