The Impact of Acute Exercise on Vascular Insulin Sensitivity

A Dissertation
Presented to
The Faculty of the Curry School of Education
and Human Development

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

---

by
Emily M Heiston, MEd
May 2020

© Copyright by
Emily Heiston
All Rights Reserved
May 2020

ABSTRACT

Purpose: Obesity is associated with decreased sensitivity to insulin, which increases risk of type 2 diabetes and cardiovascular disease. While a single session of exercise increases metabolic insulin sensitivity in adults with obesity, the effect on vascular insulin sensitivity is unknown. Therefore, the primary aim of the current study was to assess the impact of a single bout of exercise on insulin-stimulated responses in conduit arteries and capillaries. A secondary aim included acute exercise implications on insulin-stimulated arterial stiffness as well as metabolic insulin sensitivity. Finally, a tertiary aim was to understand the impact on substrate oxidation. **Methods:** Eleven sedentary adults (50.0 ± 2.42 yrs; VO_2max: 23.5 ± 1.70 ml/kg/min) with central adiposity (waist circumference: 111.8 ± 3.04 cm) completed a control and an acute exercise bout (70% VO_2max to expend 400 kcals) condition. After an overnight fast, participants underwent a 2-hr euglycemic-hyperinsulinemic clamp (40 $mU \cdot m^2 \cdot min^{-1}$) to determine vascular and metabolic insulin sensitivity. Endothelial function was assessed by brachial artery flow mediated dilation (FMD) while capillary perfusion (microvascular blood volume, MBV), filling rate (microvascular flow velocity, MFV) and blood flow (MBF=MBV*MFV) were assessed using contrast enhanced ultrasound. Systemic aortic waveform was also measured via augmentation index (AIx_{75}) to assess arterial stiffness. Metabolized glucose infusion rate and substrate oxidation was measured to understand metabolic insulin sensitivity and nutrient utilization, respectively. T-tests, repeated measures ANOVAs and correlations were used when appropriate. Significance was accepted as $P \leq 0.05$. **Results:** Exercise did not alter fasting indices of FMD, AIx_{75}, MBV, MFV. However, exercise tended to increase metabolic insulin-stimulated MBF (P=0.059) and insulin sensitivity (P=0.065) compared with control. Further, exercise increased fasting fat oxidation (P=0.03)

and insulin-stimulated carbohydrate oxidation (P=0.02) compared with control. **Conclusion:** A single bout of exercise may improve microvascular blood flow with metabolic insulin sensitivity and fuel selection the following day in adults with central adiposity. Further work is needed to determine vascular responses with different doses of exercise to design optimal lifestyle prescriptions for reducing chronic disease risk.

Emily M Heiston
Department of Kinesiology
Curry School of Education and Human Development
University of Virginia
Charlottesville, Virginia

APPROVAL OF THE DISSERTATION

This dissertation, ("The Impact of Acute Exercise on Vascular Insulin Sensitivity"), has been approved by the Graduate Faculty of the Curry School of Education and Human Development in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

_____
Steven Malin

_____
Sibylle Kranz

_____
Eugene Barrett

_____
Zhenqi Liu

Date: March 25, 2020

DEDICATION

This dissertation is lovingly dedicated to my mother, Mechelle Alger, my father, Jerry Heiston, my grandparents, Carl and Margaret Parks, and my husband, Brent Comer. Their encouragement and love have been a strong and comforting constant throughout this journey.

ACKNOWLEDGEMENTS

I am grateful to Dr. Steven Malin for his time, guidance and mentorship as well as for the support and expertise of my committee, Dr. Sibylle Kranz, Dr. Eugene Barrett and Dr. Zhenqi Liu. In addition, I am thankful for the help provided by Heidi Johnson in the Clinical Research Core Lab, Lisa Farr and Nicole Huebner in the Exercise Physiology Core Lab and members of the Applied Metabolism and Physiology Lab who aided in data collection: Stephanie Miller, Brielle Dodson, Nathan Stewart, Anna Ballentyne and Udeyvir Cheema. Finally, I am appreciative of the American College of Sports Medicine Foundation for providing funding.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

INTRODUCTION

Insulin promotes vasodilation in large conduit arteries, enhances endothelial function in resistance arterioles and increases perfusion in the microvasculature under normal physiologic conditions (Eugene J. Barrett et al., 2011). Conduit and resistance arteries are important in blood flow dynamics, whereas the microvasculature plays a critical role in the exchange of oxygen and nutrients and is essential in glucose regulation (E. J. Barrett et al., 2009). Vasodilation of the endothelium is thought to be mediated via nitric oxide (NO) (Inyard et al., 2007). However, during compromised insulin-sensitivity states, hyperinsulinemia develops and promotes vasoconstriction by increasing endothelian-1 (ET-1) (Muniyappa & Yavuz, 2013), thereby impairing glucose delivery and increasing risk for type 2 diabetes (T2D). In addition, the prolonged presence of hyperglycemia inhibits NO bioavailability (Inyard et al., 2007) and contributes to endothelial dysfunction (Zhao et al., 2015). Together, these processes demonstrate the importance of the vasculature in contributing to increased cardiovascular disease (CVD) risk.

Obesity incidence has been steadily increasing in the United States with current prevalence estimated near 42% of adults (CDC, 2020). Obesity has traditionally been linked with reduced metabolic insulin action through, in part, decreased metabolic flexibility as reflected by the blunted ability to switch from fat use in the fasted state to insulin-stimulated carbohydrate oxidation (Goodpaster & Sparks, 2017). However, recent work highlights the significance of insulin-stimulated vascular function for glucose disposal in the skeletal muscle. In fact, reduced skeletal muscle microvascular insulin sensitivity is thought to contribute to the pathogenesis of decreased metabolic insulin sensitivity (Eugene J. Barrett & Liu, 2013).

Moreover, reduced vascular insulin sensitivity across the arterial tree (i.e. large conduit arteries to the microcirculation) is clinically relevant as it increases atherogenesis (large conduit arteries), hypertension (resistance arterioles) and favors gluco-lipid toxicity (microcirculation) (Ralph A. DeFronzo et al., 1987). Together, these adverse events contribute to the development of CVD, which accounts for the majority of deaths in patients with T2D.

Exercise is established to reduce T2D and CVD risk (Penedo & Dahn, 2005). Exercise likely reduces this disease risk by increasing insulin sensitivity (Malin et al., 2014; Sjøberg et al., 2017). Indeed, several studies have highlighted that exercise increases fasting and insulin-stimulated glucose uptake (Roberts et al., 2013; Shojaee-Moradie et al., 2007; Sjøberg et al., 2017) across glucose tolerance spectrum. (Hwang & Lee, 2016). Most studies to date have studied the effect of exercise on large conduit arteries as measured by flow-mediated dilation (FMD) or arterial stiffness. Although the collective evidence supports increased FMD (Son et al., 2017) or reduced arterial stiffness (Donley et al., 2014; Madden et al., 2009), less attention has been placed on microvascular function. Moreover, no study has characterized the effect of insulin on these vascular outcomes following a single bout of exercise prior to weight loss or improved fitness. Interestingly, acute exercise increases skeletal muscle microvascular recruitment and stimulates increases in muscle insulin delivery and sensitivity for upwards of 72 hrs following a single bout (Tjønna et al., 2011). Additionally, Sjøberg et al. (Sjøberg et al., 2017) observed that single-legged exercise increased skeletal muscle perfusion of the femoral artery in response to insulin upwards of 65% in lean healthy adults when measured with contrast-enhanced ultrasound (CEU) 4 hrs post-exercise. To date, this is the only published study that has used CEU as a measure of microcirculation post-exercise. Because insulin-

induced capillary recruitment is considered to be a significant determinant of overall insulin-mediated glucose uptake, these data implicate the microvasculature as a target to improve both metabolic and vascular outcomes in adults with/at risk for T2D and CVD.

LITERATURE GAP

Currently, no study has examined the effect of an acute bout of exercise on metabolic compared to vasculature insulin sensitivity in people with increased abdominal adiposity. In addition, a majority of previous exercise studies have looked at either the conduit arteries, resistance arterioles or microcirculation separately, but not across the entire arterial tree during fasted and insulin-stimulated states. This is critical as each level may respond differentially to exercise, which would alter how exercise is prescribed based upon condition (e.g. atherosclerosis vs hypertension). Further, of the few studies that have examined vascular insulin sensitivity, measurements have been collected within 4 hrs of the exercise bout. Thus, prior work is likely not capturing insulin-dependent mechanisms as catecholamine responses and overall blood flow dynamics are altered during the immediate post-exercise period, contributing to or blunting potential effects of insulin action. Past studies have also not standardized participants' diets the day prior to testing. Dietary intake and/or composition has been shown to affect endothelial composition as well as overall blood pressure responses; therefore, it is unknown whether prior results were or were not seen due to a dietary interaction.

