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*Original Research*

# Alterations in Glycemic Variability, Vascular Health, and Oxidative Stress following a 12-Week Aerobic Exercise Intervention-A Pilot Study

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ABSTRACT

***International Journal of Exercise Science 14(3): 1334-1353, 2021.*** The state of being overweight or obese leads to an increased risk of development of cardiometabolic disease. Increases in glycemic variability have been associated with greater induction of oxidative stress and declined vascular health, which may be exacerbated by higher weight status and improved through exercise. The purpose of this study was to examine the impact of a twelve-week aerobic exercise intervention on continuous glucose monitor (CGM) assessed glucose concentrations and glycemic variability, and biomarkers of vascular health and oxidative stress in overweight or obese adults. Eight adults (Age = 48.9 ± 5.2 years; BMI = 29.4 ± 8.3 kg/m2) completed a twelve-week aerobic exercise intervention. Participants walked three times per week at moderate intensity for ~150 minutes each week. All participants wore a CGM for seven consecutive days at baseline and post-intervention. On the final day of monitoring, a fasting blood sample was collected, and an oral glucose tolerance test (OGTT) was performed. Intra- and inter-day glycemic variability was assessed as the mean amplitude of glycemic excursions, continuous overlapping net glycemic action of one-, two-, and four-hour, and the mean observation of daily differences. Plasma concentrations of nitric oxide (NO) and myeloperoxidase (MPO) were measured, and their ratio was calculated (NO:MPO). No CGM-assessed glucose concentrations or measures of glycemic variability changed from baseline to post-intervention. MPO concentration decreased (24.8 ± 8.2 ng/mL to 16.4 ± 4.6 ng/mL, $p < 0.01$), the NO:MPO ratio improved (3.5:1 to 6.4:1, $p < 0.01$) following the twelve-week intervention. Individual level changes in body weight and $VO_2$peak were found. In conclusion, twelve weeks of aerobic exercise reduced oxidative stress and improved the propensity to vasodilate but did not alter CGM-assessed glucose concentrations or glycemic variability in this group of overweight or obese non-diabetic adults. These findings may be due to individual changes in body weight or VO2peak, which necessitates further research to explore their influence on these outcomes of interest.

KEY WORDS: Cardiovascular health, continuous glucose monitoring (CGM), glycemic health, obese, overweight, vasoconstriction, vasodilation

## INTRODUCTION

The prevalence of adults in the United States who are considered overweight or obese continues to rise with ~71.6% of the population currently designated as overweight or obese with obesity

widely considered a major public health crisis of the current generation (46). Overweight and obesity-related cardiometabolic disorders lead to an increased medical care cost, such as for treatment of impaired glycemic health and diagnosed cardiovascular disease (CVD) risk factors (6).

Fasting and postprandial hyperglycemia are independent risk factors for cardiovascular disease with and without the presence of Type 2 Diabetes Mellitus and are increased in overweight and obesity (17, 31, 39). Unlike hepatic regulation of glucose in a fasted state, and how effective muscle glucose disposal is in a glucose challenged state, such as during an oral glucose tolerance test (OGTT), the measurement of glycemic variability allows for the observation of excursions that consider nadirs, peaks, and troughs of glucose concentrations rather than a single snapshot of fasting glucose and during an OGTT (2, 20, 50). Recently, increased glycemic variability has been shown to be associated with the risk of development of Type 2 Diabetes Mellitus, with overweight and obese adults having higher glycemic variability compared to normal weight (32). Additionally, it has been suggested that glycemic variability may be a more important clinical measurement of not only glucose metabolism, but cardiovascular and overall general health (41, 45, 56).

Oxidative stress is preceded by an increased exposure to reactive oxygen species, and damage to proteins, nucleic acids, and cell membranes, and has been implicated in the pathogenesis related to increased CVD risk factors, such as impaired vascular health, and subsequent CVD (9, 47). Previously, oxidative stress was believed to be a key regulator in the development of diabetic complications (4). However, evidence has suggested that an increase in oxidative stress is triggered by exacerbated oscillations in glucose concentrations (21, 40). These exacerbated glucose oscillations, known as glycemic variability, act to induce stress on the vascular endothelium and elicits endothelium-derived micro and macrovascular complications, which potentially increases CVD risk (24). In fact, the interaction between increased glycemic variability and elevated oxidative stress has recently been proposed as a mechanism for increased CVD risk and CVD progression in adults with and without diagnosed diabetes (16).

Greater glycemic variability, impaired vascular health, and increased oxidative stress have both been observed independently in overweight or obese adults in the presence and absence of diagnosed diabetes or CVD (14, 67). Exercise is often utilized as a therapeutic treatment for overweight or obesity related insulin resistance and cardiovascular strain (8, 18). Exercise training in overweight or obese adults in the presence or absence of diabetes, elicits beneficial alterations in fasting glucose concentrations, glucose tolerance during an oral glucose tolerance test (OGTT), and glucose regulation during glycemic clamp procedures independent of changes in body weight or cardiorespiratory fitness (CRF) (10, 26, 63). Yet, past studies have been largely limited to laboratory-based assessment of glucose metabolism and tolerance, while most research examining glycemic variability, vascular health, and oxidative stress collectively have focused on diabetic populations with even fewer studies examining changes due to exercise training (65). With recent advancements in continuous glucose monitor (CGM) technology, the allowance for inclusion of a free-living condition enables further determination as to how

exercise influences glucose concentrations and glycemic variability throughout the day alongside measures of vascular health and oxidative stress.

