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Relationship between continuous glucose monitor (CGM)-assessed glycemic health and biological markers of cardiovascular health in adults

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Abstract

Background Adults with overweight or obesity have a higher risk of the development of impaired glycemic health and cardiometabolic disorders. Glycemic variability acts as a more sensitive assessment of glycemic health compared to other clinical measures. Oxidative stress and diminished vascular health play a key role in the development of cardiometabolic disorders.

Objective To examine the relationship between glucose concentrations and glycemic variability with biological markers of vascular health and oxidative stress.

Methods Adults ($n = 28$; body mass index $= 32.3 \pm 6.3$ kg/m^2) completed 7-day continuous glucose monitoring. Percentage of time each day glucose concentrations were < 70 mg/dL, 70–180 mg/dL, and > 180 mg/dL was evaluated. Means of 24-h, waking and night sleep hours, maximum, minimum, and difference between maximum and minimum (Max–Min) glucose concentrations were determined. Measurements of intra- and inter-day glycemic variability were calculated. Fasting concentrations of glucose, vascular health marker nitric oxide (NO), and oxidative stress marker myeloperoxidase (MPO) were measured, and the ratio of NO concentration to MPO concentration (NO:MPO) was calculated (propensity to vasodilate).

Results MPO concentration negatively correlated with glycemic variability measured as the mean amplitude of glycemic excursion ($r^2 = 0.23$, $\beta = -0.62$, $p = 0.03$), while the NO:MPO ratio positively correlated with fasting glucose concentration ($r^2 = 0.22$, $\beta = 3.79$, $p = 0.01$).

Conclusions Our findings suggest that an increased propensity to vasodilate relates to increased fasting glucose concentration, while increased oxidative stress relates to decreased glycemic variability. These findings were unexpected and necessitates further research into the potential mechanisms of these findings for cardiometabolic health in adults having overweight or obesity.

Keywords Cardiometabolic health, Continuous glucose monitor (CGM), Free living, Myeloperoxidase (MPO), Non-diabetic, Nitric oxide (NO)

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Background

The prevalence of adults in the USA classified as having overweight or obesity continues to increase and is widely considered a major public health crisis of the current generation (Hales et al. 2018, 2020; Centers for Disease Control and Prevention (CDC) 2023). Adults who have overweight or obesity are at an increased risk of the development of cardiometabolic disorders, such as type 2 diabetes mellitus and cardiovascular disease (CVD) (Semlitch et al. 2019; NIH 2019; CDC 2023). However, having overweight or obesity may not directly translate to increased risk of CVD. Rather, a mechanism proposed is an interaction between increased body weight status and obesity-related insulin resistance causing changes in the cardiovascular system, leading to CVD (Adeva-Andany et al. 2019).

Oxidative stress has been shown to be elevated in adults having overweight and obesity and elevates the risk of development of CVD (Zheng et al. 2018; Adenan et al. 2020; Gâman et al. 2020). When accumulation exceeds detoxification of reactive oxygen species arises, an induction of oxidative stress occurs, resulting in vascular endothelium-related vasoconstriction (Sharma et al. 2018; Ramana et al. 2019). Further, chronic hyperglycemia has been mechanistically related to degraded vascular health and induction of oxidative stress, as hyperglycemia is a known cause of increased free radical activation and elevated reactive oxygen species (Luc et al. 2019).

Frequent or intensified fluctuations in glucose concentrations (i.e., glycemic variability) may contribute to deleterious complications linked to impaired glycemic health and development of CVD (Verma 2018; Kim et al. 2018). Glycemic variability has been utilized to determine glycemic control, potentially acts as a more sensitive measurement of glycemic health compared to traditional clinical assessments, and has been found to be increased in adults having overweight or obesity (Tejero et al. 2019; Rodrigues et al. 2018). Further, glycemic variability positively associates with induction of oxidative stress in type 2 diabetes (Monnier et al. 2006, 2008; Salkind et al. 2014). As technology has continued to advance, free-living glycemic variability assessment has become a minimally invasive procedure utilizing continuous glucose monitoring (CGM).

Whether a relationship exists between 24-h free-living glucose concentrations and glycemic variability with fasting biological markers of vascular health and oxidative stress in non-diabetic adults with overweight or obesity has yet to be elucidated. Therefore, the purpose of this analysis was to examine the associations between glucose concentrations and measures of glycemic variability determined by CGM with biological markers of vascular health and oxidative stress in non-diabetic adults having overweight or obesity. The overarching hypothesis was that lower glucose concentrations and glycemic variability would be associated with lower myeloperoxidase (MPO) concentration, a measure of oxidative stress (Klebanoff 2005), and higher nitric oxide (NO) concentration, a measure of vascular health (Palmer et al. 1998).

Methods

Data were obtained from baseline measures of two randomized clinical trials (NCT: 02413866 and NCT: 03162991). Both trial designs have been described in detail elsewhere (Wang et al. 2018; Sparks et al. 2020, 2021, 2022). Trial protocols were approved by the University of South Carolina Institutional Review Board, and all participants signed an informed consent form prior to participation. There were no repeat participants between the two trials. The present analysis includes 28 participants that had valid CGM data and fasting venous blood samples available for analysis at baseline, prior to either trial's intervention. All participant visits and testing were completed by the same trained research staff and took place in our research center at the University of South Carolina.