PURPOSE AND HYPOTHESES

The overall purpose of the current study was to examine how an acute bout of exercise impacts vascular and metabolic insulin sensitivity in sedentary overweight/obese adults ~16 hrs later.

Our primary hypothesis was that acute exercise would impact the vascular via increases insulin-stimulated conduit artery function and capillary blood flow. We secondarily hypothesized that acute exercise would lower insulin-stimulated arterial stiffness and increase metabolic insulin sensitivity. Lastly, we hypothesized that exercise would elevate fasting fat oxidation as well as insulin-stimulated carbohydrate oxidation. If accepted, these hypotheses will support the importance and potency of exercise as a therapy as well as distinguish the chronic versus acute effects of exercise in future work. If rejected, this work will still hold scientific relevance as it will provide insight regarding the role of acute exercise on fasted and insulin-mediated vascular health. In addition, these data will aid in understanding exercise dose for populations at risk for decreased vascular insulin sensitivity.

METHODS

Study Criteria. Adults between the ages of 40-70 years (yrs) were recruited from the community via social media and/or flyers. A prescreening questionnaire was completed over the phone to determine initial eligibility. Individuals were excluded if physically active (>60 min/wk), pregnant or nursing, on medications known to affect glucose metabolism (e.g. biguanides, insulin, TZDs, etc.), weight unstable over the prior six months (>2 kg variation), had a history of chronic disease (i.e. renal, hepatic, cardiovascular, etc.), as well as participated in smoking within the previous 2 yrs. Study protocols were approved by the University of Virginia's Institutional Review Board (IRB#19060). All individuals provided written and verbal informed consent prior to participating in the current study.

Screening. Individuals arrived to the Clinical Research Unit (CRU) following a 10 hr overnight fast to determine study eligibility. Body weight was measured to the nearest 0.01 kg on a digital scale while height was measured with a stadiometer (Seca 222, Seca GmbH, Hamburg, Germany) to determine body mass index (BMI). Systolic (SBP) and diastolic blood pressure (DBP) were measured following 5 minutes of undisturbed rest in a supine position. Three measurements were taken with a minimum of one minute between readings and averaged. A fasting blood draw was performed and analyzed by the University of Virginia Medical Laboratories. Tests included a complete blood count with differential, comprehensive metabolic panel, lipid panel, hemoglobin A1c (HbA1c) and human chorionic gonadotropin (hCG), when necessary. Individuals qualified for the current study if they had an elevated waist circumference (WC; females: ≥88 cm; males: ≥102 cm), were overweight (BMI: 27-40 kg/m$_2$) and did not have uncontrolled hypertension (SBP <160 mmHg and/or DBP <90 mm Hg).

Aerobic Fitness. Participants underwent a resting electrocardiogram and was examined by the study physician to ensure participation safety. Following physician approval, individuals completed a continuous incremental treadmill test (Carefusion, Vmax CART, Yorba Linda, CA, USA) to determine VO_2max. All tests occurred under the supervision of a certified exercise physiologist in the Exercise Physiology Core Laboratory. Following a 2 minute warm-up period at 1.8 mph, participants self-selected a comfortable walking speed that was maintained throughout the test duration. The incline was raised every 2 minute by 2.5% until volition exhaustion was reached. Throughout the test heart rate (HR), blood pressure and rating of perceived exertion (RPE) were recorded. The test was considered valid if 3 out of 4 conditions were met: 1) a plateau in VO_2 (<0.150 L/min change), 2) the maximum HR achieved

was within 10 beats of the age-predicated heart rate max (220-age), 3) RPE ≥17 AU on the Borg scale (Borg, 1982) and 4) respiratory exchange ratio ≥1.1 AU (Beltz et al., 2016).

Body Composition. Total fat and fat-free mass as well as estimated visceral adipose tissue (VAT) were assessed using dual-energy X-ray absorptiometry (DXA) (Horizon DXA System; Hologic, Marlborough, MA, USA).

Metabolic Control. Resting metabolic rate (RMR) was determined after an overnight fast using indirect calorimetry (Carefusion, Vmax CART, Yorba Linda, CA, USA). After resting supine for approximately 20 minutes, breath samples were collected for a minimum of 15 minutes. The last five minutes of steady state data were averaged during the end of testing to determine resting energy expenditure (REE). An activity factor of 1.2 was applied to the REE to determine daily caloric needs (J. A. Harris & Benedict, 1918). Food was provided to each participant the day prior to testing and comprised of 55% carbohydrates (CHO), 30% fat (<10% saturated) and 15% protein (PRO) was provided to each participant prior to the testing visit (Evert et al., 2013). The same food was provided before both testing conditions. Participants were instructed to consume the provided food and abstain from alcohol, caffeine, medication and strenuous physical activity 24 hr prior to testing.

Acute Exercise and Control. Individuals reported to the Applied Metabolism and Physiology Laboratory following a 4 hr fast between 4:00-6:30pm and completed one of two testing conditions in a counterbalanced order. Both exercise and control conditions were completed at the same time with at least 1 week between tests. Females who had a normal menstruation

cycle completed testing 1 month apart in the follicular phase. The exercise condition consisted of a treadmill walking protocol at a moderate intensity (~70% VO_2max). Time was based on of individual fitness levels to expend ~400 kcals. Oxygen consumption was measured throughout the exercise condition via a metabolic cart (Carefusion, Vmax CART, Yorba Linda, CA, USA) to confirm energy expenditure. During the control condition, individuals were instructed to remain seated for the same duration as the exercise bout. For individuals who completed the control condition first, calculations were used based on VO_2max data to estimate the total time needed to expend 400 kcals. Following either the exercise or direct rest observation period, participants were provided a standardized dinner 30 mins post exercise that was ~25% of the total daily energy expenditure. The meal was consumed in the laboratory and participants were then given a small snack to consume later at home.

Euglycemic-Hyperinsulinemic Clamp. Participants arrived to the CRU at 7:00am following a 10 hr overnight fast. Fasting CHO oxidation (CHOox; (4.55*VCO_2 (L/min) – 3.21*VO_2 (L/min)) and fat oxidation (Fox; (1.67*VO_2 (L/min) – (1.67*VCO_2 (L/min)) was determined after individuals rested supine for approximately 20 minutes as described above for RMR determination. Two intravenous lines, one in the antecubital vein and one near the wrist, were then placed for infusion and arterialized blood collection, respectively. Approximately 110 ml of 20% dextrose (McKesson Corporation, Irving, TX, USA) solution was drawn into four 140 ml syringes. Insulin (Humulin R U-500; Eli Lilly and company, Indianapolis, IN, USA) was diluted in saline containing 4% (vol/vol) of the subject's own blood. A primed (250 $mU/m^2/min^1$) constant infusion (40 $mU/m^2/min^1$) of insulin (R. A. DeFronzo et al., 1979) was administered for 120 minutes. Blood glucose was measured every 5 minutes and glucose was

infused (PHD 22/200 Syringe Pumps, Harvard Apparatus, Holliston, MA, USA) at a variable rate throughout the 120 minutes to maintain plasma glucose at 90 mg/dl. Metabolic insulin sensitivity was classified using the average glucose infused (M-value) over the last 30 minutes of the clamp. During approximately 80-100 minutes, measures of insulin-stimulated CHO metabolism and Fox were recorded. Endothelial function, microvascular function and arterial stiffness were then measured during approximately 100-120 minutes. Ambient insulin concentrations were also assessed at 90, 105 and 120 minutes of the clamp.

Flow-Mediated Dilation. Endothelial function was assessed via the brachial artery ~5 cm proximal to the antecubital fold using B-mode ultrasound and a high-frequency L12-5 linear transducer (Phillips Medical System, Andover, MA, USA). Images were collected for one cardiac beat cycle via an integrated ECG. A minimum of four images were collected to measure baseline diameter before a forearm blood pressure cuff was manually inflated to 200 mmHg. After 5 minutes the cuff was deflated and images of post-ischemic vessel diameter were recorded every 10 secs for a total of 2 minutes. Analysis was conducted by a single investigator using commercially available edge detection software (Brachial Analyzer for Research v.6, Medical Imagining Applications LLC, Coralville, IA, USA). $FMD_{unscaled}$ was calculated as a percent change: [(peak diameter – baseline diameter)/baseline diameter]*100 (R. A. Harris et al., 2010). In addition, allometrically scaled ($FMD_{allometric}$) and time to peak were determined by the above mentioned software to further understand impact of temporal kinetics of arterial diameters and blood velocities (R. A. Harris et al., 2010).

