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Glycaemic variability using continuous glucose monitoring and endothelial function in the metabolic syndrome and in Type 2 diabetes

S. Buscemi, A. Re, J. A. Batsis*†, M. Arnone, A. Mattina, G. Cerasola and S. Verga

Dipartimento di Medicina Interna, Malattie Cardiovascolari e NefroUrologiche; Facoltà di Medicina, University of Palermo, Palermo, Italy, *Section of General Internal Medicine, Dartmouth–Hitchcock Medical Center, Lebanon and †Dartmouth College, Hanover, NH, USA

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Abstract

Aims Subjects who are at increased risk of developing diabetes may have increased glycaemic variability associated with endothelial dysfunction and possibly subclinical atherosclerosis, which may lead to increased cardiovascular risk observed at the time of diabetes diagnosis. To investigate this hypothesis, we measured endothelial function, carotid intima-media thickness and glycaemic variability using 48-h continuous subcutaneous glucose monitoring in 3 groups of overweight or obese subjects – those without the metabolic syndrome, and those with the metabolic syndrome with or without newly diagnosed Type 2 diabetes.

Methods Consecutive subjects, aged 30–65 years with a body mass index ≥ 25 kg/m^2 were recruited. Patients were classified as with or without the metabolic syndrome,or as metabolic syndrome with newly diagnosed Type 2 DM. Glycaemic variability was calculated in terms of the coefficient of variation. Endothelial function was measured using brachial artery flow-mediated dilation.

Results We identified 75 subjects. Mean flow mediated dilation decreased ($P < 0.001$) and carotid intima-media thickness increased ($P < 0.05$) across groups. Flow mediated dilation predictors included mean 48-h continuous subcutaneous glucose monitoring values ($\beta = -0.022$; $P < 0.005$) and the coefficient of variation ($\beta = -0.10$; $P = 0.01$). Carotid intima-media thickness predictors included age ($\beta = 0.009$; $P < 0.001$) and flow mediated dilation ($\beta = -0.014$; $P = 0.076$). Patients re-stratified according to cut-offs for mean 48-h glycaemia and variability demonstrated that subjects with high mean glycaemia but low coefficient of variability had similar flow mediated dilation and carotid intima-media thickness to subjects with low mean glycaemia but high coefficient of variation.

Conclusions This study suggests that glycaemic variability influences endothelial function even in non-diabetic subjects. Such variability may explain the increased cardiovascular risk observed in patients prior to developing overt Type 2 diabetes

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Keywords diabetes, endothelial function, glycaemic variability, intima-media thickness, metabolic syndrome

Abbreviations CSGM, continuous subcutaneous glucose monitoring; GTN, glyceryl trinitrate; HH, high glycaemia and high glycaemic variability; HL, high glycaemia and low glycaemic variability; LH, low glycaemia and high glycaemic variability; LL, low glycaemia and low glycaemic variability; MAGE, mean amplitude of glucose excursions

Introduction

Patients with Type 2 diabetes are at increased cardiovascular risk prior to developing overt hyperglycaemia, suggesting that normal fasting, postprandial glucose or HbA_{1c} do not fully predict the risk of macrovascular complications [1–3]. Glucose level variability, even within the normal range, may independently influence the cardiovascular risk [4]. We previously demonstrated [5] that glycaemic variability measured in terms of coefficient of variability (CV) using 48-h subcutaneous continuous glucose monitoring (CSGM) may be higher in non-diabetic subjects with the metabolic syndrome, an entity known to predict both diabetes and cardiovascular disease [6,7]. Interestingly, excessive glycaemic variability induces

