Associations Between Continuous Glucose Monitoring-derived Metrics and Arterial Stiffness in Patients with Type 2 Diabetes

Satomi Wakasugi
Juntendo University: Juntendo Daigaku
Tomoya Mita (tom-m@juntendo.ac.jp)
Juntendo University https://orcid.org/0000-0002-4401-2965
Naoto Katakami
Osaka Daigaku
Yosuke Okada
Sangyo Ika Daigaku
Hidenori Yoshii
Juntendo Tokyo Koto Geriatric Medical Center: Juntendo Tokyo Koto Koreisha Iryo Center
Takeshi Osonoi
Nakakinen Clinic
Nobuichi Kuribayashi
Misaki Naika Ckinic
Yoshinobu Taneda
Taneda Clinic
Yuichi Kojima
Musashino Familiy Clinic
Masahiko Gosho
Tsukuba Daigaku
Iichiro Shimomura
Osaka Daigaku
Hirotaka Watada
Juntendo Daigaku

Original investigation

Keywords: glucose variability, continuous glucose monitoring, arterial stiffness, type 2 diabetes

Posted Date: September 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-78697/v1

Version of Record: A version of this preprint was published on January 7th, 2021. See the published version at https://doi.org/10.1186/s12933-020-01194-2.

Abstract

Background Previous studies have suggested that high mean glucose levels and various glycemic abnormalities such as glucose fluctuation and hypoglycemia accelerate the progression of atherosclerosis in patients with type 2 diabetes. Although continuous glucose monitoring (CGM) that could evaluate such glycemic abnormalities has been rapidly adopted, the associations between CGM-derived metrics and arterial stiffness are not entirely clear.

Methods This is an exploratory analysis of an ongoing prospective, multicenter, 5-year follow-up observational study. Study participants included 445 outpatients with type 2 diabetes who underwent CGM and brachial-ankle pulse wave velocity (baPWV) measurement at baseline. Associations between CGM-derived metrics and baPWV were investigated using multivariate regression models.

Results In a linear regression model, all CGM–derived metrics were significantly associated with baPWV, but HbA1c was not. Some CGM–derived metrics related to intra-day glucose variability, hyperglycemia, and hypoglycemia remained significantly associated with baPWV after adjusting for possible atherosclerotic risk factors, including HbA1c. Based on baPWV ≥1,800 cm/s as indicative of high arterial stiffness, multivariate logistic regression found that some CGM-derived metrics related to intra-day glucose variability and hyperglycemia are significantly associated with high arterial stiffness even after adjusting for possible atherosclerotic risk factors, including HbA1c.

Conclusions Multiple CGM-derived metrics are significantly associated with baPWV and high arterial stiffness in patients with type 2 diabetes who have no history of apparent cardiovascular disease. These metrics might be useful for identifying patients at high risk of developing cardiovascular disease.

This study has been registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000032325).

Background

Type 2 diabetes is an independent risk factor for cardiovascular disease (CVD), which is one of the main causes of death among patients with type 2 diabetes. Thus, achieving optimal glycemic control in patients with type 2 diabetes is indispensable to preventing CVD.

HbA1c is the gold standard for assessing glycemic control. It reflects the mean glucose level over the last 2–3 months. Some studies have demonstrated strong associations between HbA1c levels and diabetic complications [1, 2]. However, some large clinical trials have failed to show that intensive glycemic control, as assessed based on HbA1c, has beneficial effects on the onset of CVD in type 2 diabetes [3–5]. This would be most likely due to the fact that HbA1c does not provide all of the information on glycemic abnormalities, such intra-day and inter-day glucose variability and hypoglycemia, both of which may play an important role in the development of CVD.

Importantly, recent clinical studies have suggested that glucose variability is more significantly associated with a higher subsequent incidence of myocardial infarction, acute heart failure, and cardiac death than the degree of hyperglycemic exposure indicated by HbA1c levels in patients with both type 2 diabetes and acute myocardial infarction [6]. Thus, evaluating various aspects of glycemic status may help identify patients with a high probability of developing CVD. In this regard, continuous glucose monitoring (CGM) has emerged as an optimal method to obtain a more complete profile of blood glucose status that includes intra-day and inter-day glucose variations and patterns of hyperglycemia and hypoglycemia.

Recent clinical studies have demonstrated that standard deviation (SD), mean amplitude of glycemic excursion (MAGE), and time in range (TIR), which reflect intra-day glucose variation, are significantly associated with carotid artery intima-medial thickening [7, 8]. Other studies have demonstrated that MAGE is associated with the presence and severity of coronary artery disease and vascular endothelial function, respectively [9, 10].

Increased arterial wall stiffness reflects the state of atherosclerosis. The degree of arterial stiffness does not necessarily reflect arterial intima-media thickness or endothelial function. To assess arterial wall stiffness, brachial-ankle pulse wave velocity (baPWV) is a noninvasive parameter often used clinically. It is useful for evaluating the state of atherosclerosis and predicting CVD in patients with type 2 diabetes [11]. However, the relationship between CGM-derived metrics and arterial stiffness has not yet been fully clarified.

In this explanatory study, we investigated the relationship between CGM-derived metrics and arterial stiffness in 455 patients with type 2 diabetes mellitus without a history of apparent CVD.

Research Design And Methods

Study Design

This study is an exploratory sub-analysis of an ongoing, observational, prospective cohort study that aims to investigate the relationship between glucose variability evaluated with CGM and the incidence of composite cardiovascular events over a 5-year follow-up period, as described previously [12]. This study used baseline data from the cohort study. This study has been registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR), which is a non-profit organization in Japan that meets the requirements of the International Committee of Medical Journal Editors (UMIN000032325).

Study Population

The study population consists of Japanese patients with type 2 diabetes who regularly attend the outpatient diabetes clinics of 34 institutions shown in Supplementary Table 1. The study design, inclusion criteria, and exclusion criteria were published previously [12]. Briefly, consecutive subjects were screened. Patients who meet the eligibility criteria were asked to participate in the present study. A total of

1,000 outpatients with type 2 diabetes under stable control and with no history of apparent CVD was recruited between May 2018 and March 2019. One patient withdrew consent. Among 999 subjects, 445 who underwent baPWV assessment were included in this exploratory sub-analysis.

