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The relationship between glucose variability and insulin sensitivity and oxidative stress in subjects with prediabetes

Rumyana Dimova *, *Nevena Chakarova*, *Greta Grozeva*, *Georgi Kirilov*, *Tsvetalina Tankova*

Department of Endocrinology, Medical University - Sofia, Bulgaria

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ABSTRACT

Aim: The present study assessed the relationship between glucose variability (GV) and insulin levels, insulin resistance and oxidative stress at early stages of glucose intolerance.
Material and methods: A total of 50 subjects – 12 males and 38 females, mean age 55.6 ± 9.7 years, mean BMI 28.4 ± 6.4 kg/m^2, divided into 2 groups according to glucose tolerance: 32 with prediabetes and 18 with normal glucose tolerance were included. Glucose tolerance was assessed by OGTT according to WHO 2006 criteria. Plasma glucose and serum insulin were measured at fasting, 120-minute and 180-minute during the test; and oxLDL and 3-Nitrotyrosine – at fasting and 120-minute. HOMA-IR and OGIS indexes were calculated. HbA1c and lipid levels was assessed. Continuous glucose monitoring was performed with a blind sensor (FreeStyle Libre Pro) for a mean period of 13.6 ± 2.3 days.
Results: Our results demonstrate significantly increased insulin resistance in subjects with prediabetes, whereas there is no difference in oxidative stress markers between the two groups. OxLDL and 3-NT correlate positively with insulin levels and HOMA-IR and negatively with OGIS in both groups. There is a positive association between oxidative stress markers and 120-minute glucose in the prediabetes group. Insulin levels and HOMA-IR are positively related to plasma glucose and reciprocally to CV and M-Value in prediabetes, since the latter association is with borderline significance after adjustment for hypertension and smoking.
Conclusions: Our results demonstrate a significant correlation between oxidative stress and insulin resistance at early stages of glucose intolerance. Both chronic hyperglycemia and GV seem to be related to insulin levels and insulin resistance, and just postload glycaemia to oxidative stress in prediabetes.

* Corresponding author at: Division of Diabetology, Department of Endocrinology, Medical University - Sofia, 2 Zdrave Str., 1431 Sofia, Bulgaria.
E-mail address: dr.roumyana.dimova@gmail.com (R. Dimova).
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1. Introduction

Type 2 diabetes (T2D) is associated with increased morbidity and mortality for cardiovascular disease (CVD) [1], and this risk exists long before the onset of diabetes [2]. Overfeeding in combination with sedentary lifestyle predispose to induction of insulin resistance as a downstream mechanism subsequently leading to glucose dysregulation [3]. Based on cumulative evidence oxidative stress has been assumed to be the potential pathogenic mechanism linking insulin resistance with beta-cells dysfunction and eventually leading to diabetes and CVD [4]. Research in this area has revealed that the link between oxidative stress and insulin resistance is triggered by energy surplus, resulting in oxidant excess and finally leading to insulin resistance primarily in skeletal muscles [5]. This concept has been supported by the results of big studies – INSIGHT [6], HOPE [7], the West of Scotland Coronary Prevention [8], LIFE [9] and SOLVD [10], which have outlined the beneficial role of calcium channel blockers, ACE inhibitors, AT-1 receptor antagonists, and statins, all showing intracellular antioxidant activity, on the reduction of new cases of diabetes. Therefore, it seems that the prevention of diabetes and CVD may be achieved not just by directly targeting increase in insulin sensitivity, but also targeting reduction in oxidative stress [4].

Since the aforementioned pathophysiological processes occur in subjects with slightly increased glucose levels – at the prediabetes state, where traditional predictors for T2D and CVD are not reliable the attention has been drawn to the role of glucose variability (GV) on the activation of oxidative stress and the concomitant development of insulin resistance over and above solely the sustained chronic hyperglycemia [11–17].

Continuous glucose monitoring (CGM) has become a valid and reliable tool for measuring glucose fluctuations [18]. Data on GV in prediabetes and in normal glucose tolerance (NGT) are limited. An emerging issue, which is in the focus of current research, is the role of GV in the development of chronic complication and the increased cardio-metabolic risk [19,20].

This report is a continuation of our foregoing observation, which has demonstrated that the changes in glucose tolerance in prediabetes encompass not only a higher level of mean glucose, but also an increased GV [21]. These results raise the question of whether these glucose fluctuations contribute to the increased cardio-metabolic risk in this population.

Therefore, the present study aims to explore the association between glycaemia – plasma glucose levels during OGTT, HbA1c and some parameters of glucose variability, and insulin levels, indirect indexes of insulin sensitivity and insulin resistance and oxidative stress markers at early stages of impaired glucose tolerance.

2. Material and methods

A total of 50 subjects (12 males, 38 females) were enrolled in this cross-sectional study. The participants were divided into two groups according to glucose tolerance – 18 with NGT, defined as fasting plasma glucose <6.1 mmol/l and postload plasma glucose <7.8 mmol/l. and 32 with prediabetes (24 subjects with impaired fasting glucose, defined as fasting plasma glucose 6.1–6.9 mmol/l and postload plasma glucose <7.8 mmol/l, and 8 subjects with impaired glucose tolerance, defined as fasting plasma glucose <6.1 mmol/l and postload plasma glucose 7.8–11.0 mmol/l).

All participant were interview for the presence of hypertension, smoking status, medications they are taking and lifestyle. Subjects with previously diagnosed diabetes or taking anti-diabetic or anti-obesity medications, corticosteroids and antipsychotics, and with serious comorbidities were not eligible for the study.

The participants were recruited at the Division of Diabetology, Department of Endocrinology, Medical University of Sofia, within an ongoing diabetes screening and prevention program.

All participants received detailed information about the aims, methods and risks of participating in the study and signed informed consent in accordance with the Helsinki Declaration and rules of Good Clinical Practice, as the study was approved by the Ethics Committee of the Medical University of Sofia.

Anthropometric parameters were measured - height, weight, and BMI was calculated.

Arterial blood pressure was measured twice under standard conditions.

Glucose tolerance was assessed by a standard oral glucose tolerance test (OGTT) with 75 g anhydrous glucose dissolved in 250 ml of water after an overnight fast in accordance with WHO 2006 criteria.