Therefore, the primary purpose of this study was to examine glucose concentrations and glycemic variability using CGM in overweight or obese non-diabetic adults undergoing twelve-weeks of moderate-intensity aerobic exercise. A secondary purpose was to evaluate the effects of the aerobic exercise intervention on biological markers of vascular health, nitric oxide (NO), a potent vasodilator (48), and oxidative stress, myeloperoxidase (MPO), a potent vasoconstrictor (29). We also examined changes in body weight and CRF following the aerobic exercise intervention, as changes in these measures may influence outcomes of interest (15, 52).

## METHODS

The Aerobic Treadmill Exercise and Metabolism (A-TEAM; NCT: 03162991) study openly recruited from October 2017 to December 2018. The study protocol was approved by the University of South Carolina Institutional Review Board and all participants signed an informed consent form prior to participation. This research was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science (44). All participant visits, testing, and exercise sessions were completed and supervised by the same trained research staff and took place in our research center at the University of South Carolina. Three testing and measurement visits occurred at baseline and post-intervention in the same order, respectively. At the first visit, placement, and instruction for use of the CGM were performed. Following a minimum of seven days, the second visit occurred, where a fasting blood sample was collected, an OGTT was performed, and the CGM device was removed. A third visit occurred a minimum of 24 hours after the second visit for the graded exercise test.

*Participants*

Participants were not physically active (< 120 minutes of resistance or moderate-intensity endurance exercise per week during the previous three months), overweight or obese (25 ≤ BMI ≤ 40 kg/m2) males and females, age 35 - 55 years, weight stable (± 2%) during the previous three months, and, for females, eumenorrheic. Exclusion criteria included any self-reported medical conditions such as diabetes or taking medications that are known to affect metabolism (e.g., statins), CVD, chronic or recurrent respiratory conditions, including uncontrolled asthma or chronic obstructive pulmonary disease, or active cancer. Individuals self-reporting diagnosed eating or neurological disorders, and psychological issues, including but not limited to untreated depression and attention deficit disorder were also excluded. Additionally, excessive caffeine use (> 500 mg/day), smoking during the past year, pregnant or lactating females, and/or unwillingness to provide informed consent were other reasons for exclusion.

A total of 64 participants were initially screened by telephone (Figure 1). Ten participants began the exercise intervention, with eight completing the twelve-week exercise intervention. The two participants who dropped-out during the intervention completed one and eleven (~3% and 30%) of the intervention visits, respectively. These participants did not provide specific reasons why they discontinued the study, as they ceased correspondence and were unable to be contacted to

reschedule their intervention visits. Therefore, the eight participants that completed the intervention were included in this analysis.

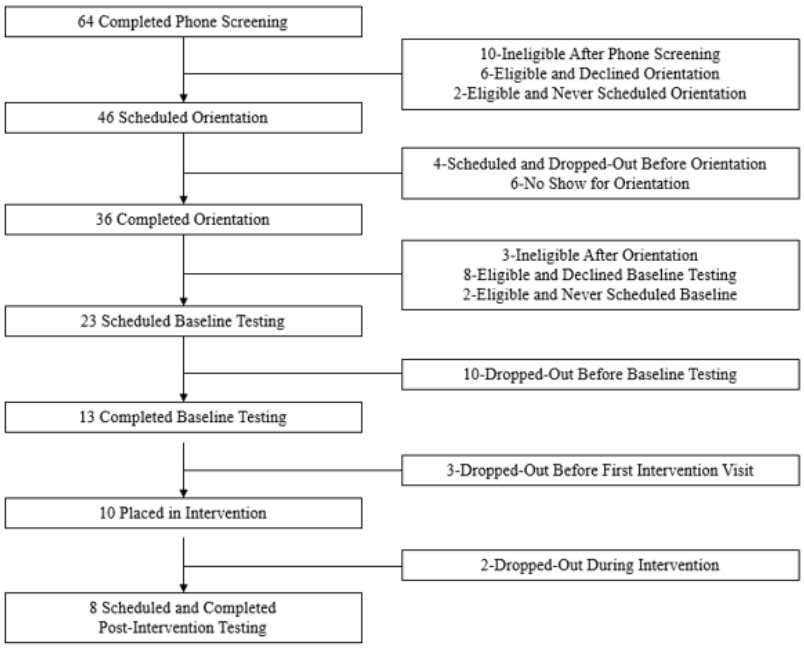


**Figure 1.** The A-TEAM Study Participant Flow Diagram.

*Protocol*

Participants walked on treadmills three times per week under research staff supervision in the Clinical Exercise Research Center (CERC) at the University of South Carolina. All exercise sessions throughout the entire twelve weeks were performed in our research center and no unsupervised sessions were performed. Each week, participants had their body weight measured, which was utilized to calculate weekly exercise volume by multiplying each participants' body weight by 10 - 12 kilocalorie per kilogram of body weight each week (KKW). As the participants in our study were not engaging in structured physical activity prior to the exercise intervention, three times per week was chosen to limit exercise-related overuse injuries. The exercise volume was achieved by varying the duration, speed, and grade to reach each participants' weekly energy expenditure goal while maintaining moderate-intensity treadmill walking, which was closely monitored throughout the twelve-week intervention. Twelve weeks of aerobic exercise training has been previously studied and is considered a duration that physiological adaptations to aerobic exercise tend to occur, such as lowered systolic blood pressure and augmentation index, which is an indirect measure of arterial stiffness and vascular health, and markers of glycemic health (22, 34, 38). The prescribed training volume, 10 - 12 KKW, is a higher volume of aerobic exercise energy expenditure compared to current physical activity guidelines, which equates to 8 KKW (42, 51, 61). To monitor adherence to the exercise prescription, the energy expenditure of each exercise session was calculated using the American College of Sports Medicine formula: {0.1 × (speed [miles per hour] × 26.8) + 1.8 × (speed [miles per hour] × 26.8) × grade (%) + 3.5} × body weight (kg) ÷ 5 (L per minute) × time (minutes) (1).