The protocol was approved by the institutional review board of each participating institution in compliance with the Declaration of Helsinki and current legal regulations in Japan. Written informed consent was obtained from all participants after a full explanation of the study.

Biochemical Tests

Blood samples were obtained at visits after overnight fasting. Renal function, lipid levels, and HbA1c (National Glycohemoglobin Standardization Program) were measured with standard techniques. Urinary albumin excretion (UAE) was measured using a latex agglutination assay on a spot urine sample. Estimated glomerular filtration rate (eGFR) was calculated using a formula [13].

CGM With The Freestyle Libre Pro Device

The FreeStyle Libre Pro CGM (FLP-CGM) device (Abbott Japan, Tokyo, Japan), which measures glucose levels every 15 minutes for up to 14 days, was used in this study as previously reported [12]. Other than FLP-CGM use, there were no restrictions on participants' daily lives. Downloaded data sets were further analyzed. Glucose variability was assessed based on MAGE [14], SD, and coefficient of variation (CV). MAGE was calculated as the arithmetic mean of the differences between consecutive peaks and nadirs, provided that the differences are greater than one SD of the mean glucose value. CV (%) was calculated by dividing SD by the mean of the corresponding glucose readings. The original statistical analysis plan (SAP) for this study was reported in the initial study protocol [12]. After the publication of the SAP, the Advanced Technologies & Treatments for Diabetes Congress proposed some CGM-derived metrics as useful clinical targets that complement HbA1c [15]. Thus, we updated the SAP by adding some CGM-derived metrics to this study prior to database lock. Mean glucose was calculated from data collected during FLP-CGM use. TIR was defined as the percentage of time spent in the target range between 3.9 and 10.0 mmol/L (time in range, $TIR^{3.9–10\ mmol/L}$), time above target glucose range ($TAR^{>10\ mmol/L}$, $TAR^{>13.9\ mmol/L}$), and time below target glucose range ($TBR^{<3.9\ mmol/L}$, $TBR^{<3.0\ mmol/L}$). Low blood glucose index (LBGI) and high blood glucose index (HBGI) formulae were implemented by converting glucose values into risk scores [16]. In addition, mean of daily differences (MODD) [17] in glucose levels and interquartile range (IQR) were calculated to assess inter-day glucose variability. MODD was calculated as the mean of the absolute difference between glucose levels measured at the same time on 2 consecutive days. IQR was calculated using values from the same time of day during the monitoring period. Since a previous study demonstrated that FLP-CGM was less accurate during the first 24 hours (from the first day to the second day) after insertion and during the last four days of its 14-day lifetime [18], we analyzed FLP-CGM data over the middle 8-day period.

Measurement Of BAPWV

At baseline, baPWV was measured using an automatic waveform analyzer (BP-203RPE form; Colin Medical Technology, Komaki, Japan), as described previously [19]. Briefly, measurement was performed in the supine position after 5 min of bed rest. Cuffs for occlusion and monitoring were placed snugly around both arms and both ankles. The pressure waveforms were then recorded simultaneously from the brachial arteries using the oscillometric method. All scans were conducted by well-trained observers at each institution. A previous study confirmed that baPWV measurements have high reproducibility [20]. Subjects with an ankle-brachial index ≤ 0.90 were considered to have peripheral artery disease; baPWV data of these individuals were excluded from this study.

Statistical Analysis

Results are presented as means ± SD for continuous variables or number (proportion) of patients for categorical variables. Comparisons between two groups were analyzed with Student's t-test and Wilcoxon's rank sum test for continuous data or the chi-square test or Fisher's exact test for categorical data as appropriate.

Subjects were categorized into two groups based on the baPWV value of 1,800 cm/s. We used this cutoff value recommended by the Japanese Circulation Society to identify subjects who are at high risk for developing CVD [21]. High arterial stiffness was defined as baPWV ≥ 1,800 cm/s and low arterial stiffness was defined as baPWV < 1,800 cm/s.

Multivariate linear regression analysis was performed to investigate whether FLP-CGM–derived metrics are associated with baPWV when it was treated as continuous variable. In addition, multivariate logistic regression analysis was performed to investigate whether FLP-CGM–derived metrics are associated with high baPWV when it was treated as a dichotomous variable. Potential conventional risk factors evaluated with clinical, biochemical, or metabolic testing were included in the models based on clinical judgment. All statistical tests were two-sided with a 5% significance level. All analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC).

Results

Baseline Clinical Characteristics

The baseline clinical characteristics of the 445 patients with type 2 diabetes are summarized in Table 1. Mean age was 65.9 ± 9.0 years, 67.6% were male, HbA1c was 7.08 ± 0.78% (53.9 ± 8.6 mmol/mol), and estimated duration of type 2 diabetes was 13.5 ± 8.3 years.

Table 1
Patient demographic and background characteristics (n = 445)

Parameter	
Age (years)	65.9 ± 9.0
Male gender (%)	301 (67.6)
Body mass index (kg/m^2)	24.5 ± 3.8
Estimated duration of diabetes (years)	13.5 ± 8.3
Current smoking (%)	92 (20.7)
Systolic blood pressure (mmHg)	132.5 ± 14.8
Diastolic blood pressure (mmHg)	77.1 ± 11.2
HbA1c (%)	7.1 ± 0.8
HbA1c (mmol/mol)	53.9 ± 8.6
Total cholesterol (mmol/L)	4.92 ± 0.78
LDL cholesterol (mmol/L)	2.77 ± 0.64
HDL cholesterol (mmol/L)	1.55 ± 0.40
Triglycerides (mmol/L)	1.40 ± 1.0
Uric acid (µmol/l)	310.6 ± 73.5
Estimated glomerular filtration rate (mL/min/ 1.73 m^2)	70.8 ± 18
FLP-CGM–derived metrics	
SD (mmol/L)	2.02 ± 0.66
CV (%)	25.6 ± 5.8
MAGE (mmol/L)	5.44 ± 2.09
TIR$^{3.9–10\ mmol/L}$ (%)	78.7 ± 19.7
TAR$^{>10\ mmol/L}$ (%)	19.6 ± 20.1
TAR$^{>13.9\ mmol/L}$ (%)	4.03 ± 9.11

Data are mean ± SD or number of patients (%).

baPWV, brachial-ankle pulse wave velocity; CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring; HBGI, high blood glucose index; HDL, high density lipoprotein; IQR, interquartile range; LBGI, low blood glucose index; LDL, low density lipoprotein; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; SD, standard deviation; SDR, simple retinopathy; TAR, time above range; TBR, time below range; TIR, time in range.