Fasting and 120 and 180-minute postload plasma glucose was measured by a hexokinase enzyme method (Roche Diagnostics). HbA1c was measured in whole blood with immunoturbidimetric NGSP-certified method (Roche Diagnostics). Serum lipid levels (total cholesterol, LDL-C, HDL-C and triglycerides) were measured by enzyme-calorimetric method (Roche Diagnostics). Serum insulin was assessed by electrochemiluminescence method.

Serum oxidized LDL (oxLDL) and serum 3-Nitrotyrosine (3-NT) were assessed at fasting and 120-minute during OGTT by ELISA method (CUSABIO BIOTECH CO., LTD) with 0.78 U/l sensitivity and intra-assay CV% <8%, and inter-assay CV% <10% for oxLDL; and 0.04 ng/ml sensitivity and intra-assay CV% <8%, and inter-assay CV% <10% for 3-NT.

Indirect index of insulin resistance – HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was calculated using the formula: $HOMA-IR = \frac{\text{plasma glucose (mmol/l)} \times \text{serum insulin (mIU/l)}}{22.5}$. Indirect index of insulin sensitivity - OGIS index (Oral Glucose Insulin Sensitivity index) was calculated using online OGIS calculator.

Continuous glucose monitoring was performed with blind sensor for professional use (FreeStyle Libre Pro, Abbot GmbH & KG) for a mean period of 13.6 ± 2.3 days in routine everyday setting. This sensor measures interstitial glucose concentration using a glucose oxidase method every 15 min for a period

of 14 days, not requiring calibration [22]. The participants were instructed to stick to their usual lifestyle to obtain data under everyday conditions.

The following GV parameters were used:

- time in target range, defined as glucose concentration between 3.0 mmol/l and 7.8 mmol/l
- standard deviation (SD), defined as the amount of dispersion of a data set and coefficient of variation (CV), defined as SD with correction for the mean glucose, calculated using a computer software (Excel, MicroSoft Office)
- CONGA (continuous overall net glycemic action), MAGE (mean amplitude of glycemic excursions), MAG (mean absolute glucose), M-value, LBGI (low blood glucose index), HBGI (high blood glucose index), J-index, L-index (lability index), GRADE (glycemic risk assessment in diabetes equation), calculated by means of a special software program – Easy GV version 9.0.R2

All GV indexes were calculated on the basis of the downloaded raw sensor data on overall measured glucose concentrations for the whole studied period.

Statistical analysis of data was performed with SPSS vs. 23.0. Histograms and curves of normal distribution were applied to determine data distribution. The variables with skewed distribution were analyzed after logarithmic transformation. Descriptive analysis, a single-factor dispersion analysis of variance (ANOVA) and correlation analysis with parametric (Pearson) coefficient and partial correlation, controlling for some confounding variables, were performed for comparison between the two 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.

3. Results

Our results show significantly increased insulin resistance indexes in subjects with prediabetes in comparison to NGT, whereas there is no difference in oxidative stress markers between the two groups (Table 1). Our findings on the differences in glucose variability indexes between the two groups has already been published [21]. There is a strong positive correlation between fasting and postload insulin levels and HOMA-IR, and fasting and postload oxLDL and 3-NT concentrations; and negative correlation between OGIS index and oxidative stress markers between the two groups - prediabetes and NGT (Table 2 and Table 3). Regarding blood and interstitial glucose parameters, oxidative stress markers positively correlate with postload plasma glucose in the prediabetes group even after adjustment for the presence of hypertension and smoking (Table 4 and Table 5). Insulin levels and HOMA-IR are positively related to plasma glucose levels during OGTT and reciprocally associated with CV and M-Value in the prediabetes group, since the latter relationship is with borderline significance after adjustment for hypertension and smoking. There is a significant negative association between OGIS index and postload plasma glucose in the pre-

Table 1 – Main characteristics of the groups according to glucose tolerance – normal glucose tolerance (NGT) and prediabetes.

Parameter	NGT	Prediabetes	p
Number	18	32	–
Male/female	4/14	8/24	–
Age (years)	54.4 ± 9.9	56.8 ± 9.6	> 0.05
Fasting glucose (mmol/l)	5.6 ± 0.3	6.4 ± 0.3	**<0.0001**
Post-load glucose (mmol/l)	5.1 ± 1.4	6.5 ± 1.9	**0.013**
HbA1c (%)	5.5 ± 0.4	5.7 ± 0.3	**0.041**
Total-C (mmol/l)	5.3 ± 1.2	5.7 ± 0.9	0.217
LDL-C (mmol/l)	3.4 ± 1.2	3.7 ± 0.8	0.309
HDL-C (mmol/l)			
in males	1.2 ± 0.8	1.1 ± 0.2	0.473
in females	1.7 ± 0.5	1.5 ± 0.4	0.556
Triglycerides (mmol/l)	0.9(0.6–1.4)	1.3(1.1–2.1)	0.059
Hypertension (%)	6	66	**0.0001**
Smoking status (%)			0.457
smoker	13	25	
non-smokers	69	66	
ex-smokers	19	9	
Insulin at fasting (mIU/l)	7.5(5.9–14.9)	14.7(10.0–19.6)	**0.005**
Insulin 120 min (mIU/l)	40.9(20.1–92.5)	57.2(30.3–140.0)	0.132
HOMA-IR	1.8(1.4–3.8)	4.3(2.9–5.7)	**<0.0001**
OGIS index	438 ± 125	347 ± 74	**0.004**
oxLDL at fasting (U/l)	43.2(34.0–61.1)	41.5(34.4–59.8)	0.777
oxLDL 120 min (U/l)	44.8(33.1–77.5)	44.8(36.8–65.7)	0.877
3-NT at fasting (ng/ml)	19.8(13.8–29.9)	24.8(14.7–37.3)	0.472
3-NT 120 min (ng/ml)	20.4(17.3–35.5)	26.1(18.8–37.8)	0.238

Data is mean ± SD and median (percentile 25–75%).
oxLDL - oxidized LDL; 3-NT – 3-Nitrotyrosine.

Table 2 – The relationship between insulin levels and indirect indexes of insulin sensitivity and insulin resistance, and oxidative stress markers.