Due to the physically inactive state of the participants, the exercise intensity and weekly energy expenditure were gradually increased to reduce risk of injury. Training intensity increased during the first 4 weeks of the exercise intervention until the target level of 50 - 55% of participant's heart rate reserve (HRR) was met, calculated as [(peak heart rate - resting heart rate) × intensity (50 - 55%)) + resting heart rate], which was determined during the baseline graded exercise test. Participants began at a weekly energy expenditure of 6 - 8 KKW during the first week of the intervention and then progressed until week four, when they attained their weekly energy expenditure of 10-12 KKW. Each exercise session began and ended with a three-minute warm-up and cool-down. Heart rate (HR) monitors (FT1; Polar, Lake Success, NY, USA) were worn to monitor exercise intensity continuously throughout each exercise session and HR was recorded every five minutes. If HR monitors were unable to detect HR, manual palpation at the radial artery was measured for 30 - 60 seconds. Blood pressure was auscultated using a standing mercury sphygmomanometer (AME-1003; AME Worldwide, United Arab Emirates) and stethoscope (Littman Classic III Stethoscope; 3M, St. Paul, MN, USA) and measured before, during warm-up, at the mid-point of the exercise session, during cool-down, and following each exercise session.

Compliance to prescribed exercise intervention (frequency, intensity, and duration) for each participant was reviewed weekly and any participant missing an exercise session without notifying study personnel was contacted via phone or e-mail to reschedule and encourage further attendance. Participants were encouraged to attend all exercise sessions and could miss a maximum of three exercise sessions throughout the twelve weeks. However, if a participant did miss an exercise session, they were prompted to attend an additional exercise session to ensure adherence to the prescribed exercise intervention.

Height and body weight were measured at the first baseline and post-intervention visit using a stadiometer and an electronic scale that was calibrated annually (CC Vaughan & Sons, Incorporated, Columbia, SC). Additionally, body weight was collected weekly throughout the twelve-week intervention. BMI was calculated at baseline and post-intervention utilizing their respective height and body weight and the following equation: BMI ($kg/m^2$) = Body Weight (kg) ÷ Height $(m)^2$.

All participants performed a maximal graded exercise test on a treadmill at baseline and post-intervention. A ramped medium protocol was determined to be ideal for these participants as they were sedentary and overweight or obese prior to the exercise intervention, and the exercise intervention itself was designed to incorporate moderate-intensity walking. The ramped medium protocol is an incremental protocol where participants self-select a comfortable, yet challenging, walking speed, which was maintained throughout the exercise test. Additionally, percent grade started at 0% and increased linearly, by 2%, every 30 - 60 seconds until each participant reached volitional fatigue. Volume of oxygen consumed (VO2) via metabolic cart (TrueOne 2400, ParvoMedics, Salt Lake City, UT) and HR using standard 12-lead electrocardiogram (Q-Stress®; Cardiac Science, Bothell, WA, USA) were monitored continuously during the progression of the test. Blood pressure was measured (instruments previously described), and rating of perceived exertion was obtained every two-minutes during the test.

Two of four generally recognizable criteria for the test to be considered satisfactory needed to be achieved: a respiratory exchange ratio ≥ 1.10; a rating of perceived exertion ≥ 17 on the Borg scale ranging from 6 - 20; achieving a maximum heart rate > 90% age-predicted maximum HR (220 - age); and/or a plateau in absolute $\dot{V}O_2$ ≤ 150 ml/min with an increase in exercise intensity. Relative peak oxygen consumption ($\dot{V}O_2$peak: ml/kg/min) was determined by the highest 30 - second average $\dot{V}O_2$ value measured during the test. All graded exercise tests took place after 12:00 hours and minimum of 24 hours after the final day of the CGM monitoring period.

A Dexcom G4 Platinum Professional CGM device (San Diego, CA, USA) was used to assess interstitial glucose concentrations over seven consecutive days at baseline and post-intervention. At the first baseline and post-intervention visit participants reported to the CERC for placement and instruction of use for the CGM device by trained research staff. At post-intervention, the CGM device was placed a minimum of 72 hours after the last exercise session to limit to influence of the last exercise session on glucose concentrations.

The Dexcom G4 Platinum Professional CGM device has been validated and proven accurate against directly evaluated blood glucose concentrations (12). The CGM device was blinded to deter any alterations in diet, physical activity, or general lifestyle, and participants were requested to maintain their normal daily routine during the seven-day monitoring period at baseline and post-intervention. On the final day of the seven-day monitoring period, participants reported back to the research center to complete a two-hour OGTT and have the device removed. Data were considered valid for analysis if data were obtained by the device for at least five days including at least one weekend day, with a minimum available glucose measure over 20 hours. Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export CGM data.

The OGTT was conducted following an overnight fast (~12 hours other than water) using the CGM device. Following a venous blood collection, participants consumed a 10-ounce, 75-gram glucose infused drink (Azer Scienmtific, Morgantown, PA). Every 30-minutes afterwards, time was recorded until two hours were complete (Fasting, 30-, 60-, 90-, and 120-minutes post-consumption). Glucose concentrations during the OGTT were determined by the CGM device rather than from blood samples. OGTT AUC was calculated utilizing the equation Glucose AUC = ½ × 30 × (yFasting + 2y30 + 2y60 + 2y90 + y120), where y represents glucose concentration at the different time points (64). All OGTTs took place between 06:00 and 09:00 and at least 72 hours after the last bout of aerobic exercise at post-intervention.