Parameter	
$TBR^{< 3.9\ mmol/L}$ (%)	1.77 ± 4.28
$TBR^{< 3.0\ mmol/L}$ (%)	0.30 ± 1.34
LBGI	1.42 ± 1.60
HBGI	5.63 ± 4.64
MODD (mmol/L)	1.72 ± 0.62
IQR (mmol/L)	2.12 ± 0.77
baPWV (cm/s)	1706 ± 367
Data are mean ± SD or number of patients (%).	
baPWV, brachial-ankle pulse wave velocity; CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring; HBGI, high blood glucose index; HDL, high density lipoprotein; IQR, interquartile range; LBGI, low blood glucose index; LDL, low density lipoprotein; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; SD, standard deviation; SDR, simple retinopathy; TAR, time above range; TBR, time below range; TIR, time in range.	

Relationship Between FLP-CGM–derived Metrics and baPWV

We investigated the relationship between FLP-CGM–derived metrics and baPaWV in patients with type 2 diabetes when baPWV was treated as continuous variable. In a linear regression model, all calculated FLP-CGM–derived metrics were significantly associated with baPWV, although no significant association was observed between HbA1c and baPWV (Model 1 in Table 2). In Model 2, which adjusted for age and gender, all the aforementioned associations remained significant. After adjusting for variables in Model 2 plus body mass index (BMI) and duration of diabetes (Model 3), the associations between FLP-CGM–derived metrics and baPWV remained significant, except for LBGI. Even after adjusting for variables in Model 3 plus HbA1c, systolic blood pressure (BP), lipid parameters, uric acid, eGFR, UAE, smoking, alcohol consumption, use of insulin therapy, use of angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin II receptor blockers (ARBs), use of statins, use of anti-platelet agents, and presence of diabetic retinopathy (Model 4), the associations between the FLP-CGM–derived metrics of mean glucose, SD, CV, MAGE, $TIR^{3.9–10\ mmol/L}$, $TAR^{> 10\ mmol/L}$, $TAR^{> 13.9\ mmol/L}$, $TBR^{< 3.9\ mmol/L}$, $TBR^{< 3.0\ mmol/L}$, and HBGI and baPWV remained significant (Table 2).

Table 2
Associations between FLP-CGM–derived metrics and branchial-ankle pulse wave velocity

Parameter	Regression coefficient (95% CI)	*P* value
Mean glucose (1 mmol/L increase)		
Model 1	25.0 (5.9–44.2)	0.011
Model 2	26.8 (9.9–43.6)	0.002
Model 3	26.7 (9.9–43.5)	0.002
Model 4	37.0 (10.9–63.1)	0.006
SD (mmol/L) (1 mmol/L increase)		
Model 1	137.2 (86.5-187.9)	< 0.001
Model 2	102.3 (56.8-147.9)	< 0.001
Model 3	93.0 (46.9–139.0)	< 0.001
Model 4	94.6 (39.4-149.8)	< 0.001
CV (%) (1% increase)		
Model 1	14.6 (8.9–20.3)	< 0.001
Model 2	8.6 (3.3–13.8)	0.001
Model 3	7.0 (1.6–12.4)	0.012
Model 4	6.1 (0.7–11.6)	0.028
MAGE (1 mmol/L increase)		
Model 1	39.7 (23.7–55.7)	< 0.001
Model 2	28.3 (13.9–42.7)	< 0.001
Model 3	26.3 (11.9–40.7)	< 0.001
Model 4	27.5 (11.4–43.7)	< 0.001
$TIR^{3.9–10\ mmol/L}$ (10% increase)		
Model 1	-33.5 (-50.6- -16.5)	< 0.001
Model 2	-29.8 (-44.9- -14.7)	< 0.001
Model 3	-28.8 (-43.9- -13.7)	< 0.001
Model 4	-37.4 (-59.8- -15.1)	0.002
$TAR^{>10\ mmol/L}$ (1% increase)		

Parameter	Regression coefficient (95% CI)	*P* value
Model 1	2.51 (0.82–4.20)	0.004
Model 2	2.36 (0.86–3.85)	0.002
Model 3	2.31 (0.82–3.80)	0.002
Model 4	3.08 (0.77–5.38)	0.009
$TAR^{>13.9\ mmol/L}$ (1% increase)		
Model 1	4.92 (1.81–8.66)	0.010
Model 2	5.50 (2.22–8.79)	0.001
Model 3	5.61 (2.35–8.87)	< 0.001
Model 4	5.81 (1.49–10.12)	0.008
$TBR^{<3.9\ mmol/L}$ (1% increase)		
Model 1	16.1 (8.2–24.0)	< 0.001
Model 2	11.7 (4.6–18.7)	0.001
Model 3	10.3 (3.21–17.3)	0.004
Model 4	8.1 (1.0-15.3)	0.026
$TBR^{<3.0\ mmol/L}$ (1% increase)		
Model 1	52.1 (28.2–77.9)	< 0.001
Model 2	45.1 (23.0-67.1)	< 0.001
Model 3	41.5 (19.4–63.6)	< 0.001
Model 4	29.9 (7.7–52.1)	0.008
LBGI (1 unit increase)		
Model 1	25.6 (4.26–46.9)	0.019
Model 2	14.3 (-4.8-33.3)	0.142
Model 3	9.1 (-10.1-28.4)	0.350
Model 4	4.0 (-16.5-24.6)	0.700
HBGI (1 unit increase)		
Model 1	12.6 (5.3–19.9)	< 0.001
Model 2	11.9 (5.5–2.2)	< 0.001