Correspondence to: Silvio Buscemi, MD, PhD U.O. di Medicina Interna, Nefrologia ed Ipertensione Policlinico ''P. Giaccone'' Via del Vespro, 129 – I-90127 Palermo, Italy. E-mail: silbus@tin.it

oxidative stress independently from the average glucose concentrations [8,9]. Endothelial dysfunction often precedes the development of atherosclerotic plaques [10] and likely precedes histological and angiographic evidence of atherosclerosis [11]. Clamp studies have recently demonstrated [12] that oscillating blood glucose concentrations have a worse effect on endothelial function than stable hyperglycaemia in diabetic, as well as in non-diabetic subjects. We also reported [13] an independent inverse relationship between endothelial function, measured *in vivo* as flow-mediated dilation of the brachial artery (FMD), and 48-h glycaemic CV using CSGM in non-diabetic subjects with or without the metabolic syndrome. Carotid intima-media thickness is increased in newly diagnosed metabolic syndrome or diabetes [14]. As this is a well-known surrogate of subclinical atherosclerosis, which predicts myocardial infarction and stroke [15,16], the possibility exists that non-diabetic subjects at increased risk of developing diabetes may have increased glycaemic variability associated with endothelial dysfunction. We therefore considered flow mediated dilation, carotid intima-media thickness and glycaemic variability using 48-h CSGM in obese or overweight subjects not classified as metabolic syndrome, and in those with the metabolic syndrome with or without Type 2 diabetes to explore these associations.

Patients and Methods

Eighty-six overweight or obese subjects (49 males, 37 females) were recruited among the Obesity and Related Diseases Outpatient Department seen at the Department of Internal Medicine, Cardiovascular and Kidney Diseases of the University of Palermo (Italy). There was no incentive provided to the participants. The study period was from January 2008 to December 2009. Inclusion criteria were ages 30–65 years, BMI [body weight (kg)/height $(m)^2$] > 25 kg/m^2, stable body weight for the preceding 3 months. Exclusion criteria included patients with previously diagnosed diabetes; cardiovascular or systemic disease, with the exception of hypertension; regular use of medications other than anti-hypertensives; pregnancy or lactation in the past 6 months. Subjects were divided into three groups according to the presence or absence of the metabolic syndrome, or as metabolic syndrome associated with Type 2 diabetes. Diabetes was diagnosed at the time of subject enrolment. Metabolic syndrome [17] and Type 2 diabetes [18] were defined according to the most recent consensus statements. The study protocol was approved by the Ethics Committee of the University of Palermo and the Committee for the Protection of Human Subjects of Dartmouth College.

Measurements

Anthropometric measurements and routine blood tests were obtained in all subjects at the beginning of the study. Subjects were tested in the morning after a 12-h overnight fast. Flow-mediated dilation of the brachial artery and carotid ultrasonography were performed by the same operator (SB); ultrasound images were video recorded and analysed by a trained examiner (SV). CSGM was performed by two operators (MA and AR). AM was responsible for body composition and fat distribution measurements.

Height and body weight were measured. Fat mass (% body weight) and fat-free mass were estimated as previously described [19] using bioelectrical impedance analysis (BIA-103; RJL Systems, Detroit, MI, USA/Akern, Florence, Italy). Measurements were obtained at the umbilicus (waist circumference), at the most prominent buttock level (hip circumference); their ratio (waist–hip ratio) was calculated. Abdominal visceral and subcutaneous adipose sizes were also estimated measuring cutis-rectis and rectis-aorta thickness using high-resolution B-mode ultrasonography (Sonoline G50; Siemens, Erlangen, Germany) as previously described [14,20]. The rectis-aorta to cutis-rectis ratio was considered an indirect measure of body fat distribution. Our laboratory intra-observer coefficient of variation for cutis-rectis is 1.2% and that for rectis-aorta is 3.9%, including subjects with a BMI range of 18–45 kg/m^2.

Images of both extracranial carotid artery walls were obtained in several projections by a high-resolution ultrasonographic 10-MHz linear array probe. End-diastolic intima-media thickness of the far wall was measured 10 mm caudal to the bulb, from the anterior, lateral and posterior approaches using two-dimensional longitudinal sections of the vessel and the distance from the first echogenic line to the second echogenic line. The mean of both sides measurements was considered for calculations [14,21].