Time spent hyperglycemic (< 70 mg/dL), time-in-range (70 - 180 mg/dL), and hyperglycemic (> 180 mg/dL) in accordance with the American Diabetes Association were calculated and presented as percent (%) of time for each day (3). Twenty-four-hour mean, diurnal, nocturnal, maximum, minimum, and maximum-minimum (Max-Min) glucose concentrations were calculated for each valid day and expressed as the average across those days. Time spent hypoglycemic, time-in-range, and hyperglycemic, and 24-hour mean, maximum, and minimum glucose concentrations was assessed from midnight to midnight for each valid day. Diurnal and

nocturnal glucose concentrations were assessed each valid day during each participants' self-reported time-in-bed and time out of bed.

The continuous overlapping net glycemic action of one-hour, two-hour, and four-hour (CONGA-1, CONGA-2, and CONGA-4) were calculated in Excel. CONGA-1, CONGA-2, and CONGA-4 were calculated as the standard deviation of the difference between each observation and the previous one-hour, two-hour, and four-hour observations (35). 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 and CONGA-1, CONGA-2, and CONGA-4 were measurements of intra-day glycemic variability for each valid day of wear time and averages of those days calculated. MAGE was calculated for each subject by taking the arithmetic mean of increased or decreased glucose concentrations (nadirs and peaks or vice-versa) when both ascending and descending concentration exceeds one standard deviation for the same 24-hour monitoring period (60). MODD served as a measurement of inter-day glycemic variability, which was calculated for each two consecutive day period and averaged to include all valid days over the seven-day monitoring period. MODD accounts for the mean of absolute differences between glucose concentrations obtained at the same time of day on consecutive days (55).

Fasting venous blood samples were collected at baseline and post-intervention and used to determine biological markers of vascular health and oxidative stress. Blood samples were collected into a BD Vacutainer EDTA plasma collection tube. Immediately following collection, blood samples were centrifuged at 3000 rpm and 4 $^{o}$C for 20 minutes immediately following collection. Plasma separated after centrifugation were aliquoted into 1.5 mL centrifugation tubes and stored at - 80$^{o}$C until all participant's samples were ready for analysis. Prior to analysis, plasma samples were thawed and re-centrifuged at 3000 rpm and 4 $^{o}$C for 20 minutes to ensure separation of any particulate.

Circulating concentrations of NO and MPO, were measured using two separate enzyme-linked immunoabsorbant assays (ELISA). These biological markers of vascular health and oxidative stress were chosen for this study as they have previously been observed to be related to measures of glycemic health in adults diagnosed with Type 2 diabetes mellitus (27, 64).

The NO ELISA kit (ThermoFicher Scientific, Waltham, MA) quantitatively determines the concentrations of nitrate and nitrite in 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. Antibodies bound to MPO were analyzed by detecting the immunocomplex and the absorbency of the sample. The ratio or NO concentration to MPO concentration (NO:MPO) was calculated by the concentration of NO divided by the concentration of MPO to examine balance between measures of vasodilation and vasoconstriction. All assays were performed and analyzed on the same day by the same trained research staff.

*Statistical Analysis*
Statistical analysis was performed using SAS version 9.4 (Cary, NC). Participant characteristics were calculated and reported as mean and standard deviation (Mean±SD). All outcome variables of interest were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Change values were calculated by subtracting baseline values from post-intervention values. Paired sample t-tests were performed to determine if any values significantly changed from baseline to post-intervention. A general linear model was utilized to adjust for change in body weight and/or change in relative $\dot{V}O_2$peak for primary outcomes of interest, including glucose concentrations, glycemic variability measures, and biological markers of vascular health and oxidative stress. A $p$ value < 0.05 was considered statistically significant.

Mikus et al. (2012) examined the influence of seven days of aerobic exercise on glycemic variability and control assessed by CGM technology in adults with Type 2 diabetes mellitus not using exogenous insulin (37). Sedentary, overweight or obese adults diagnosed with type 2 debates mellitus ($n$ = 13; age = 53.0 ± 2.0 years; BMI = 34.1 ± 1.3 kg/m2) underwent glycemic variability and control assessment utilizing CGM technology during three days of habitual activity and during the final three days of a seven day aerobic exercise training program, which consisted of 60 minutes of supervised exercise for seven consecutive days at 60 - 75% HRR, and found that "free-living" maximum blood glucose concentration decreased (maximum blood glucose: 13.6 ± 1.2 mmol/l to 10.9 ± 0.8 mmol/l, $p$ < 0.01) over the three days completing the aerobic exercise program compared to the three days of habitual activity.

Using findings from this study, post-hoc power analysis calculations were performed in G*Power 3.0.10 (Universitat Kiel, Germany). Power analysis calculation were performed for a paired sample t-test for the difference between two dependent means, determined that 8 participants would allow for > 80% power with a medium-to-large effect size (> 0.3) with alpha set to 0.05 and Cohen's $d$ = 2.55 (effect size of standardized difference score) when testing for the difference between maximum blood glucose concentration from baseline compared to post-intervention. Additionally, as this study is among the first to utilize CGM following an extended exercise intervention, findings from this study will serve to provide additional resources for future effect size estimations for examining alterations in CGM-assessed glucose concentrations following an aerobic treadmill-based exercise intervention in sedentary, overweight, or obese adults.

## RESULTS

Participant Characteristics: Participant characteristics are included in Table 1. The participants were overweight or obese, middle-aged adults, with an even distribution between males and females. The overall relative VO2peak (27.7 ± 9.0 ml/kg/min) and relative VO2peak for both men (31.1 ± 10.9 ml/kg/min) and women (24.4 ± 6.2 ml/kg/min) was determined to be lower than the standard normal values for their age and sex and thus reflective of being sedentary prior to the intervention (43).