Participants

Participants reported $<$ 120 min of resistance or endurance exercise per week during the previous 3 months, had overweight or obesity ($25 \leq BMI \leq 40$ kg/m^2), identified as males or female, age 35–55 years, weight stable ($\pm 2\%$) during the previous 3 months, and, for females, be eumenorrheic or post-menopausal for ≥ 1 year. Exclusion criteria included any self-reported medical conditions such as diabetes, CVD, chronic or recurrent respiratory conditions (e.g., uncontrolled asthma or chronic obstructive pulmonary disease), active cancer, and eating or neurological disorders, medications that affect metabolism (e.g., thyroid medications, statins), psychological issues, including but not limited to untreated depression and attention deficit disorder, excessive caffeine use (>500 mg/day), smoking during the past year, pregnant or lactating females, and/or unwillingness to provide informed consent.

Measurements

Height, body weight, and body mass index (BMI)

Height and body weight were measured at the first baseline visit using a stadiometer and an electronic scale that was calibrated annually (CC Vaughan & Sons, Incorporated, Columbia, SC). BMI was calculated using the following calculation: BMI (kg/m^2) = Body Weight (kg) $\div$ [Height (m)]^2.

Continuous glucose monitoring (CGM)

A CGM device (Dexcom G4 Platinum Professional, San Diego, CA, USA) was used to assess interstitial glucose concentrations and was worn for 7 consecutive days. Participants had a catheter inserted under the skin on the preferred side of the abdomen with an attached sensor and transmitter, approximately 2 cm to the side of the umbilicus. Participants carried a recording device which received and stored interstitial glucose concentration readings every 5 min over the 7 consecutive days. The Dexcom G4 Platinum Professional CGM device has been validated and proven accurate against directly evaluated blood glucose concentrations (Freckman et al. 2019; Facchinetti et al. 2015). The CGM device was blinded so that participants could not observe the live readings to deter any alterations in diet, physical activity, or general lifestyle, and participants were requested to maintain their normal daily routine during the 7-day monitoring period. Data were considered valid for analysis if participants wore the device for at least 5 days including at least one weekend day, with a minimum available glucose measure over 20 h each day. Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export CGM data to Excel datafiles.

Glycemia states and glucose concentrations

Percent (%) of time spent in hypoglycemia, time in range (TIR), and hyperglycemia for each valid 24-h period were calculated and expressed as the average of those valid days. Hypoglycemia, in range, and hyperglycemia were defined as glucose concentrations of < 70 mg/dL, 70–180 mg/dL, > 180 mg/dL, respectively (Battelino et al. 2019). Daily means of 24-h, waking hours, and night sleep hour glucose concentrations were calculated for each valid day, and the average of those valid days was calculated. Maximum, minimum, and the difference between maximum and minimum (Max–Min) glucose concentrations were found for each valid day, and the average of those valid days was calculated. Percent of time spent in hypoglycemia, TIR, and hyperglycemia, and 24-h mean glucose concentration were assessed from midnight to midnight for each valid day. Maximum and minimum glucose concentrations were assessed as the highest and lowest glucose concentrations measured from midnight to midnight for each valid day. Waking and night sleep hours glucose concentrations were based around each participant's self-reported time in bed at night and time out of bed the next day for each valid day, respectively.

Glycemic variability

The continuous overlapping net glycemic action of 1-h, 2-h, and 4-h (CONGA-1, CONGA-2, and CONGA-4) was calculated manually in Excel, while to calculate the mean amplitude of glycemic excursion (MAGE) and mean of daily differences (MODD) measures of glycemic variability the Excel data were transferred into the EasyGV Version 9.0.R2 (University of Oxford, Oxford, England, UK), which is an Excel-enabled workbook that utilizes macros. MAGE, CONGA-1, CONGA-2, and CONGA-4, and MODD were calculated for each participant (Service et al. 1970; McDonnell et al. 2005; Nathan et al. 2008; Kuenen et al. 2011; Rodbard et al. 2008). MAGE and CONGA-1, CONGA-2, and CONGA-4 were utilized as measurements of intra-day glycemic variability for each valid day of wear time and averages of those days calculated, while MODD was utilized as a measurement of inter-day glycemic variability for all valid days combined.

Fasting glucose concentration

For the first trial, participants had a blood sample collected following a minimum of a 12-h fast (not including water) at the first baseline visit (day 1). Fasting blood samples were collected into a BD Vacutainer serum separator collection tube. Blood samples collected into the serum separator collection tube were allowed 30-min to separate on ice, centrifuged at 3000 rpm at 4°C for 20 min, and sent to the Student Health Center for serum glucose analysis.

For the second trial, following a minimum of a 12-h fast (not including water) at the second baseline visit (day 7), participants had a blood sample collected. Time the blood sample was collected, recorded, and matched with the CGM to establish fasting glucose time point. If the fasting blood sample time point fell between CGM readings, as the CGM assesses glucose concentrations every 5 min, the average between the previous and following CGM concentration readings was calculated. The CGM-assessed glucose concentrations have been validated with venous blood glucose concentrations (Freckman et al. 2019).