Parameter	Regression coefficient (95% CI)	*P* value
Model 3	11.6 (5.2–18.0)	< 0.001
Model 4	14.1 (4.9–23.4)	0.003
MODD (1 mmol/L increase)		
Model 1	64.5 (9.1-119.9)	0.023
Model 2	69.6 (20.7-118.3)	0.005
Model 3	58.4 (8.8–108.0)	0.021
Model 4	24.8 (-38.7-88.3)	0.444
IQR (1 mmol/L increase)		
Model 1	45.7 (1.5–89.9)	0.043
Model 2	57.0 (18.1–95.8)	0.004
Model 3	48.7 (9.3–88.2)	0.015
Model 4	30.2 (-20.8-81.3)	0.245
HbA1c (1% increase)		
Model 1	7.7 (-36.2-51.4)	0.734
Model 2	22.7 (-16.0-61.4)	0.250
Model 3	20.8 (-18.2-59.8)	0.295
Model 4 (excluding HbA1c)	-15.3 (-55.7-25.1)	0.245
Model 1: crude		
Model 2: adjusted for age and gender		
Model 3: adjusted for variables in Model 2 plus BMI, and duration of diabetes		
Model 4: adjusted for variables in Model 3 plus HbA1c, systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, logarithm of urinary albumin excretion, smoker, alcohol consumption, use of insulin therapy, use of angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers, use of stains, and use anti-platelet agents		
CI, confidence interval; CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring; HBGI, high blood glucose index; IQR, interquartile range; LBGI, low blood glucose index; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; SD, standard deviation; TAR, time above range; TBR, time below range; TIR, time in range.		

Relationship Between FLP-CGM–derived Metrics and High Arterial Stiffness

Next, 445 subjects were divided into a high arterial stiffness group (n = 149) and a low arterial stiffness group (n = 296) based on the cut-off baPWV value of 1,800 cm/s. The clinical characteristics of the high and low arterial stiffness groups are summarized in Table 3. Subjects with high arterial stiffness were older, had longer duration of diabetes, lower BMI, higher systolic BP, higher UAE, higher prevalence of diabetic retinopathy, and lower eGFR. In addition, they were more frequently treated with sulfonylureas, dipeptidyl peptidase-4 inhibitors, insulin, and calcium channel blockers. There were significant differences in all FLP-CGM–derived metrics except for $TBR^{< 3.9\ mmol/L}$, $TBR^{< 3.0\ mmol/L}$, and LBGI between the two groups.

Table 3
Comparisons of clinical parameters between the higher and low arterial stiffness groups

Parameter	Low arterial stiffness (n = 296)	High arterial stiffness (n = 149)	*P* value
Age (years)	63.5 ± 9.2	70.7 ± 5.9	< 0.001
Male gender (%)	201 (67.9)	100 (67.1)	0.866
Body mass index (kg/m^2)	24.8 ± 3.9	23.8 ± 3.4	0.007
Estimated duration of diabetes (years)	12.0 ± 7.4	16.4 ± 9.3	< 0.001
Diabetic retinopathy (%)	57 (19.3)	51 (34.2)	< 0.001
Smoking (%)	71 (24)	21 (14)	0.040
Systolic blood pressure (mmHg)	129.9 ± 12.4	137.6 ± 17.7	< 0.001
Diastolic blood pressure (mmHg)	77.3 ± 10.9	76.7 ± 11.8	0.560
HbA1c (%)	7.1 ± 0.83	7.1 ± 0.69	0.532
HbA1c (mmol/mol)	53.7 ± 9.0	54.2 ± 7.5	0.532
Total cholesterol (mmol/L)	4.93 ± 0.78	4.88 ± 0.78	0.523
LDL cholesterol (mmol/L)	2.78 ± 0.63	2.73 ± 0.64	0.492
HDL cholesterol (mmol/L)	1.55 ± 0.40	1.56 ± 0.40	0.757
Triglycerides (mmol/L)	1.39 ± 1.05	1.41 ± 1.04	0.459
Uric acid (µmol/L)	309.5 ± 74.5	312.7 ± 71.6	0.668
Estimated glomerular filtration rate (mL/min/ 1.73 m^2)	72.9 ± 19	66.7 ± 17	< 0.001
Urinary albumin excretion (mg/g creatinine)	52.1 ± 145	198 ± 726	0.001
Use of oral glucose-lowering agents (%)	260 (88)	140 (94)	0.043

Data are means ± SD or number of patients (%). Continuous data were compared using Student's t-test or Wilcoxon's rank sum test. Categorical data were compared using the chi-square test or Fisher's exact test as appropriate.

CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring; HBGI, high blood glucose index; HDL, high density lipoprotein; IQR, interquartile range; LBGI, low blood glucose index; LDL, low density lipoprotein; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; SD, standard deviation; TAR, time above range; TBR, time below range; TIR, time in range.

Parameter	Low arterial stiffness (n = 296)	High arterial stiffness (n = 149)	*P* value
Metformin (%)	161 (54)	73 (49)	0.282
Sulfonylureas (%)	30 (10)	29 (20)	0.006
Glinides (%)	16 (5.4)	9 (6)	0.784
Dipeptidyl peptidase-4 inhibitors (%)	154 (52)	98 (65.8)	0.006
Sodium-glucose cotransporter 2 inhibitors (%)	73 (25)	29 (20)	0.218
Thiazolidinediones (%)	42 (14)	13 (9)	0.098
α-glucosidase inhibitors (%)	91 (31)	33 (22)	0.056
Glucagon-like peptide-1 antagonists (%)	12 (4)	8 (5.4)	0.628
Insulin (%)	31 (11)	32 (22)	0.002
Angiotensin-converting enzyme inhibitors (%)	8 (3)	10 (7)	0.071
Angiotensin II receptor blockers (%)	127 (43)	65 (44)	0.885
Calcium channel blockers (%)	64 (22)	53 (36)	0.002
Statins (%)	140 (47)	67 (45)	0.642
Antiplatelet agents (%)	10 (3)	9 (6)	0.217
FLP-CGM–derived metrics			
Mean glucose (mmol/L)	7.56 ± 1.68	8.13 ± 1.94	0.037
SD (mmol/L)	1.93 ± 0.60	2.22 ± 0.66	< 0.001
CV (%)	24.8 ± 5.62	27.4 ± 5.9	< 0.001
MAGE (mmol/L)	5.13 ± 1.88	6.06 ± 2.34	< 0.001
$TIR^{3.9–10\ mmol/L}$ (%)	80.6 ± 19.5	74.8 ± 19.9	0.004

Data are means ± SD or number of patients (%). Continuous data were compared using Student's t-test or Wilcoxon's rank sum test. Categorical data were compared using the chi-square test or Fisher's exact test as appropriate.

CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring; HBGI, high blood glucose index; HDL, high density lipoprotein; IQR, interquartile range; LBGI, low blood glucose index; LDL, low density lipoprotein; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; SD, standard deviation; TAR, time above range; TBR, time below range; TIR, time in range.

Parameter	Low arterial stiffness (n = 296)	High arterial stiffness (n = 149)	*P* value
$TAR^{>10\ mmol/L}$ (%)	17.9 ± 19.7	22.9 ± 20.6	0.013
$TAR^{>13.9\ mmol/L}$ (%)	3.33 ± 7.98	5.41 ± 10.9	0.023
$TBR^{<3.9\ mmol/L}$ (%)	1.52 ± 3.52	2.27 ± 5.46	0.085
$TBR^{<3.0\ mmol/L}$ (%)	0.23 ± 1.23	0.42 ± 1.58	0.155
LBGI	1.34 ± 1.58	1.60 ± 1.63	0.103
HBGI	5.17 ± 4.22	6.56 ± 5.27	0.003
MODD (mmol/L)	1.67 ± 0.58	1.83 ± 0.68	0.011
IQR (mmol/L)	2.06 ± 0.71	2.24 ± 0.87	0.024

Data are means ± SD or number of patients (%). Continuous data were compared using Student's t-test or Wilcoxon's rank sum test. Categorical data were compared using the chi-square test or Fisher's exact test as appropriate.

CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring; HBGI, high blood glucose index; HDL, high density lipoprotein; IQR, interquartile range; LBGI, low blood glucose index; LDL, low density lipoprotein; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; SD, standard deviation; TAR, time above range; TBR, time below range; TIR, time in range.

Using this classification of baPWV, we investigated the relationship between FLP-CGM–derived metrics and high baPWV. In a multivariate logistic regression model, all FLP-CGM–derived metrics except for LBGI were significantly associated with high arterial stiffness, although no significant association was observed between HbA1c and arterial stiffness (Model 1 in Table 4). In Models 2 and 3, all FLP-CGM–derived metrics except for LBGI remained significantly associated with high baPWV. In Model 4, SD, CV, MAGE, $TAR^{>13.9\ mmol/L}$, and HBGI remained significantly associated with high baPWV.

Table 4
Associations between FLP-CGM–derived metrics and high arterial stiffness

Parameter	Regression coefficient (95% CI)	*P* value
Mean glucose (1 mmol/L increase)		
Model 1	1.12 (1.01–1.25)	0.039
Model 2	1.16 (1.03–1.32)	0.016
Model 3	1.17 (1.03–1.32)	0.014
Model 4	1.22 (0.98–1.52)	0.074
SD (mmol/L) (1 mmol/L increase)		
Model 1	1.99 (1.46–2.71)	< 0.001
Model 2	1.86 (1.33–2.59)	< 0.001
Model 3	1.73 (1.24–2.43)	0.001
Model 4	1.95 (1.23–3.08)	0.004
CV (%) (1% increase)		
Model 1	1.08 (1.04–1.12)	< 0.001
Model 2	1.06 (1.02–1.10)	0.003
Model 3	1.05 (1.01–1.09)	0.022
Model 4	1.05 (1.01–1.10)	0.021
MAGE (1 mmol/L increase)		
Model 1	1.24 (1.12–1.36)	< 0.001
Model 2	1.21 (1.08–1.34)	< 0.001
Model 3	1.19 (1.07–1.33)	0.001
Model 4	1.24 (1.08–1.43)	0.002
$TIR^{3.9–10\ mmol/L}$ (10% increase)		
Model 1	0.87 (0.79–0.96)	0.004
Model 2	0.86 (0.77–0.96)	0.007
Model 3	0.86 (0.77–0.96)	0.009
Model 4	0.85 (0.71–1.03)	0.092
$TAR^{>10\ mmol/L}$ (1% increase)		

Parameter	Regression coefficient (95% CI)	*P* value
Model 1	1.01 (1.00-1.02)	0.014
Model 2	1.01 (1.00-1.03)	0.013
Model 3	1.01 (1.00-1.03)	0.013
Model 4	1.02 (1.00-1.04)	0.092
$TAR^{>13.9\ mmol/L}$ (1% increase)		
Model 1	1.02 (1.00-1.05)	0.029
Model 2	1.03 (1.01–1.06)	0.005
Model 3	1.04 (1.01–1.06)	0.003
Model 4	1.05 (1.01–1.09)	0.014
$TBR^{<3.9\ mmol/L}$ (1% increase)		
Model 1	1.04 (0.99–1.09)	0.098
Model 2	1.02 (0.97–1.07)	0.453
Model 3	1.01 (0.96–1.06)	0.862
Model 4	1.00 (0.94–1.07)	0.923
$TBR^{<3.0\ mmol/L}$ (1% increase)		
Model 1	1.10 (0.96–1.28)	0.179
Model 2	1.07 (0.92–1.25)	0.367
Model 3	1.04 (0.90–1.21)	0.609
Model 4	1.00 (0.83–1.20)	0.999
LBGI (1 unit increase)		
Model 1	1.10 (0.98–1.24)	0.110
Model 2	1.06 (0.93–1.21)	0.384
Model 3	1.02 (0.89–1.16)	0.790
Model 4	1.00 (0.85–1.18)	0.990
HBGI (1 unit increase)		
Model 1	1.06 (1.02–1.11)	0.004
Model 2	1.08 (1.03–1.13)	0.002

Parameter	Regression coefficient (95% CI)	*P* value
Model 3	1.08 (1.03–1.13)	0.002
Model 4	1.11 (1.02–1.20)	0.011
MODD (1 mmol/L increase)		
Model 1	1.50 (1.09–2.05)	0.012
Model 2	1.71 (1.20–2.44)	0.003
Model 3	1.59 (1.11–2.29)	0.013
Model 4	1.40 (0.83–2.36)	0.209
IQR (1 mmol/L increase)		
Model 1	1.33 (1.03–1.71)	0.026
Model 2	1.53 (1.15–2.04)	0.003
Model 3	1.45 (1.08–1.94)	0.013
Model 4	1.40 (0.92–2.15)	0.121
HbA1c (1% increase)		
Model 1	1.08 (0.84–1.39)	0.531
Model 2	1.21 (0.91–1.62)	0.193
Model 3	1.20 (0.89–1.62)	0.228
Model 4 (excluding HbA1c)	0.94 (0.65–1.35)	0.719
Model 1: crude		
Model 2: adjusted for age and gender		
Model 3: adjusted for variables in Model 2 plus BMI, and duration of diabetes		
Model 4: adjusted for variables in Model 3 plus HbA1c, systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, logarithm of urinary albumin excretion, smoker, alcohol consumption, use of insulin therapy, use of angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers, use of stains, and use anti-platelet agents		
CI, confidence interval; CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring; HBGI, high blood glucose index; IQR, interquartile range; LBGI, low blood glucose index; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; SD, standard deviation; TAR, time above range; TBR, time below range; TIR, time in range.		