Pearson Correlation Whole cohort	Ln(oxLDL at fasting)	Ln(oxLDL 120 min)	Ln(3-NT at fasting)	Ln(3-NT 120 min)
Ln(Insulin at fasting)	**r = 0.37, p = 0.014**	**r = 0.36, p = 0.018**	**r = 0.38, p = 0.013**	**r = 0.39, p = 0.009**
Ln(Insulin 120 min)	**r = 0.73, p < 0.0001**	**r = 0.77, p < 0.0001**	**r = 0.55, p < 0.0001**	**r = 0.47, p = 0.001**
Ln(HOMA-IR)	**r = 0.36, p = 0.016**	**r = 0.34, p = 0.024**	**r = 0.37, p = 0.013**	**r = 0.40, p = 0.008**
OGIS index	**r = −0.63, p < 0.0001**	**r = −0.62, p < 0.0001**	**r = −0.53, p < 0.0001**	**r = −0.55, p < 0.0001**
Pearson Correlation Prediabetes	**Ln(oxLDL at fasting)**	**Ln(oxLDL 120 min)**	**Ln(3-NT at fasting)**	**Ln(3-NT 120 min)**
Ln(Insulin at fasting)	r = 0.29, p = 0.132	r = 0.31, p = 0.112	r = 0.15, p = 0.433	r = 0.25, p = 0.203
Ln(Insulin 120 min)	**r = 0.67, p < 0.0001**	**r = 0.81, p < 0.0001**	**r = 0.38, p = 0.044**	**r = 0.50, p = 0.007**
Ln(HOMA-IR)	r = 0.29, p = 0.134	r = 0.31, p = 0.112	r = 0.15, p = 0.445	r = 0.25, p = 0.208
OGIS index	**r = −0.59, p = 0.001**	**r = −0.63, p < 0.0001**	r = −0.28, p = 0.151	**r = −0.40, p = 0.035**
Pearson Correlation NGT	**Ln(oxLDL at fasting)**	**Ln(oxLDL 120 min)**	**Ln(3-NT at fasting)**	**Ln(3-NT 120 min)**
Ln(Insulin at fasting)	**r = 0.54, p = 0.039**	**r = 0.56, p = 0.030**	**r = 0.71, p = 0.003**	r = 0.44, p = 0.099
Ln(Insulin 120 min)	**r = 0.82, p < 0.0001**	**r = 0.80, p < 0.0001**	**r = 0.81, p < 0.0001**	r = 0.44, p = 0.100
Ln(HOMA-IR)	**r = 0.55, p = 0.035**	**r = 0.57, p = 0.028**	**r = 0.72, p = 0.002**	r = 0.45, p = 0.090
OGIS index	**r = −0.78, p = 0.001**	**r = −0.78, p = 0.001**	**r = −0.88, p < 0.0001**	**r = −0.60, p = 0.017**

oxLDL - oxidized LDL; 3-NT – 3-Nitrotyrosine; HOMA-IR - Homeostatic Model Assessment for Insulin Resistance; OGIS index - Oral Glucose Insulin Sensitivity index.

Table 3 – The relationship between insulin levels and indirect indexes of insulin sensitivity and insulin resistance, and oxidative stress markers adjusted for the presence of hypertension and smoking.

Partial Correlation Whole cohort	Ln(oxLDL at fasting)	Ln(oxLDL 120 min)	Ln(3-NT at fasting)	Ln(3-NT 120 min)
Ln(Insulin at fasting)	**r = 0.45, p = 0.003**	**r = 0.42, p = 0.006**	**r = 0.40, p = 0.010**	**r = 0.38, p = 0.016**
Ln(Insulin 120 min)	**r = 0.79, p < 0.0001**	**r = 0.83, p < 0.0001**	**r = 0.57, p < 0.0001**	**r = 0.46, p = 0.002**
Ln(HOMA-IR)	**r = 0.45, p = 0.003**	**r = 0.41, p = 0.007**	**r = 0.40, p = 0.010**	**r = 0.38, p = 0.015**
OGIS index	**r = −0.70, p < 0.0001**	**r = −0.68, p < 0.0001**	**r = −0.55, p < 0.0001**	**r = −0.54, p < 0.0001**
□Partial Correlation Prediabetes	**Ln(oxLDL at fasting)**	**Ln(oxLDL 120 min)**	**Ln(3-NT at fasting)**	**Ln(3-NT 120 min)**
Ln(Insulin at fasting)	**r = 0.39, p = 0.052**	**r = 0.38, p = 0.058**	r = 0.26, p = 0.201	r = 0.29, p = 0.144
Ln(Insulin 120 min)	**r = 0.72, p < 0.0001**	**r = 0.84, p < 0.0001**	**r = 0.41, p = 0.040**	**r = 0.50, p = 0.009**
Ln(HOMA-IR)	**r = 0.38, p = 0.055**	**r = 0.38, p = 0.059**	r = 0.25, p = 0.219	r = 0.29, p = 0.153
OGIS index	**r = −0.62, p = 0.001**	**r = −0.65, p < 0.0001**	r = −0.32, p = 0.116	**r = −0.42, p = 0.034**
□Partial Correlation NGT	**Ln(oxLDL at fasting)**	**Ln(oxLDL 120 min)**	**Ln(3-NT at fasting)**	**Ln(3-NT 120 min)**
Ln(Insulin at fasting)	r = 0.53, p = 0.063	**r = 0.56, p = 0.049**	**r = 0.73, p = 0.005**	r = 0.44, p = 0.137
Ln(Insulin 120 min)	**r = 0.87, p < 0.0001**	**r = 0.80, p = 0.001**	**r = 0.77, p = 0.002**	r = 0.42, p = 0.151
Ln(HOMA-IR)	**r = 0.55, p = 0.054**	**r = 0.56, p = 0.049**	**r = 0.74, p = 0.004**	r = 0.44, p = 0.130
OGIS index	**r = −0.82, p = 0.001**	**r = −0.77, p = 0.002**	**r = −0.86, p < 0.0001**	**r = −0.59, p = 0.033**

The analysis is controlling for the presence of hypertension and smoking status.
oxLDL - oxidized LDL; 3-NT – 3-Nitrotyrosine; HOMA-IR - Homeostatic Model Assessment for Insulin Resistance; OGIS index - Oral Glucose Insulin Sensitivity index.

diabetes group. No relationship between insulin levels and oxidative stress markers, and glucose indexes is observed in NGT group (Table 4 and Table 5).

4. Discussion

Oxidative stress represents a disturbance between the production of reactive oxygen species (ROS) and antioxidant defense potential in favor of increased ROS accumulation, resulting in tissue damage [23]. Regarding oxidative stress markers, we selected to study 3-NT which is tightly involved in diabetic complications [24,25] and oxLDL – a biomarker of lipid peroxidation in circulation [26], related to metabolic syndrome and CVD [27,28], thus encompassing complementary areas of systemic oxidative stress. On the other hand, it has been shown that oxLDL, as a donor for lipid peroxidation, is linked to 3-NT production, through tyrosine oxidation, mediated by lipid-derived radicals [29].