Biological markers of vascular health and oxidative stress

Fasting venous blood samples collected were used to determine biological markers of oxidative stress. The first trial collected fasting blood samples into a BD Vacutainer serum collection tube, while the second trial collected into a BD Vacutainer EDTA plasma collection tube. Prior to analysis, serum and plasma samples were thawed and re-centrifuged at 3000 rpm and 4°C for 20 min to ensure separation of any particulate.

Two biological markers, NO and MPO, were measured using two separate enzyme-linked immunoabsorbant assays (ELISA). The NO ELISA kit (ThermoFisher Scientific, Waltham, MA) quantitatively determines the

concentrations of nitrate and nitrite, with ≥ 90% sample recovery rate, in serum and plasma samples. The MPO ELISA kit (Eagle Biosciences, Inc., Nashua, NH) quantifies MPO utilizing a two-site "sandwich" technique that binds to different epitopes of MPO. The ratio of NO to MPO was calculated by the concentration of NO divided by the concentration of MPO to examine balance between vasodilation (NO) and vasoconstriction (MPO) (NO:MPO ratio). All assays were performed and analyzed on the same day by the same trained research staff.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (Cary, NC). Participant characteristics were calculated and reported as mean and SD (Mean ± SD) as a combined sample and for each trial. Descriptive statistics were calculated for glucose concentrations, measures of glycemic variability, and biological markers of vascular health and oxidative stress. Participant characteristics for the two trials were compared utilizing independent sample t-tests, or Chi-square test when appropriate, to determine if any variables were different between the two trials. Multivariate linear regression analyses were performed to examine the associations between fasting and CGM-assessed glucose concentrations and glycemic variability with MPO, NO, and NO:MPO ratio. Adjustments for trial involvement alone or trial involvement, age, and BMI combined were performed to determine their influence on the relationship between the outcome variables of interest. Further, adjustments for race and sex were performed in addition to the initial adjustment for age, BMI, and trial involvement. Exploratory analysis for crude associations between fasting and CGM-assessed glucose concentrations and glycemic variability with MPO, NO, and NO:MPO ratio for each trial, race, and sex were performed using Pearson product moment correlations. A p value of < 0.05 was considered statistically significant.

Results

Participant characteristics

Participant characteristics are shown in Table 1 for all participants and by trial for the trials separately. Overall, participants were approximately 70% female and 57% African American with 54% considered obese (BMI ≥ 30 kg/m^2). There were no differences between the trials for participant age, sex or race composition, body weight, or BMI ($p \geq 0.58$ for all). Glycemia measurements, glucose concentrations, and measures of glycemic variability ($p \geq 0.18$ for all), as well as NO and MPO concentrations ($p \geq 0.06$ for both) were also similar. However, the ratio of NO:MPO was significantly higher in trial 2 compared to trial 1.

Association between glucose concentrations and measure of glycemic variability with biological markers of vascular health and oxidative stress

The NO:MPO ratio yielded a positive association with fasting glucose concentration with a 1 unit increase in the NO:MPO ratio relating to a 3.27 mg/dL increase in fasting glucose concentration ($p = 0.02$), which remained following adjustment for trial involvement alone ($\beta = 3.79$, $p = 0.01$) (Table 2). After adjustment for age and BMI in the model, the NO:MPO ratio no longer associated with fasting glucose concentration ($\beta = 3.1$, $p = 0.06$) and continued to be insignificant when further adjustments for race and sex in the regression modeling were performed ($\beta = 3.64$, $p = 0.06$).

We further examined the relationship between measures of glycemic variability and biological markers of vascular health and oxidative stress and found that a 1 unit increase in MPO concentration related to a 0.66 mg/dL decrease in MAGE, a 0.29 mg/dL decrease in CONGA-2, and a 0.31 mg/dL decrease in CONGA-4 ($p \leq 0.04$ for all). The relationship between MPO concentration and MAGE remained following adjustment for trial involvement only ($\beta = -0.62$, $p = 0.03$) (Table 2) and when adjusting for age and BMI in the model ($\beta = -0.63$, $p = 0.03$). However, following adjustment for age, BMI, trial involvement, race, and sex, no glycemic variability measures correlated with vascular health ($p \geq 0.08$ for all).

Association between glucose concentrations and measure of glycemic variability with biological markers of vascular health and oxidative stress by trial, race, and sex

As some of our findings were influenced by the inclusion of trial involvement, race, and sex, we performed exploratory analysis to determine the relationship by trial involvement, race, and sex (supplemental data). Positive associations between MPO concentration with percent of time spent in hypoglycemia ($r = 0.60$, $p = 0.02$) and the NO:MPO ratio with fasting glucose concentration ($r = 0.51$, $p = 0.05$) were found in trial 1 participants (Additional file 1: Table S1). However, there were no associations found between any vascular health or oxidative stress biological marker with CGM-assessed glucose concentrations or glycemic variability in the second trial participants (Additional file 2: Table S2).