Contrast-Enhanced Ultrasound. Contrast-enhanced ultrasound (CEU) was performed using a SONOS 7500 ultrasound system (Phillips Medical System, Andover, MA, USA). A L12-5 linear transducer (Phillips Medical System, Andover, MA, USA) was positioned cross-sectionally on the flexor muscles of the forearm using a clamp stand. Microbubbles (Definity, Lantheus Medical Imaging, Billerica, MA, USA) were intravenously infused at 1.5 ml/min (Medfusion 2010i Syringe Pump; Medex Inc, Duluth, GA, USA). After 4 minutes of equilibration, an ultrasound beam was used to destroy the bubbles while a pulsing-interval (time) versus video-intensity curve was generated, as described previously (Clerk et al., 2006; Eggleston et al., 2007). CEU images were analyzed off-line using commercial software (Q-Laboratory, Phillips Medical System, Andover, MA, USA) by a single investigator (EMH) to evaluate skeletal muscle capillary perfusion (microvascular blood volume, MBV), filing rate (microvascular flow velocity, MFV) and blood flow (MBF=MBV*MFV).

Pulse Wave Analysis. The SphygmaCor XCEL system (AtCor Medical, Itasca, IL, USA) was used to characterize the central arterial pressure waveform. Following a minimum of 30 minutes of rest in a supine position, individuals had a blood pressure cuff placed on the upper left arm. Three readings occurred over 10 minutes and were averaged to determine AIx_{75}, which was corrected to a standard HR of 75 bpm using the manufacture's software.

Pulse-Wave Velocity. Carotid-femoral pulse wave velocity (cfPWV) was collected using applanation tonometry (SphygmaCor XCEL; AtCor Medical, Itasca, IL, USA). A blood pressure cuff was placed around the left thigh while individuals were in a flat and supine position. The strongest point of the carotid pulse was determined and marked. Measurements

were then recorded between: 1) the top of the blood pressure cuff and the femoral artery, 2) the femoral artery and the sternal notch, 3) the sternal notch and the carotid artery. A pressure transducer was then placed on the carotid artery. Once a strong pulse was acquired over a 5 second period, the blood pressure cuff automatically inflated to obtain the femoral pulse and assess the propagation time.

Biochemical Analysis. Plasma glucose was analyzed immediately after collection using the glucose-oxidase method (YSI Instruments 2300, Yellow Springs, OH, USA). Insulin samples were centrifuged at 4°C and 1500 g and stored at -80°C until later analysis via an enzyme-linked immunosorbent assay (ALPCO, Salem, NH, USA). Blood lipids were analyzed using enzymatic colorimetric-based assays via our University Medical Laboratory and low density lipoprotein (LDL) was calculated using the Friedewald equation (Friedewald et al., 1972).

Statistical Power. It was determined from previous work (Clerk et al., 2006; Jahn et al., 2016; Sjøberg et al., 2017) that 9 individuals would be needed to reach statistical power ($\alpha = 0.05$, $\beta = 0.2$, power ($1-\beta = 0.8$)) in our primary outcomes of FMD and CEU following an acute bout of exercise. However, we expected a 30% attrition rate. Therefore, we rationalized 12 individuals would be needed to ensure adequate power to assess the main outcomes.

Statistical Analysis. Data were analyzed using the Statistical Package for Social Sciences (SPSS Software Version 26, 2019). Due to scheduling conflicts, one subject was omitted from data analysis due to incomplete data. Normality in the remaining 11 subjects was assessed using Shapiro-Wilkes test. Data that were non-normally distributed (MFV, MBV, MBF, HR)

were log-transformed for analysis. A paired t-test was used to assess fasting measurements between control and exercise while a repeated measures analysis of variances (ANOVA) was used to assess insulin-stimulated interactions (i.e. condition x time). Spearman correlations were used to determine the relationships among changes in collected outcomes. Sensitivity measures were performed to determine the impact of outliers (> 2 SD) on outcomes. Statistical significance was accepted as $P \leq 0.05$ and data are presented as mean ± SEM.

RESULTS

Participants. Eleven middle-aged (50 ± 2.4 yrs), sedentary (VO_2max: 23.5 ± 1.70 ml/kg/min) adults with central adiposity (WC: 111.8 ± 3.04; ***Table 1)*** completed the study. Participants performed a single moderate-to-high intensity exercise bout (69.6% of VO_2max) and expended nearly 397 kcals (***Table 2***). In addition, individuals adhered to the mixed-meal **(*Table 3*).**

Large Conduit Artery Function. Exercise had no effect on fasting pre-occlusion or post-occlusion diameters ***(Table 4)***, $FMD_{unscaled}$ (%), $FMD_{allometric}$ (%) or the time in which peak dimeter was reached. Exercise also did not impact insulin-stimulated $FMD_{unscaled}$ (%) (***Figure 1)*** or $FMD_{Allometric}$. However, exercise significantly increased insulin-stimulated pre-occlusion diameter (0.15 ± 0.06 mm, P=0.03) and tended to impact post-occlusion diameter (0.16 ± 0.06 mm, P=0.10) compared with control. In addition, time to peak diameter was prolonged following insulin infusion with exercise (20.3 ± 8.8 sec, P=0.10), although this did not reach statistical significance.

Microvascular Function. Exercise did not alter fasting indices of MFV (0.003 ± 0.005 sec^{-1}, *P*=0.59), MBV (0.057 ± 0.127 AI, *P*=0.85) or MBF (0.011 ± 0.16 AI/sec, *P*=0.70). Although exercise did not increase insulin-stimulated MFV (*P*=0.98) or MBV (*P*=0.41) compared to control, insulin increased MFV in both conditions (0.04 ± 0.00 sec^{-1}, *P*=0.006). There was a main effect of condition trend for MBF, suggesting exercise increased insulin-stimulated MBF (0.05 ± 0.05 AI/sec, *P*=0.059; ***Figure 2)*** when compared with control.

Blood Pressure and Arterial Stiffness. Fasting systolic (125 ± 3.99 vs 123 ± 3.67 mmHg, *P*=0.63) and diastolic blood pressure (76.4 ± 2.58 vs 76.0 ± 2.43 mmHg, *P*=0.84) were not impacted by acute exercise compared with control. Likewise, fasting HR (64 ± 2.9 vs 67 ± 2.8 bpm, *P*=0.34) and AIx_{75} was similar between control and exercise (24.4 ± 1.95 vs 19.0 ± 5.97 %, *P*=0.32). Although exercise did not differentially impact insulin-stimulated HR (Delta 120-0: -4.2 ± 3.8 vs 2.8 ± 2.2, *P*=0.21) or AIx_{75} (*P*=0.52), insulin tended to decrease AIx_{75} in both conditions (*P*=0.065; ***Figure 3)***. Because only 3 individuals had complete PWV data due to technical difficulties, PWV data were not analyzed due to inadequate sample size.

Metabolic Insulin Sensitivity and Substrate Metabolism. Exercise had no effect on fasting plasma glucose (97.8 ± 2.71 vs 99.2 ± 2.73 mg/dl, *P*=0.63) and insulin (9.14 ± 1.73 vs 9.48 ± 1.64 uU/ml). Further, there was no difference in clamp-derived insulin concentrations (70.6 ± 4.87 vs 68.9 ± 4.73 uU/ml, *P*=0.59). However, exercise tended to increase metabolic insulin sensitivity compared to control (0.58 ± 0.28 mg/kg-BW/min, *P*=0.065) ***(Figure 4)***. Although exercise did not alter fasting CHO (-1.65 ± 1.17 mg/min, *P*=0.19), it tended to increase fasting Fox (0.93 ± 0.47 mg/min, *P*=0.07) compared to control. Similarly, there were no interactions

in insulin-stimulated CHOox (P=0.10) or Fox (P=0.10). However, 1 subject had respiratory gas measurements that met outlier criteria. After removal of this subject, sensitivity analyses showed that exercise decreased CHOox (-2.52 ± 0.87 mg/min, P=0.02; n=10) and increased fasting Fox (P=0.03; n=10; ***Figure 5***). Further, exercise significantly increased insulin-stimulated CHOox when compared with control (P=0.02; n=10; ***Figure 6***).

Correlations. During the control condition, metabolic insulin sensitivity was inversely associated with WC (r=-0.63, P=0.04). Higher aerobic fitness was also associated with lower circulating fasting insulin (r=-0.70, P=0.02) and decreased body fat (%) (r=-0.66, P=0.04). Low fasting $FMD_{unscaled}$ at control correlated with higher WC (r=-0.77, P=0.005) and estimated visceral adipose tissue mass (kg) (r=-0.68, P=0.03). In addition, $FMD_{unscaled}$ and $FMD_{allometric}$ were closely correlated at fasting (r=0.66, P=0.03) and following insulin infusion (r=0.74, P=0.01). Following exercise, elevated $FMD_{allometric}$ at 120 min (i.e. Exercise – Control) was significantly related to increased metabolic insulin sensitivity (r=0.64, P=0.04, ***Figure 7***).