**Table 1.** Participant Baseline Characteristics ($n$ = 8).

| Variable | Outcome |
|---|---|
| Age (years) | 48.9±5.2 |
| Height (m) | 1.7±0.1 |
| Body Weight (kg) | 86.2±32.9 |
| BMI (kg/m$^2$) | 29.4±8.3 |
| Sex (M/F) | 4/4 |
| Race (C/AA/AAA) | 3/4/1 |
| College Graduate [n (%)] | 6 (75.0) |
| Employed for Wages [n (%)] | 7 (87.5) |
| Income ≥%80,000.00 [n (%)] | 4 (50.0) |
| Married [n (%)] | 4 (50.0) |
| Number of Children ≥1 [n (%)] | 3 (37.5) |

Outcome data presented as Mean±SD, n=number of participants, or n=number of participants and (%=proportion). m=meters, kg=kilograms, BMI=body mass index, kg/m$^2$=kilogram per meter squared, M=male, F=female, C=Caucasian, AA=African American, AAA=Asian/Asian-American

Exercise Intervention Effects: Data on the prescribed and actual completed exercise for participants that completed the exercise intervention are included in Table 2. The exercise intensity (50 - 55% HRR), volume (10 - 12 kcal/kg/week), and number of sessions (36 - 37) were set standards throughout the intervention. If participants missed ≥ 3 exercise sessions, additional sessions were included to make-up for the missed sessions. All participants walked 120 - 135 minutes per week (128.8 ± 4.4 minutes per week).

**Table 2.** Exercise Prescription and Intervention Adherence.

| Variable | Outcome |
|---|---|
| Prescribed HR Range (50 to 55% HRR, bpm) | 124.9 ± 8.8 to 129.2 ± 9.1 |
| Actual HR (bpm) | 127.7 ± 1.9 |
| Prescribed Exercise Volume Range (10 to 12 KKW) | 843.6 ± 292.6 to 1012.3 ± 351.1 |
| Actual Exercise Volume* (KKW) | 863.8 ± 225.6 |
| Prescribed Number of Exercise Sessions‡ | 36 - 37 |
| Actual Number of Exercise Session | 34.9 ± 1.1 |

Outcome data presented as Mean±SD. HR=heart rate, HRR=heart rate reserve, BPM=beats per minute, KKW=kilocalories per kilogram of body weight each week*, exercise volume calculated using the American College of Sports Medicine formula (1)‡, 36 to 37 sessions dependent on inclusion of introductory exercise intervention visit.

Body Weight, BMI, and Graded Exercise Test: Body weight, BMI, and graded exercise test measurements obtained at baseline and post-intervention are included in Table 3. Overall, there were no significant changes in body weight and BMI. Further, all participants met two of four generally recognizable criteria needed to be achieved for the graded exercise test to be considered satisfactory at baseline and post-intervention. Relative $\dot{V}O_2$peak, and maximal respiratory quotient, heart rate, and blood pressure, as well as rating of perceived exertion did not change significantly from baseline to post-intervention. Treadmill time, however, significantly increased indicating improved exercise tolerance and performance during maximal exercise.

**Table 3.** Body Weight, BMI, and Graded Exercise Test at Baseline and Post-Intervention.

| Variable | Baseline | Post-Intervention | p value |
|---|---|---|---|
| Body Weight (kg) | 86.2 ± 32.9 | 83.5 ± 27.4 | 0.27 |
| BMI (kg/m²) | 29.4 ± 8.3 | 28.5 ± 6.7 | 0.26 |
| Relative $V\dot{O}_{2Peak}$ (ml/kg/min) | 27.7 ± 9.0 | 29.6 ± 8.5 | 0.21 |
| Maximal RQ | 1.2 ± 0.1 | 1.2 ± 0.2 | 1.00 |
| Maximal RPE | 18.3 ± 1.1 | 18.3 ± 0.9 | 0.83 |
| Resting HR (bpm) | 71.3 ± 15.1 | 71.3 ± 11.4 | 1.00 |
| Resting SBP (mmHg) | 123.6 ± 11.5 | 119.3 ± 7.2 | 0.29 |
| Resting DBP (mmHg) | 79.3 ± 7.5 | 79.9 ± 7.5 | 0.82 |
| Maximal HR (bpm) | 174.0 ± 15.5 | 176.0 ± 15.5 | 0.49 |
| Maximal SBP (mmHg) | 171.0 ± 26.7 | 171.0 ± 14.9 | 1.00 |
| Maximal DBP (mmHg) | 76.5 ± 9.5 | 80.8 ± 6.8 | 0.25 |
| Treadmill Time (minutes) | 10.6 ± 2.7 | 11.8 ± 2.3 | **0.04** |

Outcome data presented as Mean±SD. kg=kilogram, BMI=body mass index, kg/m²=kilogram per meter squared $V\dot{O}_{2Peak}$=peak,volume of oxygen consumed, RQ=respiratory quotient, HR=heart rate, bpm=beats per minute, SBP=systolic blood pressure, mmHg=millimeters of mercury, DBP=diastolic blood pressure, *p* value for comparison between baseline and post-intervention, **bold** denotes statistical significance

Additionally, individual changes from baseline to post-intervention for body weight and $V\dot{O}_2$peak were examined (Figures 2 and 3, respectively). Although the data were normally distributed at baseline and post-intervention, one participant had a noticeably greater decrease in body weight (Participant 6) (Figure 2), while another participant had a noticeably greater increase in V̇O2peak (Participant 2) (Figure 3).