When examining racial differences in our sample of participants, there were a disproportionate number of participants identifying as male or female between Caucasian and African American participants ($p = 0.02$), while African American participants were, on average, shorter compared to Caucasian participants ($p = 0.002$) (Additional file 3: Table S3). Additionally, African American participants had a higher concentration of NO and greater NO:MPO ratio compared to Caucasian

Table 1 Participant characteristics combined and by trial involvement

	Combined ($n=28$)	Trial 1 ($n=15$)	Trial 2 ($n=13$)
Participant characteristics			
Age (years)	46.0 ± 6.1	45.4 ± 6.5	46.7 ± 5.8
Sex (M/F)	8/20	3/12	5/8
Race (C/AA/AAA)	11/16/1	8/7/0	3/9/1
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Body weight (kg)	92.6 ± 21.4	93.3 ± 12.0	91.8 ± 29.3
Body mass Index (BMI, kg/m^2)	32.3 ± 6.3	32.6 ± 3.9	31.9 ± 8.4
Glycemic measures, glucose concentrations, and measures of glycemic variability			
CGM observations per day (*n*; % of max)	276.2 ± 7.8; 96%	277.2 ± 7.3; 96%	275.2 ± 8.5; 95%
Hypoglycemia (% of day)	6.8 ± 8.9	5.5 ± 6.1	8.2 ± 11.3
Time in range (% of day)	91.1 ± 1.0	92.2 ± 9.3	90.0 ± 10.4
Hyperglycemia (% of day)	2.1 ± 5.3	2.3 ± 6.6	1.9 ± 3.6
Fasting (mg/dL)	93.5 ± 15.0	93.0 ± 14.2	94.1 ± 16.4
24-h mean (mg/dL)	101.2 ± 16.7	99.4 ± 12.1	103.0 ± 21.0
Wake time (mg/dL)	101.8 ± 16.0	99.0 ± 11.2	104.7 ± 20.0
Sleep hour (mg/dL)	103.0 ± 16.0	103.1 ± 10.1	102.9 ± 21.1
Maximum (mg/dL)	149.4 ± 25.7	144.3 ± 21.2	155.0 ± 29.7
Minimum (mg/dL)	69.8 ± 14.4	69.0 ± 10.8	70.7 ± 17.8
Max–Min (mg/dL)	79.0 ± 18.7	75.2 ± 20.6	83.0 ± 16.2
MAGE (mg/dL)	43.0 ± 12.1	40.3 ± 12.8	45.9 ± 11.1
CONGA-1 (mg/dL)	19.3 ± 5.0	18.6 ± 5.5	20.0 ± 4.4
CONGA-2 (mg/dL)	23.3 ± 6.3	22.5 ± 6.9	24.1 ± 5.6
CONGA-4 (mg/dL)	25.5 ± 6.6	24.4 ± 7.0	26.1 ± 6.3
MODD (mg/dL)	19.9 ± 4.5	19.5 ± 4.1	20.3 ± 4.9
Vascular health and oxidative stress biological markers			
Nitric oxide (μmol/L)	75.6 ± 40.9	62.3 ± 47.8	91.0 ± 25.0
Myeloperoxidase (ng/mL)	25.2 ± 8.2	26.8 ± 8.7	23.2 ± 7.6
NO:MPO ratio (μmol/L:ng/mL)	3.3:1	2.6:1	4.2:1*

Data presented as Mean ± SD

M, male; F, female; C, Caucasian; AA, African American; AAA, Asian/Asian American, number of observations per day (*n*; % of max) = (number of CGM observations per day/maximum observations per day) × 100; Max–Min, maximum–minimum; MAGE, mean amplitude of glycemic excursion; CONGA-1, 2, and 4, continuous overall net glycemic action of 1, 2, and 4 h; MODD, mean of daily differences, oxidative stress ratio = nitric oxide concentration (μmol/L) ÷ myeloperoxidase concentration (ng/mL)

*$p=0.03$ for the significant difference between studies when examining the NO:MPO ratio

participants ($p=0.0004$ and 0.001, respectively) (Additional file 3: Table S3). In Caucasian participants, no associations were found between any vascular health or oxidative stress biological marker with CGM-assessed glucose concentrations or glycemic variability (Additional file 4: Table S4). Yet, in African American participants NO concentration negatively associated with percent of time spent hyperglycemic ($r=-0.55$, $p=0.02$), MPO concentrations negatively associated with MAGE ($r=-0.55$, $p=0.03$) and CONGA-2 ($r=-0.53$, $p=0.03$), and the NO:MPO ratio positively associated with MAGE ($r=0.55$, $p=0.03$) (Additional file 5: Table S5).

Lastly, when examining sex differences in our sample of participants, there were a disproportionate number of participants identifying as Caucasian, African American, and Asian/Asian American between Male and Female participants ($p=0.0483$), while female participants were, on average, shorter compared to male participants ($p=0.0006$) (Additional file 6: Table S6). Additionally, female participants had higher wake time and sleep hour glucose concentrations compared to Male participants ($p=0.0025$ for both) (Additional file 6: Table S6). MPO concentration negatively associated with CONGA-1 and CONGA-4 in Male participants (Additional file 7: Table S7; $r=-0.75$, $p=0.05$ for both), while the NO:MPO ratio positively associated with fasting glucose concentration in female participants (Additional file 8: Table S8; $r=0.47$, $p=0.04$).