DISCUSSION

This is the first study to examine the arterial tree responses to insulin ~16 hrs following the acute bout of exercise. Therefore, local factors (e.g. adenosine, pH, etc.) observed during the immediate post-exercise period are not considered to be a major influence in the present study. Contrary to our primary hypothesis, it is shown herein that a single session of exercise in overweight/obese adults did not alter fasting or insulin-stimulated measures of FMD when expressed as a percentage or allometrically. Although this is somewhat surprising, we did observe that insulin increased pre-occlusion diameter following the exercise condition only.

These observations suggest that insulin increases blood vessel diameter given that occlusion did not further add to changes in post-occlusion diameter following exercise compared with control. The release of occlusion is known to induce shear stress, which is a mechanism that elicits NO-dependent vasodilation (Green et al., 2017). These findings suggest that insulin may either work to promote NO independent of shear stress or through an alternative pathway to impact arterial diameter. In either case, if blood flow and perfusion to resistance arterioles were delayed because of an increase in diameter, there may be less of a stimulus post-occlusion to promote further diameter shifts under insulin stimulation. Indeed, it was noted that exercise tended to increase the time to peak in the present study. Recognizing that the sympathetic nervous system is an important mechanism of endothelial function regulation at rest, it is possible that neural factors played a role in the diameter shift observed herein since euglycemic hyperinsulinemia increases plasma catecholamine concentrations (Emdin et al., 2001). While we did not determine muscle sympathetic drive in the present study, there were no changes in heart rate between the two conditions at fasting or following insulin-stimulation. This suggests that nervous system responses were comparable and unlikely explain the diameter change after insulin-stimulation with exercise. Future work is required to elucidate mechanisms by which exercise impacts insulin action on blood vessel diameter given that increases in insulin-stimulated FMD were directly correlated with metabolic insulin sensitivity following exercise.

Previous work has highlighted that capillaries have blunted responses to insulin in individuals with obesity (Clerk et al., 2006) and MetS (Jahn et al., 2016) when compared with lean, healthy counterparts. The current data presented herein support these findings with regard to MBV and MBF in obese adults. However, insulin increased MFV by ~25% in response to insulin during

both the control and exercise condition. Based upon this, individuals in the current study may not have been as insulin resistant at the capillary level as previous studies (Clerk et al., 2006; Jahn et al., 2016). Conversely, it appears the current study participants had lower MFV values at baseline compared to both the lean and obese groups studied by others (Clerk et al., 2006; Jahn et al., 2016). In either case, although MFV and MBV are thought to be important regarding overall blood flow and contributions to muscle glucose uptake, recent work by McClatchey et al. (2019) suggests that MFV has a greater influence than MBV on blood flow. If MFV is more important in this aspect, it may be improved by insulin to a greater extent than MBV in order to affect blood flow. However, Keske et al. (2020) noted that a limitation of the work by McClatchey et al. (2019) is that a majority of capillaries had already been perfused prior to the insulin-stimulation. Thus, MBV and MFV are both likely to play an equally important role in muscle glucose uptake. Importantly, the results of the present study show that exercise tended to increase MBF compared with control. This suggests that an acute bout of exercise may improve microcirculatory function in obese individuals. Because no differences in fasting MBF existed between exercise and the control condition, the main effect of condition trend for elevated microvascular blood flow highlights a potential role of insulin to drive this phenomenon. Additional work is warranted to confirm these observations.

Although exercise was hypothesized to decrease arterial stiffness as estimated by AIx_{75}, no effect was observed for fasting or insulin-stimulated arterial stiffness. Despite no effect of exercise, insulin tended to decrease AI_{75} in both conditions. Previous work has shown a non-response to insulin in individuals with MetS and T2D while healthy individuals decrease AIx_{75} within 30 minutes of insulin stimulation (Tamminen et al., 2002). Responses across the arterial

tree are typically hierarchical, with changes occurring in the microvasculature first, followed by the resistance arterioles and then the conduit arteries. Since there was a tendency for MBF to increase with exercise during insulin-stimulation, it would have been expected to see a slight reduction in arterial stiffness and/or blood pressure. However, both conditions tended to decrease insulin-stimulated AIx_{75} whereas only exercise saw the subsequent increase in MBF. There are some data that suggest that hyperinsulinemia inhibits endothelial function due to an imbalance between vasodilatory and vasoconstrictive substances (Arcaro Guido et al., 2002; Campia et al., 2004). While not all work support the idea of hyperinsulinemia-induced endothelial dysfunction (Perkins et al., 2015), these findings highlight that aspects of the arterial tree may respond to insulin following exercise in an unique manner.

Exercise tended to increase metabolic insulin sensitivity as corroborate by insulin-stimulated CHOox (following removal of n=1 outlier). Moreover, exercise promoted fasting Fox elevations compared with control. These collective outcomes support previous work showing that a single bout of moderate- to high-intensity exercise stimulates insulin sensitivity (Devlin et al., 1987) and Fox the following day (Schenk & Horowitz, 2007; Schrauwen et al., 1997). Skeletal muscle microvascular function has been suggested to play a key role in substrate oxidation via the successful delivery of these nutrients for utilization. However, the increase in fasting Fox observed with exercise was not associated with changes in MBF. This suggests that changes in nutrient delivery is not the primary factor explaining elevations in Fox after an overnight fast. Rather, this increase in Fox could be due to decreases in muscle glycogen following exercise. Since the ~400 kcal expended from exercise were no re-fed, it would be reasonable to consider these individuals to be in a glycogen depleted state. Previous work has

shown that when glycogen stores are low, glycogen synthesis is related to increased fasting Fox as well as whole-body insulin sensitivity (Schrauwen et al., 1997). Taken together, the rise in fasting Fox and insulin-stimulated CHOox suggest that exercise confers metabolic insulin sensitizing effects. .

There are limitations in the current study that warrant discussion. First, no control group was utilized to confirm/compare exercise responses in healthy individuals. However, the aim of the current study was to investigate whether exercise could improve insulin-mediated vascular responses within an overweight/obese population across the arterial tree. In addition, each individual acted as a subsequent control during the no exercise condition. Secondly, measures of FMD were completed without a probe stand or forearm stabilizer. Participants though were placed in a supine position and the arm was stabilized with towels to reduce forearm movement. Further, the imaging area was marked to ensure the same placement for repeated images. Analysis was also conducted using a 70% confidence threshold for each FMD image analyzed to ensure overall data quality. Finally, these data are limited to aerobic exercise. It is not clear if other modes or doses of exercise would result in similar results.

CONCLUSION

In conclusion, a single bout of exercise did not alter insulin-stimulated endothelial function or arterial stiffness. However, exercise may provide some benefit regarding insulin-stimulated arterial diameter and microvascular blood flow. In addition, exercise likely increases to metabolic insulin sensitivity and improves substrate oxidation. The impacts of acute exercise

on vascular insulin sensitivity still warrants investigation, with future research studying different acute exercise doses with, or without, dietary modifications. This is necessary for personalized medicine as these results indicate that the metabolic and vasculature system may differ regarding responses to exercise. Further research might also be conducted to determine if insulin-stimulated vascular responses require multiple bouts to see beneficial results. All of these questions are critical to designing appropriate exercise prescriptions for individuals based on evident risk factors.

SUPPLEMENTAL CHAPTER OF EXPLORATORY ENDPOINTS

Obesity is considered a chronic pro-inflammatory state characterized, in part, by increased circulating tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) levels (see the Third National Health and Nutritional Examination Survey, the Cardiovascular Health Study, the British Regional Heart Study and the Nurses' Health Study). While several inflammatory markers are known to be implicated in insulin resistance, less attention has been directed at receptor for advanced glycation end productions (RAGE) and matrix metalloproteinases (MMPs) for the metabolic and vascular impact these molecules have on insulin action.

RAGE is a multiligand receptor that acts as a key mediator in multiple biological and pathological processes such as tissue differentiation and inflammation (Riuzzi et al., 2018). Constant levels of RAGE are expressed during embryonic and early-stage life. These levels decline during development and are relatively low in differentiated cells (Sakatani et al., 2009). While RAGE levels are expressed at low levels in adulthood, upregulation is observed in disease states, including MetS, T2D and CVD (Yamagishi et al., 2005). This observation

suggests that RAGE plays a role in the development and progression of metabolic and vascular abnormalities.

RAGE exists in 2 forms: mRAGE and soluble RAGE. Soluble RAGE (sRAGE) is believed to bind advanced glycation end products (AGE) and prevent their actions and disrupt RAGE signaling (Schmidt et al., 1994; Wautier et al., 1996). Together, this is believed to lower inflammation, oxidation and glycation (Kotani et al., 2011; Prasad, 2019). Exercise tends to reduce AGE levels in conjunction, but the mechanism by which this occurs remains to be elucidated. One hypothesis is that sRAGE binding of AGEs increase in response to exercise. However, the literature on sRAGE and exercise is equivocal, with some showing decreases in sRAGE following exercise training (Kotani et al., 2011) while others show no change (Fuller et al., 2018) or even increases (Choi et al., 2012; Miranda et al., 2016). These mixed results may be driven, in part, by weight loss, aerobic fitness gains and/or health status. To date, no published data exit determining the acute submaximal effects of exercise on sRAGE in adults with obesity.