Our data demonstrate higher insulin resistance in prediabetes, whilst oxidative stress markers are comparable between the two groups. It has been established that insulin resistance contributes to the development of T2D and compensatory hyperinsulinemia is present in prediabetes and precedes clinically overt T2D by a long period of time [30].

These results are also in accordance with the reports in subjects with metabolic syndrome [31] and reflect the lack of association between oxidative stress and T2D development [22] or even with the potential protective role of oxidative stress in this high-risk population observed in a prospective study [32]. Another recent study even suggested that endogenous insulin might also exert antioxidant effects [33]. Therefore, the observed lack of difference in the levels of oxidative stress biomarkers between NGT and prediabetes group might be due to the putative protective effect of endogenous insulin. Deepening our understanding of the intimate cellular and molecular mechanisms will elucidate these issues.

In contrast to these findings it has been shown that total antioxidant capacity is decreased in subjects with prediabetes [34–36], with NGT and high HOMA-IR [37], with NGT and obesity [13], and in T2D and obesity [13,38,39], and that oxidative stress occurs early in the evolution of T2D, preceding the development of insulin resistance [35].

Based on these contradictory findings it is noteworthy to attempt to gain further insight into the respective role of insulin resistance and oxidative stress as precursors of increased cardio-metabolic risk. In this respect, we evaluated the relationship between both factors.

A strong positive correlation, controlling for some confounders such as the presence of hypertension and smoking, was observed between the oxidative stress markers and insulin levels, and insulin resistance in the whole cohort and in the two groups, and both examined biomarkers of oxidative stress showed consistent results. Since most of the participant report for a sedentary lifestyle, it is likely that this risk factor could influence oxidative stress markers in both groups equally and therefore is not taken into consideration. A common limitation of most previous studies is the small sample size when assessing the association between the degree of oxidative stress and the risk of developing insulin resistance among non-diabetic subjects and therefore the available data are scarce. The Framingham Offspring Study reported that systemic oxidative stress is associated with insulin resistance in individuals at average or elevated risk of T2D even after accounting for BMI [40], which is in accordance with our data. There are also quite a few studies evaluating the relationship between plasma oxidative stress markers and insulin resistance in high-risk non-diabetic population. Our findings are akin to the data in a large population-based observational study (Coronary Artery Risk Development in Young Adults), including a non-diabetic adult cohort, where a positive association between oxLDL and HOMA-IR was strongly evident, independently of the presence of obesity [41], and oxLDL has been shown to predict incidence of metabolic syndrome [27]. However, another study has not shown any relationship between oxLDL levels and the presence of metabolic syndrome [31]. There are few other cross-sectional studies and some have previously reported a positive correlation between different oxidative stress biomarkers and insulin resistance in prediabetes [34], in men [42] and in nonalcoholic fatty liver disease [35], whereas others have failed to find any association between oxidative stress and HOMA-IR [37,43].

Based on data from experimental models, it has been proposed that probably oxidative stress triggers the development of insulin resistance [44] and oxidative stress generation is an essential pathophysiological mechanism mediating the development of insulin resistance and its progression to prediabetes and ultimately overt T2D [4]. Beta-cells are extremely sensitive to ROS [45], glucose and lipids [46] accumulation which cause ROS-induced inhibition of glucose-mediated insulin secretion, beta-cell apoptosis and diminished beta-cell gene expression [47]. The deleterious effect of systemic oxidative stress on beta-cells is due to their high oxidative energy requirements in combination with low levels of antioxidant enzymes expression [47]. Many studies have demonstrated that the presumptive mediator of oxidative stress-induced insulin resistance is the insulin signal inhibition in insulin-targeted organs of the over nutrition and sedentary lifestyle, since ROS accumulation contributes and accelerates the development of insulin resistance [48]. The concomitant hyperinsulimenia is initially the compensatory insulin secretory response aiming to preserve normoglycemia but it additionally increases ROS production turning the vicious cycle and further increasing insulin resistance [49].

Though there is solid evidence that incessant exposure to sustained hyperglycaemia contributes to elevated oxidative stress [50], many studies in patients with T2D have shown conflicting findings with respect to glycaemic variability and the plausible underlying mechanisms are in the focus of current research.

Our data show a significant positive relationship between both 3-NT and oxLDL fasting and postload levels and 120-minute plasma glucose during OGTT, whereas there is no association between the aforementioned markers and fasting plasma glucose, HbA1c and the estimated CGM parameters of interstitial glucose variability. Since it is well known that postprandial hyperglycemia is a robust reflector of the increased risk for chronic complications and cardiovascular mortality in diabetes, many studies have confirmed its association with oxidative stress generation [51,52], which is strictly dependent on the level of glycaemia reached regarding 3-NT in both NGT and T2D [53,54]. On the other hand, chronic sustained hyperglycemia fails to show significant correlation with oxidative stress markers, meaning that glucose fluctuations in the postprandial period might exhibit a more specific triggering effect on ROS formation in both NGT and T2D [52,54]. Moreover, increased GV measured in vivo in everyday life [52], experimentally induced [54], or in animal models [55] has been shown to be equally related to oxidative stress markers in subjects with NGT or T2D [52,54–56].

It has been hypothesized that GV is a risk factor for microangiopathy in T2D via stimulation of excess oxidative stress [57], however, the effect of GV on oxidative stress in clinical settings remains controversial. It is important to notice that careful consideration should be given in regards to whether different indexes for GV derived from CGM data have the same specificity and power to estimate the link between glycemic fluctuations and ROS generation in prediabetes. Our results have replicated findings of some other studies, reporting no significant increase in oxidative stress after inducing GV in healthy subjects [58], in prediabetes [12], in T1D [59], and in T2D [60]. In contrast to our findings, some data have reported that MAGE, estimated acute GV, but not postprandial glycaemia, is a strong independent predictor of total ROS production [11,13,15,17], whereas others have

Table 4 – The relationship between blood and interstitial glucose parameters and oxidative stress markers and insulin levels and indexes.