Table 2 Adjusted regression analysis between glucose concentrations and measures of glycemic variability with biological markers of vascular health and oxidative stress

	Vascular health and oxidative stress biological markers											
	Nitric oxide (μmol/L)				Myeloperoxidase (ng/mL)				NO:MPO ratio (μmol/L:ng/mL)			
	r^2	β	SE	*p*	r^2	β	SE	*P*	r^2	β	SE	*p*
Glycemia measurements (% of day)												
Hypoglycemia	0.03	− 0.00	0.00	0.76	0.10	0.00	0.00	0.17	0.05	− 0.00	0.01	0.40
Time in range	0.04	0.00	0.00	0.43	0.02	− 0.00	0.00	0.68	0.04	0.01	0.01	0.40
Hyperglycemia	0.04	− 0.00	0.00	0.32	0.10	− 0.00	0.00	0.12	0.00	− 0.00	0.01	0.85
Glucose concentrations (mg/dL)												
Fasting	0.08	0.11	0.08	0.17	0.05	− 0.42	0.36	0.26	**0.22**	**3.79**	**1.44**	**0.01**
24-h mean	0.03	0.06	0.09	0.48	0.10	− 0.61	0.40	0.14	0.24	2.87	1.74	0.11
Wake time	0.05	0.06	0.08	0.49	0.07	− 0.38	0.39	0.34	0.09	2.12	1.68	0.22
Sleep hour	0.01	0.04	0.08	0.60	0.12	− 0.69	0.38	0.08	0.09	2.62	1.68	0.13
Maximum	0.05	0.04	0.13	0.77	0.13	− 0.93	0.60	0.14	0.10	3.33	2.69	0.23
Minimum	0.02	0.04	0.08	0.63	0.03	− 0.28	0.35	0.44	0.06	1.74	1.53	0.27
Max–Min	0.05	0.00	0.10	0.97	0.13	− 0.65	0.44	0.15	0.08	1.59	1.99	0.43
Measures of glycemic variability (mg/dL)												
MAGE	0.06	0.02	0.06	0.74	**0.23**	**− 0.62**	**0.27**	**0.03**	0.13	1.79	1.25	0.16
CONGA-1	0.02	0.01	0.03	0.76	0.13	− 0.20	0.12	0.10	0.08	0.65	0.52	0.22
CONGA-2	0.02	0.01	0.03	0.82	0.15	− 0.28	0.14	0.06	0.08	0.82	0.66	0.23
CONGA-4	0.03	0.02	0.03	0.59	0.15	− 0.30	0.15	0.06	0.09	0.96	0.69	0.18
MODD	0.07	0.03	0.02	0.24	0.08	− 0.15	0.11	0.18	0.10	0.74	0.47	0.13

This table presents findings from regression analysis adjusted for trial involvement (categorical) with dependent variable set as glycemia measurements, glucose concentrations, or measures of glycemic variability and prediction variable as nitric oxide, myeloperoxidase, or the NO:MPO ratio. Data represented as *R*-square (r^2), β estimate (β), standard error (SE), and *p* value (*p*) with bolded entries corresponding to significant values ($p < 0.05$)

Max–Min, maximum–minimum; MAGE, mean amplitude of glycemic excursions; CONGA-1, -2, -4, continuous overall net glycemic action of 1, 2, and 4 h; MODD, mean of daily differences; NO:MPO ratio, nitric oxide to myeloperoxidase ratio

Discussion

To our knowledge, this is the first analysis that examined the relationship between mean 24-h free-living glucose concentrations and glycemic variability with biological markers of vascular health and oxidative stress in non-diabetic adults having overweight or obesity. Notably, the NO:MPO ratio positively correlated with fasting glucose concentration, which suggests that higher fasting glucose concentration relates to lower vasoconstrictor and higher vasodilator properties. This was unexpected as lower fasting glucose concentration was hypothesized to relate to higher NO concentration and lower MPO concentration. A similar trend was observed when examining the relationship between MPO concentration and measures of glycemic variability, which suggests that greater glycemic oscillations relate to lower concentrations of circulating MPO. Most of these relationships persisted following adjustment for covariates. However, once adjusting for trial involvement, age, and BMI in the regression model, only MAGE remained negatively correlated with MPO concentration.

Relationship between glucose concentrations and glycemic variability with oxidative stress

The relationship between glucose concentrations and glycemic variability with vascular health and oxidative stress has only been evaluated in type 1 and type 2 diabetics (Saisho 2014). Evidence supports that vascular impediments exist in diabetic patients due to chronic hyperglycemia and greater postprandial glucose fluctuations (Giugliana et al. 1996; Monnier et al. 2006). However, no studies have examined the relationship between free-living glucose concentrations and glycemic variability with biological markers of vascular health and oxidative stress in non-diabetic adults having overweight or obesity.

Current evidence suggests contraindicatory findings when examining the relationship between fasting glucose and NO concentrations. It has been hypothesized that chronic hyperglycemia acts to uncouple receptor-mediated signal transduction and decrease bioavailability of NO synthase substrates and cofactors essential for synthesis of NO (Honing et al. 1998). Li et al. (2004) found that experimentally increased activation of the NO pathway partially contributed to skeletal muscle

glucose uptake (Li et al. 2004). Furthermore, Hoshiyama et al. (2003) found that experimentally high-level glucose exposure reduced nitrite levels and subsequently decreased NO production, but upregulated endothelial cell NO synthase protein expression (Hoshiyama et al. 2003).