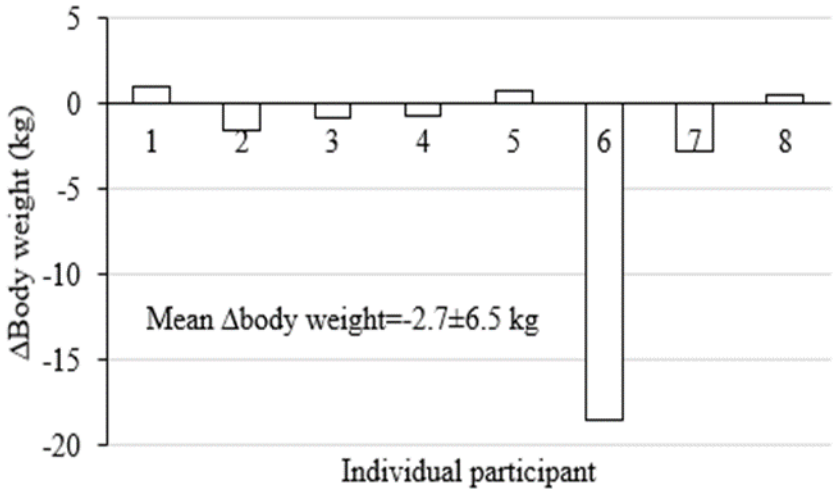


**Figure 2.** Individual Participant ΔBody Weight. Body weight changes for each individual participant from baseline to post-intervention in kilograms (kg) with mean body weight change are denoted in this figure.

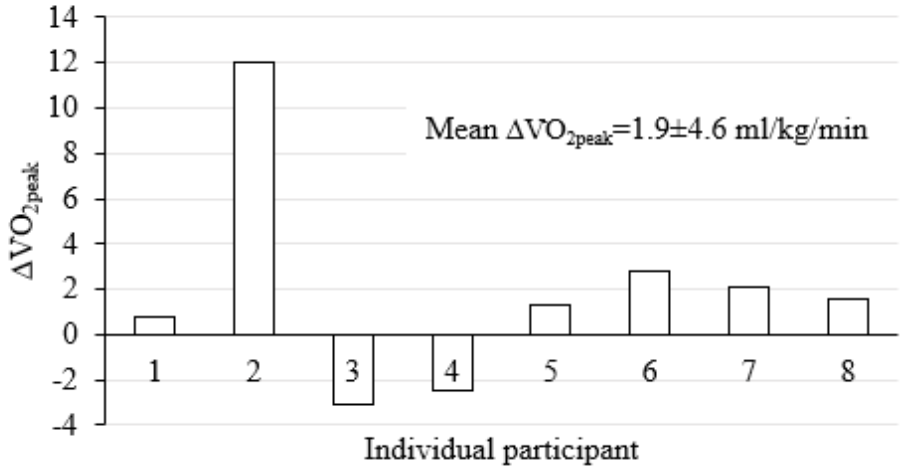


**Figure 3**. Individual Participant $\Delta VO_2peak$. $VO_2peak$ changes for each individual participants from baselines to post-intervention in milliliters per kilogram of body weight per minute (mg/kg/min) with mean $VO_2peak$ change are denoted in the figure.

Glycemic State Measurements, Glucose Concentrations, and Glycemic Variability: Glycemic state measurements, glucose concentrations, and measures of glycemic variability at baseline and post-intervention are included in Table 4. There were no significant changes in fasting and glucose concentrations during OGTT, and OGTT AUC from baseline to post-intervention. Also, there was not a significant difference from baseline to post-intervention for any glycemic state measurement, or 24-hour mean, diurnal, and nocturnal glucose concentrations. Further, no measure of intra-day glycemic variability, MAGE, CONGA-1, CONGA-2, CONGA-4, or inter-day glycemic variability, MODD, significantly changed from baseline to post-intervention. Following adjustment for body weight and/or relative $VO_2peak$, these findings persisted as all CGM-assessed glucose concentrations and glycemic variability measures did not significantly change from baseline to post-intervention ($p \geq 0.05$ for all).

Vascular Health and Oxidative Stress: Biological markers of vascular health and oxidative stress at baseline and post-intervention are included in Table 4. There was a significant decrease in MPO concentration and a significant increase in the NO:MPO ratio, with neither remaining significant following adjustment for change in body weight and/or relative $VO_2peak$ ($p \geq 0.13$ for all).

**Table 4.** Glycemia State Measurements, Glucose Concentrations, and Glycemic Variability Measures, and Vascular Health and Oxidative Stress Biological Markers at Baseline and Post-Intervention.