Pearson Correlation Prediabetes	Ln(oxLDL at fasting)	Ln(oxLDL 120 min)	Ln(3-NT at fasting)	Ln(3-NT 120 min)	Ln(Insulin at fasting)	Ln(Insulin 120 min)	Ln(HOMA-IR)	OGIS index
Blood glucose	r = 0.12	r = 0.14	r = 0.04	r = 0.10	**r = 0.36**	r = 0.18	**r = 0.45**	r = −0.23
at fasting (mmol/l)	p = 0.544	p = 0.465	p = 0.837	p = 0.625	**p = 0.047**	p = 0.352	**p = 0.011**	p = 0.222
Blood glucose	**r = 0.53**	**r = 0.57**	**r = 0.43**	**r = 0.44**	r = 0.07	**r = 0.73p < 0.0001**	r = 0.05	**r = −0.42**
120 min (mmol/l)	**p = 0.004**	**p = 0.002**	**p = 0.021**	**p = 0.021**	p = 0.722		p = 0.781	**p = 0.025**
HbA1c (%)	r = 0.22	r = 0.18	r = 0.11	r = 0.15	r = 0.31	**r = 0.45**	r = 0.30	r = −0.26
	p = 0.251	p = 0.357	p = 0.594	p = 0.434	p = 0.095	**p = 0.016**	p = 0.104	p = 0.175
Mean interstitial glucose (mmol/l)	r = 0.05	r = 0.01	r = 0.13	r = 0.24	r = 0.26	r = 0.12	r = −0.06	r = 0.24
	p = 0.810	p = 1.000	p = 0.502	p = 0.217	p = 0.154	p = 0.540	p = 0.757	p = 0.217
Ln % in target	r = 0.02	r = 0.02	r = −0.11	r = −0.12	r = 0.18	r = 0.07	r = 0.15	r = −0.20
3.0–7.8	p = 0.921	p = 0.928	p = 0.582	p = 0.562	p = 0.340	p = 0.739	p = 0.437	p = 0.301
Ln SD	r = 0.11	r = 0.17	r = 0.18	r = 0.15	r = −0.32	r = 0.11	r = −0.31	r = 0.08
	p = 0.563	p = 0.384	p = 0.354	p = 0.457	p = 0.079	p = 0.571	p = 0.093	p = 0.684
CV	r = 0.11	r = 0.17	r = 0.14	r = 0.05	**r = −0.44**	r = 0.06	**r = −0.44**	r = 0.10
	p = 0.574	p = 0.393	p = 0.464	p = 0.810	**p = 0.012**	p = 0.760	**p = 0.013**	p = 0.595
CONGA	r = 0.02	r = −0.06	r = 0.10	r = 0.20	r = 0.27	r = 0.06	r = 0.30	r = −0.01
	p = 0.935	p = 0.771	p = 0.617	p = 0.314	p = 0.137	p = 0.754	p = 0.103	p = 0.942
Ln L-index	r = 0.14	r = 0.20	r = 0.21	r = 0.22	r = −0.12	r = 0.17	r = −0.10	r = −0.07
	p = 0.485	p = 0.300	p = 0.290	p = 0.272	p = 0.531	p = 0.370	p = 0.585	p = 0.723
J-index	r = 0.08	r = 0.06	r = 0.18	r = 0.26	r = 0.11	r = 0.13	r = 0.14	r = −0.02
	p = 0.699	p = 0.762	p = 0.347	p = 0.178	p = 0.556	p = 0.491	p = 0.463	p = 0.918
Ln LBGI	r = 0.01	r = 0.13	r = −0.04	r = −0.18	**r = −0.35**	r = −0.03	**r = −0.37**	r = 0.14
	p = 0.955	p = 0.525	p = 0.852	p = 0.365	**p = 0.057**	p = 0.888	**p = 0.043**	p = 0.485
Ln HBGI	r = 0.13	r = 0.10	r = 0.18	r = 0.14	r = −0.13	r = 0.02	r = −0.11	r = −0.08
	p = 0.499	p = 0.629	p = 0.356	p = 0.493	p = 0.480	p = 0.905	p = 0.551	p = 0.672
Ln GRADE	r = 0.03	r = 0.01	r = 0.15	r = 0.19	r = −0.14	r = 0.01	r = −0.12	r = 0.22
	p = 0.864	p = 0.999	p = 0.437	p = 0.341	p = 0.441	p = 0.985	p = 0.508	p = 0.257
Ln MAGE	r = 0.15	r = 0.17	r = 0.25	r = 0.20	r = −0.29	r = 0.09	r = −0.28	r = 0.10
	p = 0.460	p = 0.393	p = 0.203	p = 0.300	p = 0.110	p = 0.655	p = 0.134	p = 0.595
Ln M-value	r = −0.03	r = 0.09	r = −0.08	r = −0.22	**r = −0.37**	r = −0.07	**r = −0.39**	r = 0.17
	p = 0.894	p = 0.633	p = 0.706	p = 0.257	**p = 0.040**	p = 0.737	**p = 0.030**	p = 0.386
MAG	r = −0.06	r = 0.01	r = 0.01	r = 0.06	r = −0.16	r = 0.06	r = −0.16	r = 0.04
	p = 0.776	p = 0.967	p = 0.970	p = 0.779	p = 0.387	p = 0.741	p = 0.394	p = 0.840
Pearson Correlation NGT	**Ln(oxLDL at fasting)**	**Ln(oxLDL 120 min)**	**Ln(3-NT at fasting)**	**Ln(3-NT 120 min)**	**Ln(Insulin at fasting)**	**Ln(Insulin 120 min)**	**Ln(HOMA-IR)**	**OGIS index**
Blood glucose	r = 0.28	r = 0.29	r = 0.49	r = 0.43	r = 0.41	r = 0.24	r = 0.47	**r = −0.52**
at fasting (mmol/l)	p = 0.318	p = 0.293	p = 0.066	p = 0.109	p = 0.115	p = 0.362	p = 0.066	**p = 0.038**
Blood glucose	r = 0.03	r = 0.08	r = 0.30	r = 0.22	r = 0.37	r = 0.20	r = 0.37	r = −0.24
120 min (mmol/l)	p = 0.616	p = 0.781	p = 0.277	p = 0.425	p = 0.154	p = 0.465	p = 0.160	p = 0.374
HbA1c (%)	r = 0.20	r = 0.21	r = 0.15	r = 0.18	r = 0.18	r = 0.02	r = 0.19	r = −0.28
	p = 0.469	p = 0.456	p = 0.604	p = 0.534	p = 0.514	p = 0.932	p = 0.486	p = 0.303
Mean interstitial glucose (mmol/l)	r = 0.24	r = 0.21	r = 0.03	r = 0.23	r = 0.16	r = 0.10	r = 0.14	r = −0.04
	p = 0.406	p = 0.472	p = 0.917	p = 0.435	p = 0.572	p = 0.729	p = 0.616	p = 0.888