Discussion

In this study, we demonstrated that most FLP-CGM–derived metrics are significantly associated with arterial stiffness, even after adjusting for various risk factors including HbA1c levels in 445 outpatients with type 2 diabetes and no history of apparent CVD. Notably, some FLP-CGM–derived metrics related to inter-day glucose variability and hyperglycemia identified subjects with high arterial stiffness who were at high risk for developing CVD.

In patients with type 2 diabetes, the main cause of arterial stiffness is damage to vascular walls caused by prolonged hyperglycemia. In fact, previous cross-sectional studies have indicated that higher HbA1c levels are associated with increased arterial stiffness in patients with type 2 diabetes [22, 23]. In contrast, HbA1c was not associated with arterial stiffness in this study, a finding that was consistent with a different previous study [24]. On the other hand, this study found significant associations between FLP-CGM derived metrics related to hyperglycemia such as $TAR^{>13.9\ mmol/L}$ and HBGI. Unlike HbA1c, these parameters reflect remarkable hyperglycemia and were not affected by hypoglycemia. Thus, our data do not contradict the fact that the main cause of arterial stiffness is damage to vascular walls caused by prolonged hyperglycemia in patients with type 2 diabetes.

Previous cross-sectional studies demonstrated that CGM-derived metrics related to intra-day glucose variability are associated with carotid atherosclerosis, coronary atherosclerosis, or endothelial dysfunction in patients with type 2 diabetes [7–10]. This study also found that SD, CV, and MAGE are significantly associated with arterial stiffness; these variables reflect another aspect of atherosclerosis in patients with type 2 diabetes. Various factors are involved in the progression of arterial stiffness in patients with type 2 diabetes. Indeed, previous cross-sectional studies have demonstrated that conventional atherosclerotic risk factors such as age, BMI, duration of type 2 diabetes, glycemic control, dyslipidemia, systolic BP, eGFR, elevated uric acid levels, and albuminuria [25–27] are associated with arterial stiffness in patients with type 2 diabetes. Intriguingly, in this study FLP-CGM–derived metrics related to intra-day glucose variability were significantly associated with degree of arterial stiffness, even after adjusting for those possible conventional atherosclerotic risk factors. Taken together, this study highlighted the importance of intra-day glucose variability in terms of assessing the risk of arterial stiffness.

A recent study demonstrated that the incremental glucose peak, an indicator of glucose variability, during an oral glucose tolerance test is associated with arterial stiffness in patients with type 2 diabetes [28]. However, whether oral glucose tolerance test-derived incremental glucose peak reflects the pattern of glucose variability during usual living conditions has not yet been clarified. In this regard, this is the first study to provide evidence that FLP-CGM–derived metrics related to intra-day glucose variability evaluated during usual living conditions are significantly associated with arterial stiffness. Although the exact mechanism of how glucose variability contributes to arterial stiffness remains unclear, we propose the following possible scenarios. Previous studies have shown that glucose variability induces inflammation and overproduction of oxidative stress to a greater extent than chronic persistent hyperglycemia [29, 30], leading to advanced AGE formation. The formation of AGEs is considered to be involved in arterial stiffness through cross-linking of collagen molecules and a subsequent loss of collagen elasticity [31].

Accordingly, vascular walls may be damaged by glucose variability more than by chronic persistent hyperglycemia.

In this study, FLP-CGM–derived metrics related to hypoglycemia were associated with arterial stiffness. Similarly, some recent studies have demonstrated that the acute effects of hypoglycemia include inflammation and endothelial injury in patients with type 1 diabetes [32, 33]. In addition, a cross-sectional study demonstrated that repeated episodes of hypoglycemia are associated with preclinical atherosclerosis as evaluated by carotid and femoral ultrasonography and measurement of flow-mediated brachial dilatation in patients with type 1 diabetes [34]. Furthermore, we previously reported that a higher frequency of hypoglycemic episodes is associated with progression of carotid atherosclerosis in patients with type 2 diabetes [35]. Accordingly, physicians need to help prevent hypoglycemic episodes through assessing the risks of hypoglycemia with CGM in order to minimize arterial stiffness, especially in patients who have difficulty detecting or who are completely unaware of hypoglycemia.

As discussed above, this study demonstrated that FLP-CGM–derived metrics are significantly associated with arterial stiffness. On the other hand, it is more important to screen for patients with a high potential of developing CVD in order to reduce the incidence of CVD. To achieve this goal, the Japanese Circulation Society proposed baPWV of 1,800 cm/sec as the cutoff value to identify subjects who are at high risk for developing CVD based on the results of several studies [36]. Based on this cutoff value, approximately 33% of study participants were defined as being at high risk for CVD. Subjects with high arterial stiffness were older, had longer duration of diabetes, lower BMI, higher systolic BP, higher UAE, higher prevalence of diabetic retinopathy, and lower eGFR. They were more frequently treated with insulin and calcium channel blockers. Even after adjusting for these confounding factors, FLP-CGM–derived metrics related to intra-day glucose variability such as SD, CV, MAGE, and metrics related to hyperglycemia such as $TAR^{>13.9\ mmol/L}$ and HBGI were significantly associated with high arterial stiffness. Given that FLP-CGM–derived metrics related to hypoglycemia, such as $TIR^{3.9–10\ mmol/L}$ and $TAR^{>10\ mmol/L}$ were not associated with high arterial stiffness, high postprandial glucose excursion amplitude may be a major contributor to increased arterial stiffness. Thus, based on FLP-CGM–derived metrics, focusing on reducing the amplitude of postprandial glucose excursion may be important to reducing the risk of both arterial stiffness and CVD development.