Forty-eight-hour CGSM

All subjects underwent CSGM. We targeted a 48-h period (range 46–50 h) by means of a microdialytic system (Glucoday; Menarini Diagnostics, Florence, Italy) as described elsewhere [5,22–24]. Briefly, a subtle semipermeable microdialytic fibre is placed in the subcutaneous adipose tissue of the abdominal wall by means a subtle catheter guide. The membrane is therefore in contact with the interstitial space; serum concentrations of glucose are roughly similar to those of the interstitial fluids despite a latency between the two compartments of 10–15 min. The microdialytic system is connected to a small peristaltic pump through two subtle Teflon draining tubes. A saline solution is continuously pushed from a small bag and crosses the microdialytic system so that a gradient of concentration of glucose between the saline and the interstitial fluid is continuously achieved. The glucose moves across the dialytic membrane from the interstitial fluid to the saline solution and the dialysate-containing glucose is drained towards the device. The glucose concentration of the dialysate is measured in the device by means of a biosensor based on the glucose–oxidase reaction. Once dressed, this device allows the routine daily activities to be attended to and it can be performed on an outpatient basis. Subjects are requested to maintain their usual dietary habits.

This system is generally employed for approximately 48 h, but it does allow accurate measurements for longer. At the end of the test, the registered data are downloaded into a computer and analysed by means of dedicated software. The final report gives the analysis of the glycaemic values registered every 3 min. Each test was accepted if at least 700 glycaemic values were obtained. Patients remained blinded throughout the test to glycaemic values.

The mean 48-h glycaemia, the standard deviation, the area under the curve of the 48-h glycaemic values, the coefficient of variability [CV% = (SD/mean) × 100] and the mean amplitude of glucose excursions (MAGE) [25] were computed for each CSGM test. Both the coefficient of variation and MAGE were surrogate measures of glycaemic variability.

Endothelial function

Endothelium-dependent reactivity in the macrocirculation was measured by brachial artery flow mediated dilation using a high-resolution vascular ultrasound (Sonoline G50; Siemens) with a 10-MHz linear array transducer [26–29]. A video processing system computed the brachial artery diameter in real time by analysing B-mode ultrasound images (FMD Studio; Institute of Physiology CNR, Pisa, Italy). The flow mediated dilation was calculated as the maximum percentage of increase of brachial artery diameter over baseline. Endothelium-independent dilation was assessed after the administration of 300 μg sublingual glyceryl–trinitrate. All flow mediated dilation and glyceryl trinibrate (GTN) dilation assessments were performed by the same operator; ultrasound images were video recorded and analysed by a trained reader. The intra-observer coefficient of variation for flow mediated dilation was 2.9% in our laboratory.

Laboratory analysis

Plasma glucose concentrations were measured using the glucose oxidase method (Instrumentation Laboratory, Milan, Italy); serum triglycerides, cholesterol and HDL cholesterol concentrations were measured with spectrophotometric assays (IL Test CHOL; IL Test HDL-CHOL; IL Test Triglycerides; Instrumentation Laboratory). LDL cholesterol concentration was calculated according to the Friedewald formula [30]. HbA_{1c} was measured by a commercially available kit (DCA 2000 Analyzer; Bayer Diagnostics, Milan, Italy).

Statistical analysis

All data are presented as means ± SEM. The cohort was divided into four subgroups according to both the median values of 48-h CSGM glycaemia and the coefficient of variation of glycaemia that were, respectively, 6.4 mmol/l and 26.7%. Variables were normally distributed on the basis of skewness and kurtosis. Groups were classified as follows: low glycaemia and low glycaemic variability (LL); low glycaemia and high glycaemic variability (LH); high glycaemia and low glycaemic variability (HL); high glycaemia and high glycaemic variability (HH). One-way ANOVA compared the group effect and, when statistically significant, pairwise comparisons were tested using the Fisher's least significant difference test. Linear regression analysis assessed the relationships between variables. Multiple regression analysis (stepwise forward selection) was performed to assess the strength and independency of associations between variables. Correlations are expressed by the Pearson's correlation coefficient. A two-tailed $P < 0.05$ was considered significant. All analyses were performed using Systat (Windows version 11.0; Systat Software Inc., San Jose, CA, USA).