Table 4 – (continued)

Pearson Correlation Prediabetes	Ln(oxLDL at fasting)	Ln(oxLDL 120 min)	Ln(3-NT at fasting)	Ln(3-NT 120 min)	Ln(Insulin at fasting)	Ln(Insulin 120 min)	Ln(HOMA-IR)	OGIS index
Ln % in target (3.0–7.8 mmol/l)	r = 0.01 p = 0.965	r = −0.05 p = 0.863	r = 0.21 p = 0.467	r = 0.32 p = 0.267	r = −0.11 p = 0.696	r = 0.15 p = 0.588	r = −0.10 p = 0.725	r = −0.22 p = 0.437
Ln SD	r = −0.05 p = 0.866	r = 0.02 p = 0.956	r = −0.34 p = 0.235	r = −0.27 p = 0.346	r = −0.14 p = 0.619	r = −0.24 p = 0.390	r = −0.14 p = 0.620	r = 0.28 p = 0.313
CV	r = −0.30 p = 0.303	r = −0.18 p = 0.537	r = −0.46 p = 0.102	r = −0.51 p = 0.064	r = −0.28 p = 0.309	r = −0.41 p = 0.133	r = −0.27 p = 0.336	r = 0.39 p = 0.151
CONGA	r = 0.23 p = 0.421	r = 0.21 p = 0.462	r = 0.08 p = 0.786	r = 0.23 p = 0.423	r = 0.20 p = 0.470	r = 0.15 p = 0.603	r = 0.18 p = 0.522	r = −0.09 p = 0.744
Ln L-index	r = 0.11 p = 0.720	r = 0.11 p = 0.701	r = −0.23 p = 0.420	r = −0.01 p = 0.996	r = −0.13 p = 0.645	r = −0.22 p = 0.429	r = −0.13 p = 0.652	r = 0.22 p = 0.429
J-index	r = 0.19 p = 0.514	r = 0.18 p = 0.547	r = −0.05 p = 0.861	r = 0.13 p = 0.655	r = 0.11 p = 0.700	r = 0.02 p = 0.950	r = 0.09 p = 0.740	r = 0.04 p = 0.877
Ln LBGI	r = −0.32 p = 0.270	r = −0.30 p = 0.305	r = −0.18 p = 0.533	r = −0.35 p = 0.218	r = −0.23 p = 0.414	r = −0.21 p = 0.455	r = −0.20 p = 0.467	r = 0.16 p = 0.577
Ln HBGI	r = 0.29 p = 0.321	r = 0.30 p = 0.295	r = −0.02 p = 0.956	r = 0.10 p = 0.744	r = 0.03 p = 0.919	r = 0.04 p = 0.894	r = 0.04 p = 0.900	r = −0.08 p = 0.783
Ln GRADE	r = −0.11 p = 0.704	r = −0.10 p = 0.741	r = −0.05 p = 0.863	r = −0.14 p = 0.644	r = 0.06 p = 0.823	r = −0.10 p = 0.714	r = 0.07 p = 0.803	r = 0.18 p = 0.515
Ln MAGE	r = −0.10 p = 0.747	r = 0.02 p = 0.941	r = −0.42 p = 0.131	r = −0.29 p = 0.324	r = −0.27 p = 0.326	r = −0.26 p = 0.350	r = −0.26 p = 0.341	r = 0.28 p = 0.320
Ln M-value	r = −0.33 p = 0.251	r = −0.31 p = 0.280	r = −0.20 p = 0.495	r = −0.39 p = 0.170	r = −0.20 p = 0.486	r = −0.23 p = 0.404	r = −0.17 p = 0.543	r = 0.19 p = 0.458
MAG	r = −0.01 p = 0.992	r = 0.01 p = 0.986	r = −0.35 p = 0.219	r = −0.11 p = 0.711	r = −0.11 p = 0.710	r = −0.24 p = 0.386	r = −0.11 p = 0.711	r = 0.33 p = 0.236

oxLDL - oxidized LDL; 3-NT – 3-Nitrotyrosine; HOMA-IR - Homeostatic Model Assessment for Insulin Resistance; OGIS index - Oral Glucose Insulin Sensitivity index; SD - standard deviation; CV - coefficient of variation; CONGA - continuous overall net glycemic action; L-index - lability index; LBGI - low blood glucose index; HBGI - high blood glucose index; GRADE - glycemic risk assessment in diabetes equation; MAGE - mean amplitude of glycemic excursions; MAG - mean absolute glucose.

Table 5 – The relationship between blood and interstitial glucose parameters and oxidative stress markers and insulin levels and indexes, adjusted for the presence of hypertension and smoking.