However, Cosentino et al. (1997) found that experimentally increased glucose exposure increased NO synthase and subsequent NO release (Cosentino et al. 1997). More recently, Adela et al. (2015) observed a significant positive relationship between fasting blood glucose and NO concentration in patients diagnosed with type 2 diabetes mellitus, while also providing evidence that experimentally high glucose exposure increases NO production (Adela et al. 2015). These findings suggest that the mechanisms by which high glucose concentration simultaneously increases NO synthase expression and production may be due to an increase in glucose-induced endothelial cell NO gene expression and activation related to diminished vascular function in those with impaired glycemic health. Based on previous findings, along with the findings in our trial, it may be hypothesized that, even in the absence of glycemic dysfunction, participants with higher fasting glucose concentration may have higher activation of NO synthase and in-turn greater NO production compared to those with lower fasting glucose concentration.

MPO concentration, a potent vasoconstrictor and measure of declined vascular health, negatively associated with MAGE, CONGA-2, and CONGA-4 measures of glycemic variability. The relationship between glycemic health and MPO has been previously investigated but have arrived at contradictory conclusions. Zhang et al. (2015) found that fasting glucose and MPO concentrations were positively associated with one another, with increases in glucose concentration directly relating to increases in MPO concentration in non-diabetic adults with acute coronary syndrome (Zhang et al. 2015).

Yet, Uchimura et al. (1999) found that fasting glucose concentration was higher and MPO concentration was lower in non-insulin dependent type 2 diabetes mellitus compared to non-diabetic controls but found no relationship between fasting glucose and MPO concentrations (Uchimura et al. 1999). Furthermore, a previous trial in adult patients with type 2 diabetes mellitus found that those with poor glycemic control had lower MPO activity compared to those with optimal glycemic control (Unubol et al. 2015). Poor glycemic control in type 2 diabetes patients may contribute to hyperglycemic-associated loss of physiological function, which may subsequently lead to decreased circulating MPO (Brownlee 2001; de Souza Ferreira et al. 2012). Thus, our results are consistent with some of the literature; however, our participants were non-diabetic and most previous literature have suggested these findings in adults with cardiovascular complications or diagnosed with type 2 diabetes mellitus.

Strengths and limitations

The primary strengths of this trial include the use of CGM technology to assess glucose concentrations and glycemic variability, which allows for the observation of a free-living environment as opposed to standard clinical measures. The primary limitation to this analysis is that the participants were from a convenience sample of adults (35–55 years of age) having overweight or obesity and not diagnosed with type 2 diabetes or taking any diabetic medications. Other limitations include the small sample size, which was not assessed for a priori statistical power analysis. As shown in our regression analysis, adjusting for sex and race as categorical covariates in the model eliminated previous significant findings, thus necessitating further research in sex and race differences in these outcomes of interest. However, crude examination by sex and race expressed there are differences within these examinations, which may warrant further investigation with larger sample sizes. Lastly, the difference in measurement time point and method of obtaining fasting glucose concentration between the trials, as well as sample type (serum versus plasma) utilized for measurement of biological markers of vascular health and oxidative stress, may cause potential error. However, many of these relationships persisted when adjusting for trial involvement and previous literature has established that glucose concentrations assessed by CGM are valid against venous blood samples (Freckman et al. 2019).

Conclusions

NO:MPO ratio was found to be positively associated with fasting glucose concentration, while MPO concentration was negatively associated with MAGE, CONGA-2, and CONGA-4. After adjustment for trial involvement, age, and BMI, many of these relationships remained significant, which leads us to support our claims that glycemic variability is related to oxidative stress. However, this relationship may be different than previous literature suggests in type 1 and type 2 diabetic adults as our findings were unexpected, yet remain speculative based on previous contraindicatory findings.

Abbreviations

BMI	Body mass index
CVD	Cardiovascular disease
CGM	Continuous glucose monitor
CONGA	Continuous overlapping net glycemic action
ELISA	Enzyme-linked immunoabsorbant assay
Max–Min	Maximum–minimum
MAGE	Mean amplitude of glycemic excursions

MODD Mean of daily differences
MPO Myeloperoxidase
NO Nitric oxide
TIR Time in range

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42269-023-01040-x.

Additional file 1. Supplemental Table 1. Pearson product correlations between glucose concentrations and measures of glycemic variability with biological markers of vascular health and oxidative stress for trial 1 participants.

Additional file 2. Supplemental Table 2. Pearson product correlations between glucose concentrations and measures of glycemic variability with biological markers of vascular health and oxidative stress for trial 2 participants.

Additional file 3. Supplemental Table 3. Participant characteristics combined and by race (excluding Asian/Asian American).

Additional file 4. Supplemental Table 4. Pearson product correlations between glucose concentrations and measures of glycemic variability with biological markers of vascular health and oxidative stress for Caucasian participants.

Additional file 5. Supplemental Table 5. Pearson product correlations between glucose concentrations and measures of glycemic variability with biological markers of vascular health and oxidative stress for African American participants.

Additional file 6. Supplemental Table 6. Participant characteristics combined and by sex.

Additional file 7. Supplemental Table 7. Pearson product correlations between glucose concentrations and measures of glycemic variability with biological markers of vascular health and oxidative stress for Male participants.

Additional file 8. Supplemental Table 8. Pearson product correlations between glucose concentrations andmeasures of glycemic variability with biological markers of vascular health and oxidative stress for Female participants.