Results

Eleven patients were excluded because the CSGM was reported as failed: in four cases this was because of elevated pressures in the apparatus; in seven cases it was as a consequence of an accidental broken fibre being placed in the subcutaneous tissue, leading to seventy-five subjects being included in our analysis. Baseline characteristics are reported in Table 1. Both the rectis-aorta to cutis-rectis ratio and the carotid intima-media thickness ratio significantly increased from the non-metabolic syndrome group to the metabolic syndrome with Type 2 diabetes group. Similarly, the 48-h mean glycaemia and the 48-h AUC of glycaemia increased. The 48-h glycaemic variability was higher in the netabolic syndrome with Type 2 diabetes group when considered in terms of coefficient of variation but not in terms of MAGE. Flow mediated dilation was lower in the metabolic syndrome with Type 2 diabetes group; a progressive reduction of GTN dilation was observed from the non-metabolic syndrome group to the metabolic syndrome with Type 2 diabetes group.

Multiple stepwise linear regression analysis demonstrated that flow mediated dilation was exclusively and independently predicted by both 48-h mean glycaemia ($\beta = -0.022$, $P < 0.005$) and the coefficient of variation ($\beta = -0.10$, $P < 0.01$); similarly, carotid intima media thickness was independently predicted by age ($\beta = 0.009$, $P < 0.001$) and flow mediated dilation ($\beta = -0.014$, $P = 0.076$). Data relative to the four subgroups based on the median values of mean 48-h glycaemia and coefficient of variation are reported in Table 2. In particular, both flow mediated and GTN dilation progressively decreased, moving from the LL to the HH group, but no significant difference was observed between the LH and the HL groups. Simple correlations between variables are reported in the Supporting Information (Appendix).

Discussion

We observed that glycaemic variability, expressed in terms of the coefficient of variation of 48-h CSGM, may be elevated even in non-diabetic subjects with or without the metabolic syndrome, and is also independently correlated with endothelial function. Our mean CSGM glycaemia is comparable with another study which investigated glucose exposure for normal glucose-tolerant

Table 1 Characteristics of the study population

	MetS– $n = 30$	MetS+ $n = 23$	MetS+/Type 2 DM $n = 22$	P* ANOVA	MetS– vs. MetS+	MetS– vs. MetS+/Type 2 DM	MetS+ vs. MetS+/Type 2 DM
Males/females	18/12	13/10	13/9				
Age (years)	47 ± 2	49 ± 2	57 ± 2	< 0.005	NS	< 0.001	< 0.01
Smokers (%)	30.4	13.6	26.7	NS			
Systolic blood pressure (mmHg)	129 ± 3	140 ± 4	139 ± 3	< 0.05	< 0.01	NS	NS
Diastolic blood pressure (mmHg)	78 ± 2	88 ± 3	80 ± 2	< 0.001	< 0.001	NS	< 0.005
Hypertension (%)	26.7	47.8	54.5	< 0.001			
Subjects on anti-hypertensives (%)	16.7	30.4	22.7	NS			
Biochemical parameters							
Cholesterol (mmol/l)	5.0 ± 0.2	5.4 ± 0.4	5.3 ± 0.3	NS			
HDL cholesterol (mmol/l)	1.33 ± 0.08	1.01 ± 0.05	1.22 ± 0.08	< 0.005	< 0.001	NS	NS
LDL cholesterol (mmol/l)	3.20 ± 0.16	3.48 ± 0.29	3.12 ± 0.21	NS			
Triglycerides (mmol/l)	1.11 ± 0.07	2.18 ± 0.26	2.27 ± 0.28	< 0.001	< 0.001	< 0.001	NS
Uric acid (mmol/l)	297 ± 24	351 ± 18	315 ± 24	NS			
Glucose (mmol/l)	4.8 ± 0.2	5.4 ± 0.1	9.1 ± 0.9	< 0.001	NS	< 0.001	< 0.001
HbA_{1c} (%)	5.3 ± 0.2	5.9 ± 0.1	7.9 ± 0.4	< 0.001	NS	< 0.001	< 0.001
Ultrasonographic parameters							
RA (mm)	88 ± 4	94 ± 6	94 ± 4	NS			
CR (mm)	32 ± 2	32 ± 2	26 ± 2	NS			
RA/CR	2.9 ± 0.2	3.3 ± 0.3	4.1 ± 0.4	< 0.05	NS	< 0.005	NS
C-IMT (mm)	0.71 ± 0.04	0.74 ± 0.04	0.88 ± 0.05	< 0.05	NS	< 0.01	< 0.05
Anthropometric measurements							
Body weight (kg)	95.3 ± 6.2	94.8 ± 3.1	86.5 ± 5.5	NS			
BMI (kg/m^2)	34.6 ± 2.2	35.0 ± 1.5	32.2 ± 1.4	NS			
Fat mass (%)	32.3 ± 2.5	35.4 ± 3.8	37.3 ± 3.0	NS			
Waist circumference (cm)	114 ± 5	110 ± 4	106 ± 5	NS			
Hip circumference (cm)	119 ± 5	116 ± 3	110 ± 4	NS			
48h-CSGM							
Mean glycaemia (mmol/l)	5.9 ± 0.2	6.4 ± 0.2	9.4 ± 0.7	< 0.001	NS	< 0.001	< 0.001
Standard deviation (mmol/l)	1.7	1.8	3.2				
95% CI	1.1–2.0	1.4–2.1	2.6–3.8				
CV%	28.3	26.7	33.4				
95% CI	25.1–31.5	23.0–30.3	29.6–37.2				
MAGE	209 ± 66	139 ± 14	222 ± 44	NS			
AUC of glycaemia	111 106 ± 9199	114 619 ± 5677	168 329 ± 16 405	< 0.001	NS	< 0.001	< 0.001
Endothelial function							
Flow-mediated dilation (%)	7.6 ± 0.6	7.9 ± 0.5	5.1 ± 0.5	< 0.001	NS	< 0.005	< 0.001
GTN dilation (%)	21.3 ± 1.1	18.1 ± 0.9	16.2 ± 0.8	< 0.005	< 0.05	< 0.001	NS