One strength of this study is its multicenter study design. Our study had certain limitations. First, the study was an exploratory study with a relatively small sample size. Second, the cross-sectional study design made it impossible to evaluate whether there was a causal relationship between FLP-CGM–derived metrics and arterial stiffness. We only used arterial stiffness as a marker for the risk of CVD development. In this regard, we are currently conducting a long-term follow-up study in the same cohort that focuses on FLP-CGM–derived metrics and onset of primary CVD or changes in arterial stiffness. Third, FLP-CGM–derived metrics were evaluated based on FLP-CGM measurements during a limited time. Due to this limitation, FLP-CGM–derived metrics related to inter-day glucose variability may be not associated with arterial stiffness after adjusting for various atherosclerotic risk factors. Repeated FLP-CGM measurements may be required to clarify the relationship between inter-day glucose variability and

arterial stiffness. Fourth, we only recruited Japanese patients with type 2 diabetes. These constraints may limit the generalizability of our results.

Conclusion

In this study, we demonstrated that FLP-CGM–derived metrics related to intra-day glucose variability, hyperglycemia, and hypoglycemia are significantly associated with arterial stiffness, even after adjusting for various risk factors in patients with type 2 diabetes with no history of apparent CVD. In addition, based on FLP-CGM–derived metrics, high postprandial glucose excursion amplitude can identify subjects who are at high risk for developing CVD. Thus, these metrics could provide medical professionals with useful information for assessing the risk of CVD.

Declarations

Acknowledgments

The authors wish to thank the study investigators listed in Supplementary Table 1 and the participants for their contributions to this study. **Funding**

This study was financially supported by the Japan Agency for Medical Research and Development (AMED) under Grant Number JP20ek0210105 (to H.W.) and the Manpei Suzuki Diabetes Foundation (to H.W.).

Ethics approval and consent to participate.

The protocol was approved by the institutional review board of each participating institution in compliance with the Declaration of Helsinki and current legal regulations in Japan. Written informed consent was obtained from all participants after a full explanation of the study.

Availability of data and materials.

The analyzed datasets are available from the corresponding author on reasonable request.

Consent for publication.

Not applicable.

Competing interests.

T.O. and H.W. have received research funds from Abbott Japan. H.W. is a member of the advisory board of Abbott Japan. All other authors (S.W., T.M., N.K., Y.O., H.Y., K.N., T.S., K.T., A.K., M.G., and I.S.) declare no conflicts of interest.

Author Contributions

All authors contributed to the study design and were involved in all stages of manuscript development. S.W. and T.M. drafted the manuscript. M.G., a statistician, was primarily responsible for data analysis. S.W, T.M., N.K., Y.O., T.O., H.Y., N.K., Y.T., Y.K., M.G., I.S., and H.W. also collected, analyzed, and interpreted the data; reviewed and edited the manuscript; and approved the final manuscript. H.W. is the principal guarantor of this work; he has full access to all study data and takes responsibility for the integrity of the data and the accuracy of data analysis. All authors have read and agreed to the publication of the manuscript.

Additional Information

Data Availability All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

References

1. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR: **Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study**. *Bmj* 2000, **321**(7258):405-412.
2. **The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial**. *Diabetes* 1995, **44**(8):968-983.
3. Action to Control Cardiovascular Risk in Diabetes Study G, Gerstein HC, Miller ME, Byington RP, Goff DC, Jr., Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F *et al*: **Effects of intensive glucose lowering in type 2 diabetes**. *The New England journal of medicine* 2008, **358**(24):2545-2559.
4. Group AC, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P *et al*: **Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes**. *The New England journal of medicine* 2008, **358**(24):2560-2572.
5. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R *et al*: **Glucose control and vascular complications in veterans with type 2 diabetes**. *The New England journal of medicine* 2009, **360**(2):129-139.
6. Gerbaud E, Darier R, Montaudon M, Beauvieux MC, Coffin-Boutreux C, Coste P, Douard H, Ouattara A, Catargi B: **Glycemic Variability Is a Powerful Independent Predictive Factor of Midterm Major Adverse Cardiac Events in Patients With Diabetes With Acute Coronary Syndrome**. *Diabetes care* 2019, **42**(4):674-681.
7. Mo Y, Zhou J, Li M, Wang Y, Bao Y, Ma X, Li D, Lu W, Hu C, Li M *et al*: **Glycemic variability is associated with subclinical atherosclerosis in Chinese type 2 diabetic patients**. *Cardiovascular diabetology* 2013, **12**:15.
8. Xin Z, Zhu Y, Wang S, Liu S, Xu M, Wang T, Lu J, Chen Y, Zhao Z, Wang W *et al*: **Associations of subclinical atherosclerosis with nonalcoholic fatty liver disease and fibrosis assessed by non-invasive score**. *Liver international : official journal of the International Association for the Study of the Liver* 2020, **40**(4):806-814.