MMPs are responsible for the degradation of extracellular matrix (ECM) proteins and play a role in regulating ECM remodeling. MMPs can be activated by phosphorylation and by oxidative stress, such as hydrogen sulfide and NO. However, the exact mechanism regarding NO and MMP regulation is not completely understood. MMP-1 is a collengenase believed to be involved in plaque burden and pro-inflammation as elevated MMP-1 levels are associated with myocardial infarction and coronary artery disease. MMP-7 is a matrilysin that targets MMP-1, -2 and -9, all of which are related to atherosclerosis and hypertension (Jaoude & Koh,

2016). This highlights that MMP-7 has a cardioprotective role in disease risk. Additionally, MMP-7 has been linked to increases in the bioavailability of insulin-like growth factor 1 (IGF-1). This relationship is important because low levels of IGF-1 are associated with T2D risk (Kotani et al., 2011).

Given the effects of MMP-1 and MMP-7 on arterial remodeling, it is not surprising that lifestyle or medical interventions have targeted MMPs as a modulator of vascular function. In fact, bariatric surgery induced weight loss increases MMP-7 levels in conjunction with improved blood glucose and lipid metabolism (Ress et al., 2010). However, 8 weeks of aerobic exercise reduced both circulating MMP-1 and -7 levels in adults with MetS but not healthy controls (Donley et al., 2014). This divergent response between bariatric surgery and exercise training in MMP-1 and -7 highlight that either intervention specific effects, magnitude of weight loos, fitness gains or disease risk of the population may alter responses of MMPs. Thus, while both MMP-1 and -7 are known to have structural effects on the vasculature, it is possible that the length of the intervention also affects these MMPs to influence vascular function. Currently, there are no published studies in adults with obesity on the impact of a single bout of exercise on plasma MMP-1 and MMP-7.

Taken together, no research has investigated the effect of acute exercise on RAGE or MMPs in relation to metabolic and vascular insulin sensitivity in adults with obesity. Given the tendency for exercise to promote microvascular blood flow and metabolic insulin sensitivity, an exploratory analysis was conducted regarding the current study to determine effects of acute exercise on fasting and insulin-stimulated RAGE, MMP-1 and MMP-7 responses.

METHODS

Inflammation: Using the same subjects (see *Methods, Study Criteria* above) blood samples were collected prior to and at 120 minutes of the euglycemic-hyperinuslinemic clamp to determine RAGE, MMP-1 and MMP. Samples were centrifuged and frozen until biochemical analysis. Enzyme-linked immunosorbent assays were used to measure serum sRAGE (R&D Systems, INC, Minneapolis, MN, USA), MMP-1 (R&D Systems, INC, Minneapolis, MN, USA) and MMP-7 (R&D Systems, INC, Minneapolis, MN, USA). All samples were batch analyzed and run in duplicate to minimize variance.

Statistical Analysis: Data were analyzed using the Statistical Package for Social Sciences (SPSS Software Version 26, 2019). Normality was assessed using Shapiro-Wilkes test. Data that were non-normally distributed (sRAGE, MMP-7) were log-transformed for analysis. A paired t-test was used to assess fasting measurements between control and exercise, while a repeated measures analysis of variances (ANOVA) was used to assess insulin-stimulated interactions (i.e. condition x time). Spearman correlations were used to determine the relationships among changes in collected outcomes. Sensitivity measures were performed to determine the impact of outliers (> 2 SD) on outcomes. Statistical significance was accepted as $P \leq 0.05$ and data are presented as mean ± SEM.

RESULTS

Inflammation. Exercise did not statistically alter fasting MMP-1 or MMP-7 circulating levels. However, it did tend to decrease insulin-stimulated MMP-1 concentrations compared with control (-0.59 ± 0.27 ng/ml, P=0.056; ***Table 5***). While initial analysis showed no effects of

exercise on fasting sRAGE (*P*=0.13), 1 subject met criteria as an outlier. In turn, sensitivity analysis revealed that exercise significantly decreased sRAGE (1484 ± 119 vs 1636 ± 117 pg/ml, *P*=0.05) after removal of this subject. However, there was no effect of exercise on insulin-stimulated sRAGE concentrations compared to control (Delta 120-0: 28.4 ± 64.2 vs -101 ± 76.4 pg/ml, *P*=0.28).

Correlations. Fasting control measures of MFV (r=0.62, *P*=0.04), MBV(r=0.81, *P*=0.003) and MBF (r=0.67, *P*=0.02) were all related to high fasting circulating MMP-7. Further, exercise-induced increases in 120 min MBV (r=-0.66, *P*=0.03) and MBF (r=-0.71, *P*=0.02; ***Figure 8***) were associated with decreased 120 min sRAGE levels.

DISCUSSION

In the present exploratory work, a single bout of exercise decreased fasting sRAGE concentrations in people with obesity by approximately 8.8% and had no further effects during insulin stimulation. This finding on fasting sRAGE corroborates work in older adults where 24 weeks of light to moderate-intensity exercise decreased sRAGE in older adults by 6.6%. (Kotani et al., 2011). However, our data contradict work done in healthy males, in which fasting sRAGE levels were unaltered ~16-18 hrs following exercise (Fuller et al., 2018). Fuller et al. (2018) prescribed cycle ergometry for 45 minutes at 65% VO_2peak in healthy individuals and did not measure caloric expenditure. Interestingly, healthy control sRAGE concentrations were lower compared to obese individuals observed herein. This highlights that the level of inflammation may have been higher in our subjects with obesity prior to exercise and explain the differential exercise effects between studies. Regardless, the lowering of sRAGE may be

physiologically relevant following a single bout of exercise. We observed that insulin-stimulated decreases in sRAGE following exercise were related to insulin-stimualted MBF. It is beyond the scope of the study to determine how exercise lower sRAGE,. However, iinteraction of AGEs with their receptors promote vascular damage via induction of coagulation on cellular endothelium surface and impair microcirculatory blood flow (Payne, 2006). Although we did not directly measure AGE is the current study, prior work suggests that exercise with weight loss lowers AGEs (Deo et al., 2017; Macías-Cervantes et al., 2015). Thus, exercise may relate to improved insulin-stimulated MBF through, in part, a complex interaction of the RAGE pathway.

Despite no changes in fasting MMP-1, exercise tended to decrease insulin-stimulated MMP-1 concentrations. This suggests a direct effect of insulin on lowering MMP-1. Previous work reported that 8 weeks of aerobic exercise (3x/wk, 60 min/d, 60-85% heart rate reserve) decreased fasting MMP-1 levels by 18.5% (Donley et al., 2014). Recent work suggests that MMP-1 stimulates tissue remodeling during adipose tissue expansion (Jaoude & Koh, 2016). This connection to adipose tissue may indicate that fasting MMP-1 levels change in response to weight loss. While no study has examined the effects of insulin on MMP-1, some have observed insulin reduced MMP-2 and -9 (Boden et al., 2008; Guo et al., 2013). This was likely due to the insulin-induced increase of tissue inhibitor of metalloproteinases (TIMPs). Indeed, insulin infusion increases TIMP-1 and -2 concentrations, both of which are believed to regulate MMP-1 (Boden et al., 2008). While TIMPs were not measured in the current study, TIMPs have been observed to increase in response to exercise (Tayebjee et al., 2005). Therefore, it is possible that exercise promoted a greater increase of TIMPs in response to insulin and

subsequently lowered MMP-1 concentrations. Surprisingly, this decrease in MMP-1 did not relate to any improvements in vascular or metabolic function. However, previous work noted that MMP-1 is associated with PWV and may contribute to arterial stiffness (Carrick-Ranson et al., 2019). Unfortunately, no PWV data are available in the current study to test this association.

Despite no changes in fasting or insulin-stimulated MMP-7 levels following exercise, fasting levels of MMP-7 were positively associated with microvascular function. In contrast to MMP-1, MMP-7 levels are decreased in obesity (Chavey et al., 2003; Ress et al., 2010) and have been associated with improvements in glucolipid metabolism following bariatric surgery (Ress et al., 2010). Taken together with the data from the current study, it appears that increases in MMP-7 may be beneficial for individuals with obesity. However, MMP-7 serves as an activator for MMP-1, -2 and -9, all of which are associated with arterial dysfunction. While this challenges the idea of the beneficial roles MMP-7 may play, it is hypothesized that the increase in MMP-7 may be in response to acute and/or chronic injury and stimulates repair and remodeling of tissues. More research is needed to better understand MMP-7 functions in relation to exercise-induced vascular outcomes.