Partial Correlation Prediabetes	Ln(oxLDL at fasting)	Ln(oxLDL 120 min)	Ln(3-NT at fasting)	Ln(3-NT 120 min)	Ln(Insulin at fasting)	Ln(Insulin 120 min)	Ln(HOMA-IR)	OGIS index
Blood glucose	r = 0.24	r = 0.25	r = 0.10	r = 0.14	**r = 0.42**	r = 0.24	**r = 0.52**	r = −0.26
at fasting (mmol/l)	p = 0.256	p = 0.225	p = 0.643	p = 0.519	**p = 0.035**	p = 0.252	**p = 0.008**	p = 0.208
Blood glucose	**r = 0.49**	**r = 0.54**	r = 0.37	**r = 0.41**	r = 0.15	**r = 0.75p < 0.0001**	r = 0.14	**r = −0.45**
120 min (mmol/l)	**p = 0.014**	**p = 0.006**	p = 0.071	**p = 0.042**	p = 0.467		p = 0.513	**p = 0.023**
HbA1c (%)	r = 0.34	r = 0.25	r = 0.18	r = 0.17	r = 0.14	**r = 0.44**	r = 0.14	r = −0.28
	p = 0.111	p = 0.222	p = 0.388	p = 0.409	p = 0.508	**p = 0.029**	p = 0.517	p = 0.174
Mean interstitial glucose (mmol/l)	r = 0.09	r = 0.01	r = 0.19	r = 0.25	r = 0.25	r = 0.06	r = 0.29	r = −0.07
	p = 0.674	p = 0.953	p = 0.357	p = 0.226	p = 0.226	p = 0.791	p = 0.158	p = 0.742
Ln % in target	r = 0.07	r = 0.05	r = −0.05	r = −0.10	r = 0.18	r = 0.07	r = 0.14	r = −0.19
3.0–7.8	p = 0.759	p = 0.820	p = 0.800	p = 0.648	p = 0.389	p = 0.753	p = 0.507	p = 0.363
Ln SD	r = −0.08	r = 0.05	r = −0.02	r = 0.07	r = −0.22	r = 0.08	r = −0.19	r = 0.04
	p = 0.723	p = 0.819	p = 0.942	p = 0.733	p = 0.292	p = 0.705	p = 0.354	p = 0.837
CV	r = −0.12	r = 0.04	r = −0.11	r = −0.06	**r = −0.39**	r = 0.07	**r = −0.39**	r = 0.08
	p = 0.579	p = 0.850	p = 0.586	p = 0.795	**p = 0.053**	p = 0.738	**p = 0.057**	p = 0.709
CONGA	r = 0.07	r = −0.04	r = 0.19	r = 0.22	r = 0.26	r = −0.01	r = 0.30	r = −0.01
	p = 0.729	p = 0.898	p = 0.357	p = 0.287	p = 0.211	p = 0.981	p = 0.149	p = 0.980
Ln L-index	r = 0.02	r = 0.14	r = 0.06	r = 0.17	r = −0.02	r = 0.15	r = 0.01	r = −0.14
	p = 0.931	p = 0.521	p = 0.773	p = 0.424	p = 0.934	p = 0.475	p = 0.955	p = 0.496
J-index	r = 0.05	r = 0.03	r = 0.16	r = 0.24	r = 0.13	r = 0.07	r = 0.17	r = −0.04
	p = 0.822	p = 0.897	p = 0.435	p = 0.256	p = 0.542	p = 0.746	p = 0.427	p = 0.850
Ln LBGI	r = −0.12	r = 0.07	r = −0.20	r = −0.24	r = −0.31	r = 0.03	r = −0.34	r = 0.14
	p = 0.556	p = 0.746	p = 0.340	p = 0.239	p = 0.136	p = 0.900	p = 0.100	p = 0.850
Ln HBGI	r = 0.01	r = −0.01	r = 0.05	r = 0.09	r = 0.04	r = 0.01	r = 0.07	r = −0.12
	p = 0.982	p = 0.975	p = 0.816	p = 0.677	p = 0.859	p = 0.969	p = 0.756	p = 0.570
Ln GRADE	r = −0.01	r = −0.05	r = 0.13	r = 0.15	r = −0.17	r = −0.08	r = −0.13	r = 0.22
	p = 0.978	p = 0.832	p = 0.547	p = 0.462	p = 0.431	p = 0.705	p = 0.535	p = 0.297
Ln MAGE	r = −0.04	r = 0.04	r = 0.07	r = 0.14	r = −0.18	r = 0.05	r = −0.14	r = 0.08
	p = 0.836	p = 0.867	p = 0.755	p = 0.502	p = 0.403	p = 0.820	p = 0.498	p = 0.720
Ln M-value	r = −0.17	r = 0.03	r = −0.25	r = −0.30	**r = −0.36**	r = −0.02	**r = −0.39**	r = 0.18
	p = 0.415	p = 0.886	p = 0.234	p = 0.148	**p = 0.075**	p = 0.936	**p = 0.052**	p = 0.380
MAG	r = −0.19	r = −0.08	r = −0.15	r = −0.01	r = −0.12	r = 0.04	r = −0.12	r = −0.01
	p = 0.363	p = 0.708	p = 0.475	p = 0.984	p = 0.557	p = 0.860	p = 0.578	p = 0.985
□Partial Correlation NGT	**Ln(oxLDL at fasting)**	**Ln(oxLDL 120 min)**	**Ln(3-NT at fasting)**	**Ln(3-NT 120 min)**	**Ln(Insulin at fasting)**	**Ln(Insulin 120 min)**	**Ln(HOMA-IR)**	**OGIS index**
Blood glucose	r = 0.34	r = 0.27	r = 0.53	r = 0.42	r = 0.46	r = 0.31	r = 0.51	**r = −0.57**
at fasting (mmol/l)	p = 0.254	p = 0.378	p = 0.061	p = 0.154	p = 0.112	p = 0.301	p = 0.078	**p = 0.042**
Blood glucose	r = −0.04	r = −0.06	r = 0.07	r = 0.16	r = 0.36	r = −0.05	r = 0.34	r = 0.01
120 min (mmol/l)	p = 0.906	p = 0.857	p = 0.824	p = 0.596	p = 0.229	p = 0.866	p = 0.251	p = 0.988
HbA1c (%)	r = 0.24	r = 0.22	r = 0.20	r = 0.18	r = 0.19	r = 0.08	r = 0.19	r = −0.32
	p = 0.433	p = 0.469	p = 0.504	p = 0.558	p = 0.530	p = 0.794	p = 0.529	p = 0.286

Table 5 – The relationship between blood and interstitial glucose parameters and oxidative stress markers and insulin levels and indexes, adjusted for the presence of hypertension and smoking.