All values expressed as mean ± SEM or count (%).
C-IMT, carotid intima-media thickness; CSGM, continuous subcutaneous glucose monitoring; CR, cutis-rectis thickness; GTN, brachial artery glyceryl–trinitrate dilation; MAGE, mean amplitude of glucose excursions; MetS, metabolic syndrome; NS, not significant; RA, rectis-aorta thickness; Type 2 DM, Type 2 diabetes.
*P-values calculated using ANOVA; when ANOVA was significant ($P < 0.05$), pairwise comparisons among groups were tested using the Fisher's least significant difference test.

individuals using continuous glucose monitoring [31]; however, to our knowledge, no other study has considered glycaemic variability from CSGM and endothelial function in terms of flow mediated dilation in non-diabetic subjects. The concept that glycaemic variability influences endothelial function independently from constant hyperglycaemia has already been demonstrated both *in vitro* in human renal cells [32] and in animal studies [33,34]. Our results support the hypothesis that this mechanism may exist in humans *in vivo* even in the presence of normal blood glucose concentrations.

Reclassifying our cohort on the basis of median 48-h glycaemia and coefficient of variation illustrated the progressive decline of flow mediated dilation from the LL group through to the HH groups. Our data provide important information on the role glycaemic variability may play on influencing cardiovascular risk even in subjects with both mean 48-h glycaemia and HbA_{1c}

Table 2 Characteristics of patients re-stratified according to cut-offs for mean 48-h CSGM mean glycaemia and CV%

	Low 48-h CSGM mean glycaemia		High 48-h CSGM mean glycaemia		
	Low CV% LL	High CV% LH	Low CV% HL	High CV% HH	ANOVA (*P*)
Subjects (*n*)	24	14	14	23	
HbA_{1c} (%)	5.9 ± 0.1	6.1 ± 0.1	6.7 ± 0.3	7.6 ± 0.4*†‡	< 0.001
48-h CSGM					
Mean glycaemia (mmol/l)	5.6 ± 0.1	5.6 ± 0.1	7.7 ± 0.4‡§	9.3 ± 0.6†‡¶	< 0.001
CV%	22.1	33.6	22.3	38.5	
95% CI	20.5–23.7	31.6–35.5	20.2–24.5	35.6–41.4	
MAGE	207 ± 79	144 ± 44	119 ± 16	247 ± 41	NS
AUC of glycaemia	114 407 ± 10 576	95 793 ± 7681	123 435 ± 4451	167 727 ± 16 097*†‡	< 0.001
Flow-mediated dilation (%)	8.7 ± 0.5	7.3 ± 0.8	6.7 ± 0.8**	5.1 ± 0.5‡††	< 0.001
GTN (%)	21.1 ± 1.3	19.8 ± 0.8	17.1 ± 1.4**	16.8 ± 0.9§§	< 0.05
C-IMT (mm)	0.71 ± 0.04	0.67 ± 0.03	0.78 ± 0.04	0.89 ± 0.06†‡‡	< 0.005