In summary, these exploratory data highlight the potential of a single bout of exercise to improve the inflammatory profile. In fact, improvements in sRAGE and MMP-7 were associated with improved insulin-stimulated microvascular function. Future work is also needed to assess how acute exercise impacts the inflammatory profile. This will help not only

identify potential associations with improvements in vascular and metabolic insulin sensitivity but also a lowering of chronic disease risk.

REFERENCES

Arcaro Guido, Cretti Anna, Balzano Sara, Lechi Alessandro, Muggeo Michele, Bonora Enzo, & Bonadonna Riccardo C. (2002). Insulin Causes Endothelial Dysfunction in Humans. *Circulation*, *105*(5), 576–582. https://doi.org/10.1161/hc0502.103333

Barrett, E. J., Eggleston, E. M., Inyard, A. C., Wang, H., Li, G., Chai, W., & Liu, Z. (2009). The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia*, *52*(5), 752. https://doi.org/10.1007/s00125-009-1313-z

Barrett, Eugene J., & Liu, Z. (2013). The Endothelial Cell: An "Early Responder" in the Development of Insulin Resistance. *Reviews in Endocrine & Metabolic Disorders*, *14*(1), 21–27. https://doi.org/10.1007/s11154-012-9232-6

Barrett, Eugene J., Wang, H., Upchurch, C. T., & Liu, Z. (2011). Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. *American Journal of Physiology. Endocrinology and Metabolism*, *301*(2), E252-263. https://doi.org/10.1152/ajpendo.00186.2011

Beltz, N. M., Gibson, A. L., Janot, J. M., Kravitz, L., Mermier, C. M., & Dalleck, L. C. (2016). Graded Exercise Testing Protocols for the Determination of VO2max: Historical Perspectives, Progress, and Future Considerations. *Journal of Sports Medicine*, *2016*. https://doi.org/10.1155/2016/3968393

Boden, G., Song, W., Kresge, K., Mozzoli, M., & Cheung, P. (2008). Effects of hyperinsulinemia on hepatic metalloproteinases and their tissue inhibitors. *American Journal of Physiology - Endocrinology and Metabolism*, *295*(3), E692–E697. https://doi.org/10.1152/ajpendo.90370.2008

Borg, G. (1982). Psychophysical Bases of Perceived Exertion. *Medicine and Science in Sports and Exercise*, *14*, 377–381. https://doi.org/10.1249/00005768-198205000-00012

Campia, U., Sullivan, G., Bryant, M. B., Waclawiw, M. A., Quon, M. J., & Panza, J. A. (2004). Insulin impairs endothelium-dependent vasodilation independent of insulin sensitivity or lipid profile. *American Journal of Physiology. Heart and Circulatory Physiology*, *286*(1), H76-82. https://doi.org/10.1152/ajpheart.00539.2003

Carrick-Ranson, G., Spinale, F. G., Bhella, P. S., Sarma, S., Shibata, S., Fujimoto, N., Hastings, J. L., & Levine, B. D. (2019). Plasma matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs and aging and lifelong exercise adaptations in ventricular and arterial stiffness. *Experimental Gerontology*, *123*, 36–44. https://doi.org/10.1016/j.exger.2019.05.004

CDC. (2020, February 27). *Obesity is a Common, Serious, and Costly Disease*. Centers for Disease Control and Prevention. https://www.cdc.gov/obesity/data/adult.html

Chavey, C., Mari, B., Monthouel, M.-N., Bonnafous, S., Anglard, P., Obberghen, E. V., & Tartare-Deckert, S. (2003). Matrix Metalloproteinases Are Differentially Expressed in Adipose Tissue during Obesity and Modulate Adipocyte Differentiation. *Journal of Biological Chemistry*, *278*(14), 11888–11896. https://doi.org/10.1074/jbc.M209196200

Choi, K. M., Han, K. A., Ahn, H. J., Hwang, S. Y., Hong, H. C., Choi, H. Y., Yang, S. J., Yoo, H. J., Baik, S. H., Choi, D. S., & Min, K. W. (2012). Effects of exercise on sRAGE levels and cardiometabolic risk factors in patients with type 2 diabetes: A randomized controlled trial. *The Journal of Clinical Endocrinology and Metabolism*, *97*(10), 3751–3758. https://doi.org/10.1210/jc.2012-1951

Clerk, L. H., Vincent, M. A., Jahn, L. A., Liu, Z., Lindner, J. R., & Barrett, E. J. (2006). Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes*, *55*(5), 1436–1442.

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. *The American Journal of Physiology*, *237*(3), E214-223. https://doi.org/10.1152/ajpendo.1979.237.3.E214

DeFronzo, Ralph A., Sherwin, R. S., & Kraemer, N. (1987). Effect of Physical Training on Insulin Action in Obesity. *Diabetes*, *36*(12), 1379–1385. https://doi.org/10.2337/diab.36.12.1379

Deo, P., Keogh, J. B., Price, N. J., & Clifton, P. M. (2017). Effects of Weight Loss on Advanced Glycation End Products in Subjects with and without Diabetes: A Preliminary Report. *International Journal of Environmental Research and Public Health*, *14*(12). https://doi.org/10.3390/ijerph14121553

Devlin, J. T., Hirshman, M., Horton, E. D., & Horton, E. S. (1987). Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. *Diabetes*, *36*(4), 434–439. https://doi.org/10.2337/diab.36.4.434

Donley, D. A., Fournier, S. B., Reger, B. L., DeVallance, E., Bonner, D. E., Olfert, I. M., Frisbee, J. C., & Chantler, P. D. (2014). Aerobic exercise training reduces arterial stiffness in metabolic syndrome. *Journal of Applied Physiology*, *116*(11), 1396–1404. https://doi.org/10.1152/japplphysiol.00151.2014

Eggleston, E. M., Jahn, L. A., & Barrett, E. J. (2007). Hyperinsulinemia Rapidly Increases Human Muscle Microvascular Perfusion but Fails to Increase Muscle Insulin Clearance: Evidence That a Saturable Process Mediates Muscle Insulin Uptake. *Diabetes*, *56*(12), 2958–2963. https://doi.org/10.2337/db07-0670

Emdin, M., Gastaldelli, A., Muscelli, E., Macerata, A., Natali, A., Camastra, S., & Ferrannini, E. (2001). Hyperinsulinemia and autonomic nervous system dysfunction in obesity: Effects of weight loss. *Circulation*, *103*(4), 513–519. https://doi.org/10.1161/01.cir.103.4.513

Evert, A. B., Boucher, J. L., Cypress, M., Dunbar, S. A., Franz, M. J., Mayer-Davis, E. J., Neumiller, J. J., Nwankwo, R., Verdi, C. L., Urbanski, P., & Yancy, W. S. (2013). Nutrition Therapy Recommendations for the Management of Adults With Diabetes. *Diabetes Care*, *36*(11), 3821–3842. https://doi.org/10.2337/dc13-2042

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*(6), 499–502.

Fuller, K. N. Z., Valentine, R. J., Miranda, E. R., Kumar, P., Prabhakar, B. S., & Haus, J. M. (2018). A single high-fat meal alters human soluble RAGE profiles and PBMC RAGE expression with no effect of prior aerobic exercise. *Physiological Reports*, *6*(14). https://doi.org/10.14814/phy2.13811

Goodpaster, B. H., & Sparks, L. M. (2017). Metabolic Flexibility in Health and Disease. *Cell Metabolism*, *25*(5), 1027–1036. https://doi.org/10.1016/j.cmet.2017.04.015

Green, D. J., Hopman, M. T. E., Padilla, J., Laughlin, M. H., & Thijssen, D. H. J. (2017). Vascular Adaptation to Exercise in Humans: Role of Hemodynamic Stimuli. *Physiological Reviews*, *97*(2), 495–528. https://doi.org/10.1152/physrev.00014.2016

Guo, J., Dhaliwall, J. K., Chan, K. K., Ghanim, H., Al Koudsi, N., Lam, L., Madadi, G., Dandona, P., Giacca, A., & Bendeck, M. P. (2013). In vivo effect of insulin to decrease matrix metalloproteinase-2 and -9 activity after arterial injury. *Journal of Vascular Research*, *50*(4), 279–288. https://doi.org/10.1159/000351611

Harris, J. A., & Benedict, F. G. (1918). A Biometric Study of Human Basal Metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, *4*(12), 370–373.