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Author contributions
JRS designed, acquired, analyzed, and/or interpreted data for the studies, and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Data may be made available upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate
Both trials were approved by the University of South Carolina Institutional Review Board. All participants were informed of the appropriate trial protocol and signed an informed consent prior to participation. Human rights and protection of research participants were ensured in accordance with the World Medical Association's Declaration of Helinski (World Medical Association 1964) and Department of Health and Human Services Belmont Report (The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research 1979).

Consent for publication
Not applicable.

Competing interests
The author does not have any conflicts of interest to report.

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References

Adela R, Nethi SK, Bagul PK, Baui AK, Mattapally S, Kuncha M, Patra CR, Reddy PNC, Banerjee SK (2015) Hyperglycaemia enhances nitric oxide production in diabetes: a study from South Indian patients. PLoS ONE 10(4):e0125270

Adenan DM, Jaafar Z, Jayapalan JJ, Aziz AA (2020) Plasma antioxidants and oxidative stress status in obese women: correlation with cardiopulmonary response. PeerJ 8:e9230

Adeva-Andany MM, Martínex-Rodríguez J, Gonzálex-Lucán M, Fernández-Fernández C, Castro-Quintela E (2019) Insulin resistance is a cardiovascular risk factor in humans. Diabetes Metab Syndr 13(2):1449–1455

Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, Bosi E, Buckingham B, Cefalu WT, Close KL, Cobelli C, Dassau E, DeVries JH, Donaghue KC, Dovc K, Doyle FJ, Garg S, Grunberger G, Heller S, Heinemann L, Hirsch IB, Hovorka R, Jia W, Kordonouri O, Kovatchev B, Kowalski A, Laffel L, Levine B, Mayorov A, Mathieu C, Murphy HR, Nimri R, Nørgaard K, Parkin CG, Renard E, Rodbard D, Saboo B, Schatz D, Stoner K, Urakami T, Weinzimer SA, Phillip M (2019) Clinical targets for continuous glucose monitoring data interpretation: recommendations from the International Consensus on Time in Range. Diabetes Care 42(8):1593–1603

Brownlee M (2001) Biochemistry and molecular biology of diabetic complications. Nature 414:813–820

Centers for Disease Control and Prevention: Division of Nutrition, Physical Activity, and Obesity, National Center for Chronic Disease Prevention and Health Promotion (2023) Physical activity data and statistics. https://www.cdc.gov/physicalactivity/data/index.html. Accessed 28 Feb 2023

Cosentino F, Hishikawa K, Katusic ZS, Lüscher TF (1997) High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 96:25–28

de Souza FC, Araújo TH, Ángelo ML, Pennacchi PC, Okada SS, de Araújo Paula FB, Migliorini S, Rodrigues MR (2012) Neutrophil dysfunction induced by hyperglycemia: modulation of myeloperoxidase activity. Cell Biochem Funct 30(7):604–610

Facchinetti A, Favero SD, Sparacubi G, Cobelli C (2015) Model of glucose sensor error components: Identification and assessment for new Dexcom G4 generation devices. Med Biol Eng Comput 53(12):1259–1269

Freckman G, Pleus S, Grady M, Setford S, Levy B (2019) Measures of accuracy for continuous glucose monitoring and blood glucose monitoring devices. J Diabetes Sci Technol 13(3):575–583

Gâman M-A, Epîngeac ME, Diaconu CC, Gâman AM (2020) Evaluation of oxidative stress levels in obesity and diabetes by the free oxygen radical test and free oxygen radical defence assays and correlations with anthropometric and laboratory parameters. World J Diabetes 11(5):193–201

Giugliana D, Ceriello A, Paolisso G (1996) Oxidative stress and diabetic vascular complications. Diabetes Care 19(3):257–267

Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL (2018) Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007–2008 to 2015–2016. JAMA 319(16):1723–1725

Hales CM, Carroll MD, Fryar CD, Ogden CL (2020) Prevalence of obesity among adults and youth: United States, 2017–2018. NCHS Data Brief 288:1–8