| Variable | Baseline | Post-Intervention | p value |
|---|---|---|---|
| CGM Observations per Day (% of Max) | 271.3 ± 11.2 (94.2%) | 270.4 ± 9.1 (93.9%) | 0.92 |
| Glycemia State (% of Time of Day) | | | |
| Hypoglycemic | 0.3 ± 0.4 | 1.5 ± 1.0 | 0.06 |
| Time-In-Range | 91.6 ± 0.1 | 95.5 ± 0.1 | 0.08 |
| Hyperglycemic | 8.1 ± 8.0 | 3.0 ± 5.0 | 0.21 |
| Oral Glucose Tolerance Test Concentration (mg/dL) | | | |
| Fasting | 88.1 ± 9.0 | 92.9 ± 11.3 | 0.38 |
| 30-Minute | 125.2 ± 28.8 | 138.4 ± 33.8 | 0.31 |
| 60-Minute | 139.1 ± 25.6 | 137.6 ± 29.2 | 0.92 |
| 90-Minute | 126.5 ± 19.5 | 130.7 ± 19.7 | 0.73 |
| 120-Minute | 105.3 (102.0-153.0) | 117.0 ± 18.0 | 0.95* |
| AUC (mg/dL·2-hour) | 14,810.4 ± 2,334.8 | 15,353.6 ± 2,603.5 | 0.69 |
| Free-Living Glucose Concentration (mg/dL) | | | |
| 24-Hour Mean | 100.5±13.5 | 103.4±9.7 | 0.29 |
| Diurnal | 101.1±13.9 | 106.4±7.8 | 0.19 |
| Nocturnal | 98.7±12.5 | 100.8±11.8 | 0.26 |
| Maximum | 148.6±11.7 | 160.0±10.0 | 0.05 |
| Minimum | 64.6±11.2 | 71.7±6.3 | 0.07 |
| Maximum-Minimum | 83.9±8.3 | 88.3±8.1 | 0.26 |
| Glycemic Variability (mg/dL) | | | |
| MAGE | 42.3 (36.8-60.9) | 43.3 ± 7.6 | 0.67* |
| CONGA-1 | 18.7 ± 3.9 | 20.3 ± 3.4 | 0.56 |
| CONGA-2 | 21.7 ± 4.6 | 23.0 ± 3.6 | 0.68 |
| CONGA-4 | 22.9 ± 4.8 | 25.4 ± 3.3 | 0.34 |
| MODD | 19.4 ± 3.5 | 22.5 ± 6.2 | 0.31 |
| Vascular Health and Oxidative Stress | | | |
| Nitric Oxide (µmol/L) | 83.4 ± 23.8 | 102.9 ± 33.7 | 0.09 |
| Myeloperoxidase (ng/mL) | 24.8 ± 8.2 | 16.4 ± 4.6 | **<0.01** |
| NO:MPO Ratio | 3.5 ± 1.1 | 6.4 ± 1.7 | **<0.01** |

Outcome data presented as Mean±SD, non-normally distributed data presented as median (lower quartile-upper quartile) and compared using log-transformed data*, mg/dL=milligram per deciliter, AUC=area under the curve, mg/dL·2-hour=milligram per deciliter per 2-hour, MAGE=mean amplitude of glycemic excursions, CONGA-1, -2, and -4=continuous overlapping net glycemic action of 1-hour, 2-hour, and 4-hour, MODD=mean of daily differences, µmol/L=micromole per liter, ng/mL=nanogram per milliliter, NO:MPO=nitric oxide to myeloperoxidase, *p* value for comparison between baseline and post-intervention, **bold** denotes statistical significance

## DISCUSSION

The primary purpose of this study was to examine the effect of aerobic exercise on CGM-assessed glucose concentrations and glycemic variability and biological markers of vascular

health and oxidative stress in overweight or obese, non-diabetic adults. The primary findings were that MPO concentration significantly decreased while the ratio of nitric oxide concentration to MPO concentration, significantly increased, suggesting reduced oxidative stress and improved ability to vasodilate compared to vasoconstrict following the intervention. These findings were no longer significant following adjustment for change in body weight and/or V̇O2peak, suggesting changes in these outcomes may influence our primary findings. Additionally, there were no observable changes in CGM-assessed glucose concentrations or glycemic variability.

Effect of Aerobic Exercise Intervention on Biological Markers of Vascular Health and Oxidative Stress: A purpose of our study was to examine the effect of chronic exercise on circulating concentrations of NO and MPO. Our study found a significant decrease in MPO concentration and a significant increase in the NO:MPO ratio following exercise training. Our findings are in-line with previous evidence to suggest that aerobic exercise training alters circulating biological markers of vascular health and oxidative stress and increasing the potential propensity to vasodilate. A previous study aimed to assess whether a twelve-week endurance exercise program could reduce biological markers of oxidative stress in adults at an increased risk of coronary events and found that MPO concentration significantly decreased after training (54). Another study examined the effect of 16 weeks of aerobic exercise, which incorporated two different intensities (30 - 40% V̇O$_2$max or 55 - 65% V̇O$_2$max) for 30 minutes each session, three times per week in sedentary, obese males that were non-diabetic (30 - 40% V̇O$_2$max: $n$ = 6; 55 - 65% V̇O$_2$max: $n$ = 6) or Type 2 diabetics (30 - 40% V̇O2max: $n$ = 6; 55 - 65% V̇O$_2$max: $n$ = 7) (30). This study found that skeletal muscle NO expression, but not circulating serum NO concentration, increased following the 16 weeks of aerobic exercise at 55 - 65% V̇O2peak in only non-diabetic participants, with no alterations in those performing lower-intensity aerobic exercise, or in adults diagnosed with Type 2 diabetes following either exercise intensity. Our findings, along with the findings of others, highlight that aerobic exercise improves vascular health and decreases oxidative stress. Mechanistically, inferences may be made that these alterations are due to increased skeletal muscle mass and improved mitochondrial function (7), which have further implications on cardiometabolic health (58). However, as the present study did not measure body composition or cellular respiration, future research needs to be performed to identify these as contributing factors.