Harris, R. A., Nishiyama, S. K., Wray, D. W., & Richardson, R. S. (2010). ULTRASOUND ASSESSMENT OF FLOW-MEDIATED DILATION: A TUTORIAL. *Hypertension*, *55*(5), 1075–1085. https://doi.org/10.1161/HYPERTENSIONAHA.110.150821

Hwang, M.-H., & Lee, S. (2016). Insulin resistance: Vascular function and exercise. *Integrative Medicine Research*, *5*(3), 198–203. https://doi.org/10.1016/j.imr.2016.06.001

Inyard, A. C., Clerk, L. H., Vincent, M. A., & Barrett, E. J. (2007). Contraction Stimulates Nitric Oxide–Independent Microvascular Recruitment and Increases Muscle Insulin Uptake. *Diabetes*, *56*(9), 2194–2200. https://doi.org/10.2337/db07-0020

Jahn, L. A., Hartline, L., Rao, N., Logan, B., Kim, J. J., Aylor, K., Gan, L.-M., Westergren, H. U., & Barrett, E. J. (2016). Insulin Enhances Endothelial Function Throughout the Arterial Tree in Healthy But Not Metabolic Syndrome Subjects. *The Journal of Clinical Endocrinology & Metabolism*, *101*(3), 1198–1206. https://doi.org/10.1210/jc.2015-3293

Jaoude, J., & Koh, Y. (2016). Matrix metalloproteinases in exercise and obesity. *Vascular Health and Risk Management*, *12*, 287–295. https://doi.org/10.2147/VHRM.S103877

Keske, M. A., Barrett, E. J., Lindner, J. R., Richter, E. A., Liu, Z., McConell, G. K., Askew, C. D., Serné, E. H., Premilovac, D., Richards, S. M., Rattigan, S., & Eringa, E. C. (2020). Perfusion controls muscle glucose uptake by altering the rate of glucose dispersion in vivo. *American Journal of Physiology-Endocrinology and Metabolism*, *318*(3), E311–E312. https://doi.org/10.1152/ajpendo.00430.2019

Kotani, K., Caccavello, R., Sakane, N., Yamada, T., Taniguchi, N., & Gugliucci, A. (2011). Influence of Physical Activity Intervention on Circulating Soluble Receptor for Advanced Glycation end Products in Elderly Subjects. *Journal of Clinical Medicine Research*, *3*(5), 252–257. https://doi.org/10.4021/jocmr704w

Macías-Cervantes, M. H., Rodríguez-Soto, J. M. D., Uribarri, J., Díaz-Cisneros, F. J., Cai, W., & Garay-Sevilla, M. E. (2015). Effect of an advanced glycation end product-restricted diet and exercise on metabolic parameters in adult overweight men. *Nutrition (Burbank, Los Angeles County, Calif.)*, *31*(3), 446–451. https://doi.org/10.1016/j.nut.2014.10.004

Madden, K. M., Lockhart, C., Cuff, D., Potter, T. F., & Meneilly, G. S. (2009). Short-term aerobic exercise reduces arterial stiffness in older adults with type 2 diabetes, hypertension, and hypercholesterolemia. *Diabetes Care*, *32*(8), 1531–1535. https://doi.org/10.2337/dc09-0149

Malin, S. K., del Rincon, J. P., Huang, H., & Kirwan, J. P. (2014). Exercise-induced lowering of fetuin-A may increase hepatic insulin sensitivity. *Medicine and Science in Sports and Exercise*, *46*(11), 2085–2090. https://doi.org/10.1249/MSS.0000000000000338

McClatchey, P. M., Williams, I. M., Xu, Z., Mignemi, N. A., Hughey, C. C., McGuinness, O. P., Beckman, J. A., & Wasserman, D. H. (2019). Perfusion controls muscle glucose uptake by altering the rate of glucose dispersion in vivo. *American Journal of Physiology-Endocrinology and Metabolism*, *317*(6), E1022–E1036. https://doi.org/10.1152/ajpendo.00260.2019

Miranda, E. R., Mey, J. T., Blackburn, B. K., Farabi, S. S., Quinn, L., & Haus, J. M. (2016). Production of Soluble Receptor for Advanced Glycation End-Products Following Acute Aerobic Exercise is Gender Specific. *The FASEB Journal*, *30*(1_supplement), 734.8-734.8. https://doi.org/10.1096/fasebj.30.1_supplement.734.8

Muniyappa, R., & Yavuz, S. (2013). Metabolic actions of angiotensin II and insulin: A microvascular endothelial balancing act. *Molecular and Cellular Endocrinology*, *378*(1–2), 59–69. https://doi.org/10.1016/j.mce.2012.05.017

Payne, G. W. (2006). Effect of Inflammation on the Aging Microcirculation: Impact on Skeletal Muscle Blood Flow Control. *Microcirculation*, *13*(4), 343–352. https://doi.org/10.1080/10739680600618918

Penedo, F. J., & Dahn, J. R. (2005). Exercise and well-being: A review of mental and physical health benefits associated with physical activity. *Current Opinion in Psychiatry*, *18*(2), 189.

Perkins, J. M., Joy, N. G., Tate, D. B., & Davis, S. N. (2015). Acute effects of hyperinsulinemia and hyperglycemia on vascular inflammatory biomarkers and endothelial function in overweight and obese humans. *American Journal of Physiology. Endocrinology and Metabolism*, *309*(2), E168-176. https://doi.org/10.1152/ajpendo.00064.2015

Prasad, K. (2019). Is there any evidence that AGE/sRAGE is a universal biomarker/risk marker for diseases? *Molecular and Cellular Biochemistry*, *451*(1–2), 139–144. https://doi.org/10.1007/s11010-018-3400-2

Ress, C., Tschoner, A., Ciardi, C., Laimer, M. W., Engl, J. W., Sturm, W., Weiss, H., Tilg, H., Ebenbichler, C. F., Patsch, J. R., & Kaser, S. (2010). Influence of significant weight loss on serum matrix metalloproteinase (MMP)-7 levels. *European Cytokine Network*, *21*(1), 65–70. https://doi.org/10.1684/ecn.2009.0177

Riuzzi, F., Sorci, G., Sagheddu, R., Chiappalupi, S., Salvadori, L., & Donato, R. (2018). RAGE in the pathophysiology of skeletal muscle. *Journal of Cachexia, Sarcopenia and Muscle*, *9*(7), 1213–1234. https://doi.org/10.1002/jcsm.12350

Roberts, C. K., Hevener, A. L., & Barnard, R. J. (2013). Metabolic Syndrome and Insulin Resistance: Underlying Causes and Modification by Exercise Training. *Comprehensive Physiology*, *3*(1), 1–58. https://doi.org/10.1002/cphy.c110062

Sakatani, S., Yamada, K., Homma, C., Munesue, S., Yamamoto, Y., Yamamoto, H., & Hirase, H. (2009). Deletion of RAGE causes hyperactivity and increased sensitivity to auditory stimuli in mice. *PloS One*, *4*(12), e8309. https://doi.org/10.1371/journal.pone.0008309

Schenk, S., & Horowitz, J. F. (2007). Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid–induced insulin resistance. *The Journal of Clinical Investigation*, *117*(6), 1690–1698. https://doi.org/10.1172/JCI30566

Schmidt, A. M., Hori, O., Brett, J., Yan, S. D., Wautier, J. L., & Stern, D. (1994). Cellular receptors for advanced glycation end products. Implications for induction of oxidant

stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology*, *14*(10), 1521–1528. https://doi.org/10.1161/01.atv.14.10.1521

Schrauwen, P., van Marken Lichtenbelt, W. D., Saris, W. H., & Westerterp, K. R. (1997). Role of glycogen-lowering exercise in the change of fat oxidation in response to a high-fat diet. *The American Journal of Physiology*, *273*(3 Pt 1), E623-629. https://doi.org/10.1152/ajpendo.1997.273.3.E623

Shojaee-Moradie, F., Baynes, K. C. R., Pentecost, C., Bell, J. D., Thomas, E. L., Jackson, N. C., Stolinski, M., Whyte, M., Lovell, D., Bowes, S. B., Gibney, J., Jones, R. H., & Umpleby, A. M. (2007). Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia*, *50*(2), 404–413. https://doi.org/10.1007/s00125-006-0498-7

Sjøberg, K. A., Frøsig, C., Kjøbsted, R., Sylow, L., Kleinert, M., Betik, A. C., Shaw, C. S., Kiens, B., Wojtaszewski, J. F. P., Rattigan, S., Richter, E. A., & McConell, G. K. (2017). Exercise Increases Human Skeletal Muscle Insulin Sensitivity via Coordinated Increases in Microvascular Perfusion and Molecular Signaling. *Diabetes*, *66*(6), 1501–1510. https://doi.org/10.2337/db16-1327

Son, Y., Kim, K., Jeon, S., Kang, M., Lee, S., & Park, Y. (2017). Effect of Exercise Intervention on Flow-Mediated Dilation in Overweight and Obese Adults: Meta-Analysis. *International Journal of Vascular Medicine*, *2017*. https://doi.org/10.1155/2017/7532702

Tamminen, M., Westerbacka, J., Vehkavaara, S., & Yki-Järvinen, H. (2002). Insulin-Induced Decreases in Aortic Wave Reflection and Central Systolic Pressure Are Impaired in Type 2 Diabetes. *Diabetes Care*, *25*(12), 2314–2319. https://doi.org/10.2337/diacare.25.12.2314