Honing MLH, Morrison PJ, Banga JD, Stoes ESG, Rabelink T (1998) Nitric oxide availability in diabetes mellitus. Diabetes Metab Rev 14:241–249
Hoshiyama M, Li B, Yao J, Harada T, Morioka T, Oite T (2003) Effect of high glucose on nitric oxide production and endothelial nitric oxide synthase protein expression in human glomerular endothelial cells. Nephron Exp Nephrol 95(2):e62–e68
Kim JA, Lee JS, Chung HS, Roh E, Lee Y-B, Hong S-H, Kim NH, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi KM (2018) Impact of visit-to-visit fasting plasma glucose variability on the development of type 2 diabetes: a nationwide population-based cohort study. Diabetes Care 41(12):2610–2616
Klebanoff SJ (2005) Myeloperoxidase: friend and foe. J Leukoc Biol 77(5):598–625
Kuenen JC, Borg R, Zheng H, Schoenfeld D, Heine RJ, Nathan DM (2011) Does glucose variability influence the relationship between average glucose and HbA1c levels in type 1 and type 2 diabetic patients? Diabetes Care 34(8):1843–1847
Li J, Hu X, Selvakumar P, Russell RR III, Cushman SW, Holman GD, Young LH (2004) Role of the nitric oxide pathway in AMPK-mediated glucose uptake and GLUT4 translocation in heart muscle. Am J Physiol Endocrinol Metab 287(5):E835–E841
Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP (2019) Oxidative stress and inflammatory markers in prediabetes and diabetes. J Physiol Pharmacol 70(6):809–824
McDonnell CM, Donath SM, Vidmar SI, Werther GA, Cameron FJ (2005) A novel approach to continuous glucose analysis utilizing glycemic variation. Diabetes Technol Ther 7(2):253–263
Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C (2006) Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 295(14):1681–1687
Monnier L, Colette C, Owens DR (2008) Glycemic variability: the third component of the dysglycemia in diabetes: Is it important? How to measure It? J Diabetes Sci Technol 2(6):1094–1100
Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ (2008) Translating the A1C assay into estimated average glucose values. Diabetes Care 31(8):1473–1478
National Institutes of Health (2019) Managing overweight and obesity in adults: systematic evidence review from the obesity expert panel. https://www.nhlbi.nih.gov/health-topics/managing-overweight-obesity-in-adults. Accessed 28 Feb 2023
Obesity and Overweight-Centers for Disease Control and Prevention-National Center for Health Statistics [Internet]. Centers for Disease Control and Prevention, Atlanta. Cited 3 April 2023. https://www.cdc.gov/nchs/fastats/obesityoverweight.htm
Palmer RMJ, Ashton DS, Moncada S (1998) Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333(6174):664–666
Ramana KV, Srivatava S, Singhal SS (2019) Lipid peroxidation products in human health and disease 2019. Oxid Med Cell Longev. Article ID 7147235
Rodbard D, Matsubara B, Nakamura K, Bailey T, Jovanovic L, Zisser H, Kaplan R, Garg SR (2008) Improved quality of glycemic control and reduced glycemic variability with use of continuous glucose monitoring. Diabetes Technol Ther 11(11):717–723
Rodrigues R, de Medeiros LA, Cunha LM, Garrote-Filho MDS, Bernardino Neto M, Jorge PT, Resende ES, Penha-Silva N (2018) Correlations of the glycemic variability with oxidative stress and erythrocytes membrane stability in patients with type 1 diabetes under intensive treatment. Diabetes Res Clin Pract 144:153–160
Saisho Y (2014) Importance of beta cell function for the treatment of type 2 diabetes. J Clin MEd 3(3):923–943
Salkind SJ, Huizenga R, Fonda SJ, Walker MS, Vigersky RA (2014) Glycemic variability in nondiabetic morbidly obese persons: results of an observational study and review of literature. J Diabetes Sci Technol 8(5):1042–1047
Semlitch T, Stigler FL, Jeitler K, Horvath K, Siebenhofer A (2019) Management of overweight and obesity in primary care—a systematic overview of international evidence-based guidelines. Obes Rev 20(9):1218–1230
Service JF, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF (1970) Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes 19(9):644–655
Sharma GN, Gupta G, Sharma P (2018) A comprehensive review of free radicals, antioxidants, and their relationship with human ailments. Crit Rev Eukaryot Gene Expr 28(2):139–154
Sparks JR, Porter RR, Youngstedt SD, Bowyer KP, Durstine JL, Wang X (2020) Effects of moderate sleep restriction during 8-week calorie restriction on lipoprotein particles and glucose metabolism. Sleep Adv 1(1):zpab001
Sparks JR, Sarzynski MA, Davis JM, Grandjean PW, Wang X (2021) Alterations in glycemic variability, vascular health, and oxidative stress following a 12-week aerobic exercise intervention—a pilot study. Int J Exerc Sci 14(3):1334–1353
Sparks JR, Sarzynski MA, Davis JM, Grandjean PW, Wang X (2022) Cross-sectional and individual relationships between physical activity and glycemic variability. TJACSM. 7:1–12
Tejero J, Shiva S, Gladwin MT (2019) Sources of vascular nitric oxide and reactive oxygen species and their regulation. Physiol Rev 99(1):311–379
Uchimura K, Nagasaka A, Hayashi R, Makino M, Nagata M, Kakizawa H, Kobayashi T, Fujiwara K, Kato T, Iwase K, Shinohara R, Kato K, Itoh M (1999) Changes in superoxide dismutase activities and concentrations and myeloperoxidase activities in leukocytes from patients with diabetes mellitus. J Diabetes Complicat 13(5–6):264–270
Unubol M, Yavasoglu I, Kacar F, Guney E, Omurlu IK, Ture M, Kadikoylu G, Bolaman Z (2015) Relationship between glycemic control and histochemical myeloperoxidase activity in neutrophils in patients with type 2 diabetes. Diabetol Metab Syndr 7:Article 15
Verma N (2018) Glycemic variability and its clinical implications. Natl Ther Interv Diabetes Metab Syndr 2:263–268
Wang X, Sparks JR, Bowyer KP, Youngstedt SD (2018) Influence of sleep restriction on weight loss outcomes associated with calorie restriction. Sleep 41(5):zsy027
Zhang X, Dong L, Wang Q, Xie X (2015) The relationship between fasting plasma glucose and MPO in patients with acute coronary syndrome. BMC Cardiovasc Disord 15:93
Zheng Y, Ley SH, Hu FB (2018) Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol 14:88–98

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