Effect of Aerobic Exercise Intervention on CGM-Assessed Glucose Concentrations and Glycemic Variability: Unexpectedly, there were no significant changes in any glucose concentration or measure of glycemic variability from baseline to post-intervention. Therefore, findings from previous studies examining the influence of exercise on glucose concentrations and glycemic variability are not in line with findings from our study in overweight or obese non-diabetic adults undergoing chronic exercise training. Mikus et al. (2012) examined the differences in glycemic variability during three days of habitual PA and during the final three days of a seven-day aerobic exercise program in obese adults diagnosed with Type 2 diabetes mellitus that were not currently taking exogenous insulin (37). Their study found that 24-hour mean glucose concentration was not different during the final three days of aerobic exercise compared to the 3 days of habitual exercise. Further, a randomized control trial examined the effects of a four-

month free-living continuous walking or interval-walking training on CGM-assessed glycemic control in adults diagnosed with Type 2 diabetes (25). Their study found elevated 24-hour mean glucose concentration in the control group, who underwent no exercise, while 24-hour mean glucose concentration decreased in the interval-walking group with no changes noted in the continuous-walking group. A more recent clinical trial in Type 2 diabetics examined the effects of twelve weeks of hybrid low-volume high-intensity interval training (HIIT), including both aerobic- and resistance-based exercise, on cardiometabolic outcomes (13). Their study found significant decreases in 24-hour mean glucose concentration and MAGE following the twelve weeks of HIIT training. These studies provide evidence that 24-hour glucose concentration and glycemic variability are potentially lowered by acute and chronic exercise in Type 2 diabetics. Although exercise training is traditionally thought to improve clinic-based assessments of glucose metabolism and glycemic health, outcomes such as glucose disposal rate utilizing a hyperinsulinemic-euglycemic clamp have been shown to improve due to exercise training in the absence of alterations in more traditional measurements (28) and is considered the gold standard for assessing insulin sensitivity in humans (11). As such, and in-line with previous inferences that exercise training would increase skeletal muscle mass and improve cellular respiration, our lack of significant findings could be due to assessment technique and not necessarily imply a lack of metabolic adaptation. Future studies should consider more sensitive measures of glycemic health assessment in addition to traditional assessments, such as the OGTT performed in our study.

Confounders to Our Findings and Lessons Learned: Our study was initially designed for the CGM monitoring period to begin, fasting blood sample collection, and OGTT to occur ≥ 72 hours after the last exercise session. This timeframe was determined to limit the acute effect of the last bout of exercise on glucose concentrations, glycemic variability, and oxidative stress, as even a single bout of exercise could influence our outcomes of interest (36, 53). Although this timing was within the time frame before potential detraining effects on biological markers of vascular health and oxidative stress occur (33), the effects of exercise on glucose homeostasis-related insulin sensitivity begins to be lost within 5 - 10 days following cessation of exercise training (19). Therefore, timing of CGM placement and subsequent first monitoring day may be a limiting factor as to why we observed no changes in glucose concentration or glycemic variability.

Further, individual changes in body weight and relative $\dot{V}O_2$peak may have contributed to our null findings. Although exercise is not often a prescribed means of weight loss (5), but a promoter of improved body composition (23), there were noted individual level changes to participant body weight following aerobic exercise training. However, exercise is traditionally utilized to improve CRF ($\dot{V}O_2$) regardless of modality (67), yet intensity of exercise throughout an intervention often determines improvements in $\dot{V}O_2$ (49). As such, there were noted individual level changes to participant VO2peak following aerobic exercise training. Previous evidence from the Studies of Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE) trials have shown that varying doses and intensities of exercise influences body weight loss and cardiorespiratory fitness differentially (57, 61). As our exercise prescription was of moderate-intensity and dose of exercise energy expenditure, 50 - 55% HRR and 10 - 12 KKW,

compared to previous trials, the intervention responses for body weight and $\dot{V}O_2$peak are not surprising and should be accounted for in future research studies.

Strengths and Limitations*:* The primary strength of this study was that all exercise sessions were monitored by trained research staff in the CERC, which controlled for the structure and environment of the exercise intervention. Another strength was the use of CGM to assess free-living glucose concentrations and glycemic variability for multiple days. Further, evaluation of changes in biological markers of vascular health and oxidative stress in conjunction with glycemic variability, while also exploring the relationship between changes in the primary outcomes of interest, were strengths of this study.

However, there exist several limitations. The primary limitation was the small sample size ($n$ = 8). Yet, there were significant changes observed in MPO concentration and the NO:MPO ratio. Our study was initially designed to serve as a pilot and feasibility study to use CGM to assess glucose concentrations and glycemic variability in exercise training studies. We successfully used CGM in our study. However, the timing of placement of the CGM and collection of fasting blood samples may have influenced our findings. Even though we were attempting to limit the impact of the last bout of exercise on our outcomes of interest, we may have limited ourselves to evaluation of glucose concentrations during a de-training state as opposed to a trained state. Thus, placement of the CGM device earlier, even the same day as the last exercise bout, may provide greater insight into the effect of exercise training on free-living glucose concentrations and glycemic variability. Additionally, the exercise intensity (50 - 55% HRR), volume (10 - 12 KKW), or duration (twelve weeks) may not have been sufficient to observe changes in clinical or free-living measures of glucose concentrations and glycemic variability. Lastly, the inclusion of a free-living condition where we did not test the results of a standardized diet on glucose concentrations was a potential limitation. Yet, this was also believed to be a strength, as not completely controlling for diet allows for a greater insight and potential dietary changes in adults incorporating exercise training into their daily life.

Although we found a significant decrease in MPO concentration and an improvement in the NO:MPO ratio, there were no changes in any clinical-based or free-living glucose concentrations or measures of glycemic variability. This may partially be explained by timing of placement and subsequent monitoring of CGM glucose concentrations and glycemic variability following exercise training, exercise intensity, volume, and duration of the intervention, as well as individual level changes in body weight and VO2peak following the intervention. Future studies should consider timing of the CGM device and a higher intensity of exercise to determine changes in glucose concentrations and glycemic variability in sedentary overweight or obese adults undergoing aerobic exercise training.

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