9. Torimoto K, Okada Y, Mori H, Tanaka Y: **Relationship between fluctuations in glucose levels measured by continuous glucose monitoring and vascular endothelial dysfunction in type 2 diabetes mellitus**. *Cardiovascular diabetology* 2013, **12**:1.
10. Su G, Mi S, Tao H, Li Z, Yang H, Zheng H, Zhou Y, Ma C: **Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes**. *Cardiovascular diabetology* 2011, **10**:19.
11. Maeda Y, Inoguchi T, Etoh E, Kodama Y, Sasaki S, Sonoda N, Nawata H, Shimabukuro M, Takayanagi R: **Brachial-ankle pulse wave velocity predicts all-cause mortality and cardiovascular events in patients with diabetes: the Kyushu Prevention Study of Atherosclerosis**. *Diabetes care* 2014, **37**(8):2383-2390.
12. Mita T, Katakami N, Okada Y, Yoshii H, Osonoi T, Nishida K, Shiraiwa T, Torimoto K, Kurozumi A, Wakasugi S *et al*: **Protocol of a Prospective Observational Study on the Relationship Between Glucose Fluctuation and Cardiovascular Events in Patients with Type 2 Diabetes**. *Diabetes therapy : research, treatment and education of diabetes and related disorders* 2019, **10**(5):1565-1575.
13. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A: **Revised equations for estimated GFR from serum creatinine in Japan**. *Am J Kidney Dis* 2009, **53**(6):982-992.
14. Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF: **Mean amplitude of glycemic excursions, a measure of diabetic instability**. *Diabetes* 1970, **19**(9):644-655.
15. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, Bosi E, Buckingham BA, Cefalu WT, Close KL *et al*: **Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range**. *Diabetes care* 2019, **42**(8):1593-1603.
16. Kovatchev BP, Cox DJ, Kumar A, Gonder-Frederick L, Clarke WL: **Algorithmic evaluation of metabolic control and risk of severe hypoglycemia in type 1 and type 2 diabetes using self-monitoring blood glucose data**. *Diabetes technology & therapeutics* 2003, **5**(5):817-828.
17. Hill NR, Oliver NS, Choudhary P, Levy JC, Hindmarsh P, Matthews DR: **Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups**. *Diabetes technology & therapeutics* 2011, **13**(9):921-928.
18. Boscari F, Galasso S, Acciaroli G, Facchinetti A, Marescotti MC, Avogaro A, Bruttomesso D: **Head-to-head comparison of the accuracy of Abbott FreeStyle Libre and Dexcom G5 mobile**. *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2018, **28**(4):425-427.
19. Osonoi Y, Mita T, Osonoi T, Saito M, Tamasawa A, Nakayama S, Someya Y, Ishida H, Kanazawa A, Gosho M *et al*: **Morningness-eveningness questionnaire score and metabolic parameters in patients with type 2 diabetes mellitus**. *Chronobiology international* 2014, **31**(9):1017-1023.
20. Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, Koji Y, Hori S, Yamamoto Y: **Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity**

measurement. *Hypertension research : official journal of the Japanese Society of Hypertension* 2002, **25**(3):359-364.

21. Munakata M: **Brachial-ankle pulse wave velocity in the measurement of arterial stiffness: recent evidence and clinical applications**. *Current hypertension reviews* 2014, **10**(1):49-57.
22. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FU, Protogerou AD *et al*: **Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity**. *Journal of hypertension* 2012, **30**(3):445-448.
23. Hvelplund Kristiansen M, Banghoj AM, Laugesen E, Tarnow L: **Arterial stiffness in people with Type 2 diabetes and obstructive sleep apnoea**. *Diabetic medicine : a journal of the British Diabetic Association* 2018, **35**(10):1391-1398.
24. Hamamura M, Mita T, Osonoi Y, Osonoi T, Saito M, Tamasawa A, Nakayama S, Someya Y, Ishida H, Gosho M *et al*: **Relationships Among Conventional Cardiovascular Risk Factors and Lifestyle Habits With Arterial Stiffness in Type 2 Diabetic Patients**. *Journal of clinical medicine research* 2017, **9**(4):297-302.
25. Taniwaki H, Kawagishi T, Emoto M, Shoji T, Kanda H, Maekawa K, Nishizawa Y, Morii H: **Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. Vessel wall properties in type 2 diabetes**. *Diabetes care* 1999, **22**(11):1851-1857.
26. Ferreira MT, Leite NC, Cardoso CR, Salles GF: **Correlates of aortic stiffness progression in patients with type 2 diabetes: importance of glycemic control: the Rio de Janeiro type 2 diabetes cohort study**. *Diabetes care* 2015, **38**(5):897-904.
27. Fukui M, Tanaka M, Shiraishi E, Harusato I, Hosoda H, Asano M, Kadono M, Hasegawa G, Yoshikawa T, Nakamura N: **Serum uric acid is associated with microalbuminuria and subclinical atherosclerosis in men with type 2 diabetes mellitus**. *Metabolism: clinical and experimental* 2008, **57**(5):625-629.
28. Foreman YD, Brouwers M, Berendschot T, van Dongen M, Eussen S, van Greevenbroek MMJ, Henry RMA, Houben A, van der Kallen CJH, Kroon AA *et al*: **The oral glucose tolerance test-derived incremental glucose peak is associated with greater arterial stiffness and maladaptive arterial remodeling: The Maastricht Study**. *Cardiovascular diabetology* 2019, **18**(1):152.
29. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C: **Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes**. *Jama* 2006, **295**(14):1681-1687.
30. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, Boemi M, Giugliano D: **Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients**. *Diabetes* 2008, **57**(5):1349-1354.
31. Zieman SJ, Melenovsky V, Kass DA: **Mechanisms, pathophysiology, and therapy of arterial stiffness**. *Arteriosclerosis, thrombosis, and vascular biology* 2005, **25**(5):932-943.

32. Wright RJ, Newby DE, Stirling D, Ludlam CA, Macdonald IA, Frier BM: **Effects of acute insulin-induced hypoglycemia on indices of inflammation: putative mechanism for aggravating vascular disease in diabetes**. *Diabetes care* 2010, **33**(7):1591-1597.
33. Gogitidze Joy N, Hedrington MS, Briscoe VJ, Tate DB, Ertl AC, Davis SN: **Effects of acute hypoglycemia on inflammatory and pro-atherothrombotic biomarkers in individuals with type 1 diabetes and healthy individuals**. *Diabetes care* 2010, **33**(7):1529-1535.
34. Gimenez M, Gilabert R, Monteagudo J, Alonso A, Casamitjana R, Pare C, Conget I: **Repeated episodes of hypoglycemia as a potential aggravating factor for preclinical atherosclerosis in subjects with type 1 diabetes**. *Diabetes care* 2011, **34**(1):198-203.
35. Mita T, Katakami N, Shiraiwa T, Yoshii H, Kuribayashi N, Osonoi T, Kaneto H, Kosugi K, Umayahara Y, Gosho M *et al*: **Relationship between frequency of hypoglycemic episodes and changes in carotid atherosclerosis in insulin-treated patients with type 2 diabetes mellitus**. *Scientific reports* 2017, **7**:39965.
36. Ohkuma T, Tomiyama H, Ninomiya T, Kario K, Hoshide S, Kita Y, Inoguchi T, Maeda Y, Kohara K, Tabara Y *et al*: **Proposed Cutoff Value of Brachial-Ankle Pulse Wave Velocity for the Management of Hypertension**. *Circulation journal : official journal of the Japanese Circulation Society* 2017, **81**(10):1540-1542.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supTablewakasugietal.doc