Partial Correlation Prediabetes	Ln(oxLDL at fasting)	Ln(oxLDL 120 min)	Ln(3-NT at fasting)	Ln(3-NT 120 min)	Ln(Insulin at fasting)	Ln(Insulin 120 min)	Ln(HOMA-IR)	OGIS index
Mean interstitial glucose (mmol/l)	r = 0.26	r = 0.22	r = 0.07	r = 0.24	r = 0.17	r = 0.14	r = 0.15	r = −0.07
	p = 0.393	p = 0.465	p = 0.827	p = 0.438	p = 0.572	p = 0.641	p = 0.620	p = 0.825
Ln % in target	r = −0.05	r = −0.11	r = 0.09	r = 0.31	r = −0.16	r = −0.01	r = −0.13	r = −0.16
(3.0–7.8 mmol/l)	p = 0.873	p = 0.732	p = 0.776	p = 0.298	p = 0.595	p = 0.994	p = 0.672	p = 0.612
Ln SD	r = 0.06	r = 0.12	r = −0.16	r = −0.29	r = −0.09	r = −0.01	r = −0.12	r = 0.16
	p = 0.856	p = 0.709	p = 0.593	p = 0.343	p = 0.768	p = 0.975	p = 0.706	p = 0.602
CV	r = −0.27	r = −0.14	r = −0.31	**r = −0.62**	r = −0.30	r = −0.24	r = −0.31	r = 0.32
	p = 0.374	p = 0.654	p = 0.297	**p = 0.024**	p = 0.321	p = 0.439	p = 0.304	p = 0.282
CONGA	r = 0.23	r = 0.21	r = 0.07	r = 0.23	r = 0.20	r = 0.14	r = 0.18	r = −0.09
	p = 0.447	p = 0.488	p = 0.815	p = 0.448	p = 0.512	p = 0.642	p = 0.560	p = 0.779
Ln L-index	r = 0.24	r = 0.22	r = −0.04	r = 0.05	r = −0.09	r = −0.01	r = −0.11	r = 0.10
	p = 0.435	p = 0.465	p = 0.896	p = 0.860	p = 0.769	p = 0.991	p = 0.714	p = 0.741
J-index	r = 0.23	r = 0.21	r = 0.03	r = 0.15	r = 0.14	r = 0.12	r = 0.12	r = −0.02
	p = 0.443	p = 0.492	p = 0.919	p = 0.617	p = 0.646	p = 0.704	p = 0.702	p = 0.953
Ln LBGI	r = −0.30	r = −0.28	r = −0.14	r = −0.35	r = −0.21	r = −0.17	r = −0.19	r = 0.12
	p = 0.317	p = 0.348	p = 0.650	p = 0.249	p = 0.488	p = 0.584	p = 0.534	p = 0.696
Ln HBGI	r = 0.31	r = 0.32	r = 0.04	r = 0.11	r = 0.03	r = 0.13	r = 0.02	r = −0.11
	p = 0.297	p = 0.282	p = 0.908	p = 0.724	p = 0.930	p = 0.683	p = 0.946	p = 0.718
Ln GRADE	r = −0.06	r = −0.06	r = 0.09	r = −0.12	r = 0.11	r = 0.04	r = 0.10	r = 0.11
	p = 0.837	p = 0.846	p = 0.783	p = 0.707	p = 0.721	p = 0.893	p = 0.737	p = 0.730
Ln MAGE	r = −0.03	r = 0.09	r = −0.31	r = −0.28	r = −0.31	r = −0.11	r = −0.32	r = 0.23
	p = 0.927	p = 0.776	p = 0.299	p = 0.352	p = 0.311	p = 0.773	p = 0.285	p = 0.452
Ln M-value	r = −0.31	r = −0.30	r = −0.14	r = −0.38	r = −0.17	r = −0.18	r = −0.15	r = 0.14
	p = 0.303	p = 0.327	p = 0.640	p = 0.198	p = 0.570	p = 0.559	p = 0.617	p = 0.638
MAG	r = 0.16	r = 0.14	r = −0.14	r = −0.08	r = −0.01	r = 0.04	r = −0.03	r = 0.18
	p = 0.603	p = 0.658	p = 0.638	p = 0.807	p = 0.988	p = 0.896	p = 0.923	p = 0.554

The analysis for the prediabetes group is controlling for the presence of hypertension and smoking status; and for the NGT group is controlling just for smoking status, since 1/18 of subjects with NGT is with hypertension.

oxLDL - oxidized LDL; 3-NT – 3-Nitrotyrosine; HOMA-IR - Homeostatic Model Assessment for Insulin Resistance; OGIS index - Oral Glucose Insulin Sensitivity index; SD - standard deviation; CV - coefficient of variation; CONGA - continuous overall net glycemic action; L-index - lability index; LBGI - low blood glucose index; HBGI - high blood glucose index; GRADE - glycemic risk assessment in diabetes equation; MAGE - mean amplitude of glycemic excursions; MAG - mean absolute glucose.

observed significant correlations between mean glucose, HbA1c and the area under the curve for postprandial glucose levels and plasma oxidant capacity in T2D [61]. Results regarding the effect of fasting plasma glucose reduction on a potential decrease in oxidative stress level in T2D are rather conflicting [61,62].

The discrepancy among the numerous studies might have several explanations – related to the study design, based on the specific characteristics of the studied cohort, being a result of the choice of oxidative stress biomarkers [15], or different methods used for oxidative stress measurement [63]. Another reason might be the assessed GV parameters. For instance, MAGE reflects relatively large glycemic excursions and ignores smaller glucose fluctuations, which are more common in prediabetes. It is a reasonable hypothesis that this is the main reason why MAGE has been shown to be closely related to oxidative stress in T2D, but this measurement of GV has not shown any association with 3-NT and oxLDL in the present study, focused on high-risk non-diabetic population, characterized with mild fluctuations of blood glucose levels [15].

In respect to insulin levels and insulin resistance parameters, our study succeeded to reaffirm literature data for their strong relationship with fasting and postload plasma glucose levels during OGTT, chronic hyperglycemia [16], and some indexes of GV, namely CV (the gold standard) and M-value. CV is accepted as the most simple and accurate parameter of GV independent of mean glucose value and M-value reflects GV, affected by mean glucose value and puts greater emphasis on hypoglycemia [15]. These two different measurements of GV reflect a wide spectrum of glucose levels and therefore, show the influence of insulin concentrations and insulin resistance on all aspects of glycaemia. These results are in line with the assumption that insulin resistance increases GV even in subjects without diabetes [14,16] and GV is a consequence of β-cell dysfunction in prediabetes [12]. Thus, aiming to improve oxidative stress and insulin resistance might reduce GV in the high-risk subjects and diminish the overall cardio-metabolic risk in prediabetes.

Contrary to the above, no correlation between GV and insulin resistance has been reported in a non-diabetic population as well [64].

5. Conclusion

Our data confirm the close relationship between oxidative stress and insulin resistance at early stages of glucose intolerance. Chronic hyperglycemia parameters and some GV indexes seem to be related to insulin levels and insulin resistance, and just postload glycaemia to be related to oxidative stress in prediabetes. GV should be considered an additional parameter in the evaluation of glycaemia even in a high-risk non-diabetic population in order to assess the increased cardio-metabolic risk.

Our data adds to what is known about the relationship between GV and oxidative stress and insulin resistance in high-risk subjects with NGT or prediabetes. Although keeping the average blood glucose tightly controlled is of paramount importance, these data suggests that it may be equally essential to avoid large fluctuations in blood glucose, and even at early stages of glucose dysmetabolism efforts should be concentrated on the control of acute glycemic spikes.

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Declaration of Competing Interest

None.

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