Data are means ± SEM.
Fischer's least significant difference test, significance level of *P*: **P* < 0.05 vs. HL; †*P* < 0.001 vs. LH; ‡*P* < 0.001 vs. LL; §*P* < 0.005 vs. LH; ¶*P* < 0.01 vs. HL; ***P* < 0.05 vs. LL; ††*P* < 0.05 vs. LH; ‡‡*P* < 0.005 vs. LL; §§*P* < 0.01 vs. LL .
C-IMT, carotid intima-media thickness; CSGM, continuous subcutaneous glucose monitoring GTN, brachial artery glyceryl–trinitrate dilation; HH, high glycaemia and high glycaemic variability; HL, high glycaemia and low glycaemic variability; LH, low glycaemia and high glycaemic variability; LL, low glycaemia and low glycaemic variability; MAGE, mean amplitude of glucose excursions ; NS, not significant.

within the normal range. Measured glycaemic variability may explain the increased cardiovascular risk already observed at the diagnosis of Type 2 diabetes, whereby elevated glycaemic variability prior to the diagnosis of diabetes may unfavourably affect endothelial function. This study provides additional credence to subclinical structural changes existing before a diagnosis of Type 2 diabetes is established. In fact, both carotid intima media thickness and GTN brachial artery dilation change accordingly, not only from the non metabolic syndrome to the metabolic syndrome with Type 2 groups, but also from the LL to the LH to the HL to the HH subgroups (Table 2), suggesting the presence of a continuum, and that increased glycaemic variability, particularly as evidenced by the correlation between carotid intima media thickness and flow mediated dilation, may precede established hyperglycaemia and be associated with endothelial dysfunction.

We observed significant results when glycaemic variability was considered in terms of the coefficient of variation, not by MAGE, which were not surprising. Despite the use of MAGE in assessing daily glycaemic variability, this measure was originally implemented to assess glycaemic variability of few pieces of data performed daily or quotidian with calculations designed to quantitate major fluctuations and exclude minor ones [35,36]. However, minor variations included in the calculation of both standard deviation and the coefficient of variation are probably able to trigger deleterious effects by the activation of oxidative stress [12,36], thereby explaining their negative effect on endothelial function in non-diabetic subjects. The coefficient of variation has already been used to describe the variability of other variables, including blood pressure obtained from non-invasive ambulatory blood pressure monitoring [37]. Despite standard deviation being a measure of variability, it appears correlated with the mean glycaemic value and hence is inappropriate to use when comparing diabetic and non-diabetic groups. Therefore, the coefficient of variation has the advantage of normalizing the variability expressed by the standard deviation by the mean value of glycaemia, allowing a more appropriate comparison between groups of subjects with different glucose tolerance [36]. This does not exclude the need to identify other more appropriate expressions to describe glycaemic variability from a larger number of observations as compared with those we obtained (more than 700) from the 48-h CSGM profile [38].

Our study has inherent limitations. The limited number of subjects was not homogeneous as far as age, but was accounted for in our multiple regression analysis. In addition, the small cohort may not have external validity; however, this number is considered high for studies using the microdialytic system of CSGM. Moreover, our results are based on more than 60 000 glycaemic values. We do acknowledge that larger cohorts are needed to confirm these preliminary results.

In conclusion, this study provides additional evidence that glycaemic variability influences endothelial function even in non-diabetic subjects. The 48-h CSGM test may have a future promise in identifying and classifying subjects with varying glycaemic variability to better stratify their cardiovascular risk and possibly target individualized treatments and preventive therapies. However, further studies are necessary to address these issues.

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Competing interests

Nothing to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Matrix of univariate correlations.

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