Tayebjee, M. H., Lip, G. Y. H., Blann, A. D., & MacFadyen, R. J. (2005). Effects of age, gender, ethnicity, diurnal variation and exercise on circulating levels of matrix metalloproteinases (MMP)-2 and -9, and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMP)-1 and -2. *Thrombosis Research*, *115*(3), 205–210. https://doi.org/10.1016/j.thromres.2004.08.023

Thankamony, A., Capalbo, D., Marcovecchio, M. L., Sleigh, A., Jørgensen, S. W., Hill, N. R., Mooslehner, K., Yeo, G. S. H., Bluck, L., Juul, A., Vaag, A., & Dunger, D. B. (2014). Low Circulating Levels of IGF-1 in Healthy Adults Are Associated With Reduced β-Cell Function, Increased Intramyocellular Lipid, and Enhanced Fat Utilization During Fasting. *The Journal of Clinical Endocrinology & Metabolism*, *99*(6), 2198–2207. https://doi.org/10.1210/jc.2013-4542

Tjønna, A. E., Rognmo, Ø., Bye, A., Stølen, T. O., & Wisløff, U. (2011). Time Course of Endothelial Adaptation After Acute and Chronic Exercise in Patients With Metabolic

Syndrome. *The Journal of Strength & Conditioning Research*, *25*(9), 2552. https://doi.org/10.1519/JSC.0b013e3181fb4809

Wautier, J. L., Zoukourian, C., Chappey, O., Wautier, M. P., Guillausseau, P. J., Cao, R., Hori, O., Stern, D., & Schmidt, A. M. (1996). Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. *Journal of Clinical Investigation*, *97*(1), 238–243.

Yamagishi, S., Nakamura, K., & Imaizumi, T. (2005). Advanced glycation end products (AGEs) and diabetic vascular complications. *Current Diabetes Reviews*, *1*(1), 93–106. https://doi.org/10.2174/1573399052952631

Zhao, Y., Vanhoutte, P. M., & Leung, S. W. S. (2015). Vascular nitric oxide: Beyond eNOS. *Journal of Pharmacological Sciences*, *129*(2), 83–94. https://doi.org/10.1016/j.jphs.2015.09.002

TABLES

Table 1: Participant demographics.

N	11 (5M/6F)
Age (yrs)	50 ± 2.4
Body Composition	
Weight (kg)	100 ± 4.50
BMI (kg/m2)	34.4 ± 1.34
WC (cm)	112 ± 3.04
Total Fat (%)^	40.5 ± 2.36
Estimated VAT (kg)^	0.87 ± 0.05
FFM (kg)^	58.7 ± 3.66
Aerobic Fitness	
VO2max (ml/kg/min)	23.5 ± 1.70

^n=10. Data are mean ± SEM.

Table 2: Acute exercise session descriptives.

Energy expended (kcals)	397 ± 1.98
Energy Intake (kcals)	1640 ± 92.56
Total energy expended (%)	24.8 ± 1.31
VO2max (%)	69.6 ± 1.35
Duration (min)	55.5 ± 4.53
HR (bpm)	125 ± 4.42
%HRmax	76.7 ± 1.61
RPE (AU)	13.5 ± 0.34
RER	0.90 ± 0.01

Data are mean ± SEM.

Table 3: Predicted and actual food intake.

	Predicted	Actual	Δ	*P-Value*
Total Food Intake (kcals)	1644 ± 95.88	1640 ± 92.56	-4.2 ± 4.6	0.43
CHO (kcals)	904 ± 52.7	927 ± 54.5	22.8 ± 21.8	0.20
PRO (kcals)	247 ± 14.3	254 ± 30.0	7.0 ± 29	0.81
Fat (kcals)	493 ± 28.8	470 ± 39.8	-23.1 ± 24.3	0.27

Δ = Actual - Predicted. Data are mean ± SEM

Table 4: Effect of control and exercise on fasting and insulin-stimulated brachial artery diameter and endothelial function.

	Control		Exercise		*P*-Values		
	0min	120min	0 min	120min	Time	Condition	Interaction
Pre-Occlusion Diameter (mm)	4.22 ± 0.21	4.22 ± 0.17	4.15 ± 0.23	4.30 ± 0.21	0.97	0.26	0.03
Post-Occlusion Diameter (mm)	4.26 ± 0.22	4.30 ± 0.18	4.19 ± 0.23	4.35 ± 0.21	0.90	0.10	0.10
$FMD_{Allometric}$ (%)	6.71 ± 0.24	6.86 ± 0.30	6.84 ± 0.24	6.98 ± 2.24	0.82	0.67	0.64
Time to Peak (s)	31.6 ± 6.24	41.1 ± 7.53	28.9 ± 4.50	49.2 ± 10.1	0.66	0.10	0.26

Data are mean ± SEM.

Table 5: Effect of control and exercise on fasting and insulin-stimulated blood biochemistries.

	Control		Exercise		*P*-Values		
	0min	120min	0 min	120min	Time	Condition	Interaction
MMP-1 (ng/ml)	4.71 ± 1.07	4.36 ± 0.99	4.25 ± 0.88	3.66 ± 0.74	0.39	0.056	0.65
MMP-7 (ng/ml)	3.51 ± 0.39	3.55 ± 0.42	3.48 ± 0.45	3.41 ± 0.36	0.51	1.00	0.98

Data are mean ± SEM.

FIGURES

Figure 1: Effect of control and acute exercise on fasting and insulin-stimulated endothelial function. Data are mean ± SEM.

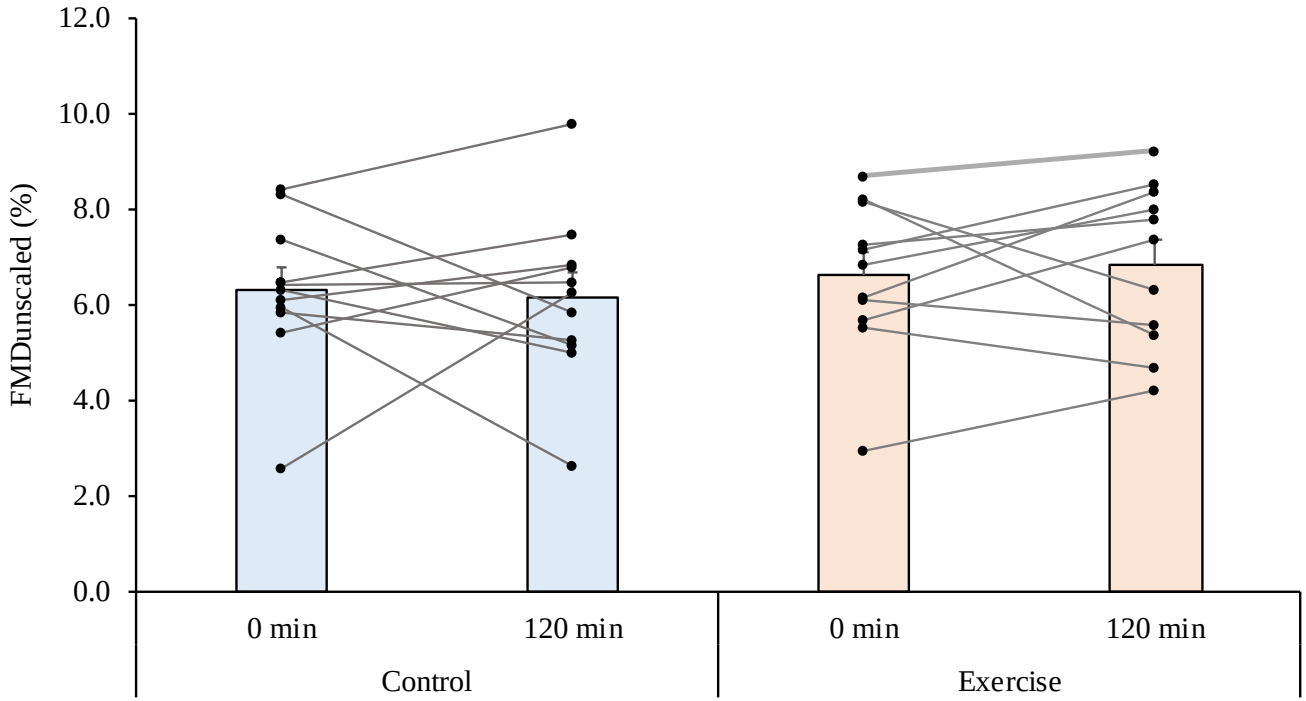


Figure 2: Effect of control and acute exercise on fasting and insulin-stimulated microvasculature responses. A) MFV, B) MBV and C) MBF. Data are mean ± SEM. Main effect of time: *P=0.006. Main effect of condition: †P=0.059.

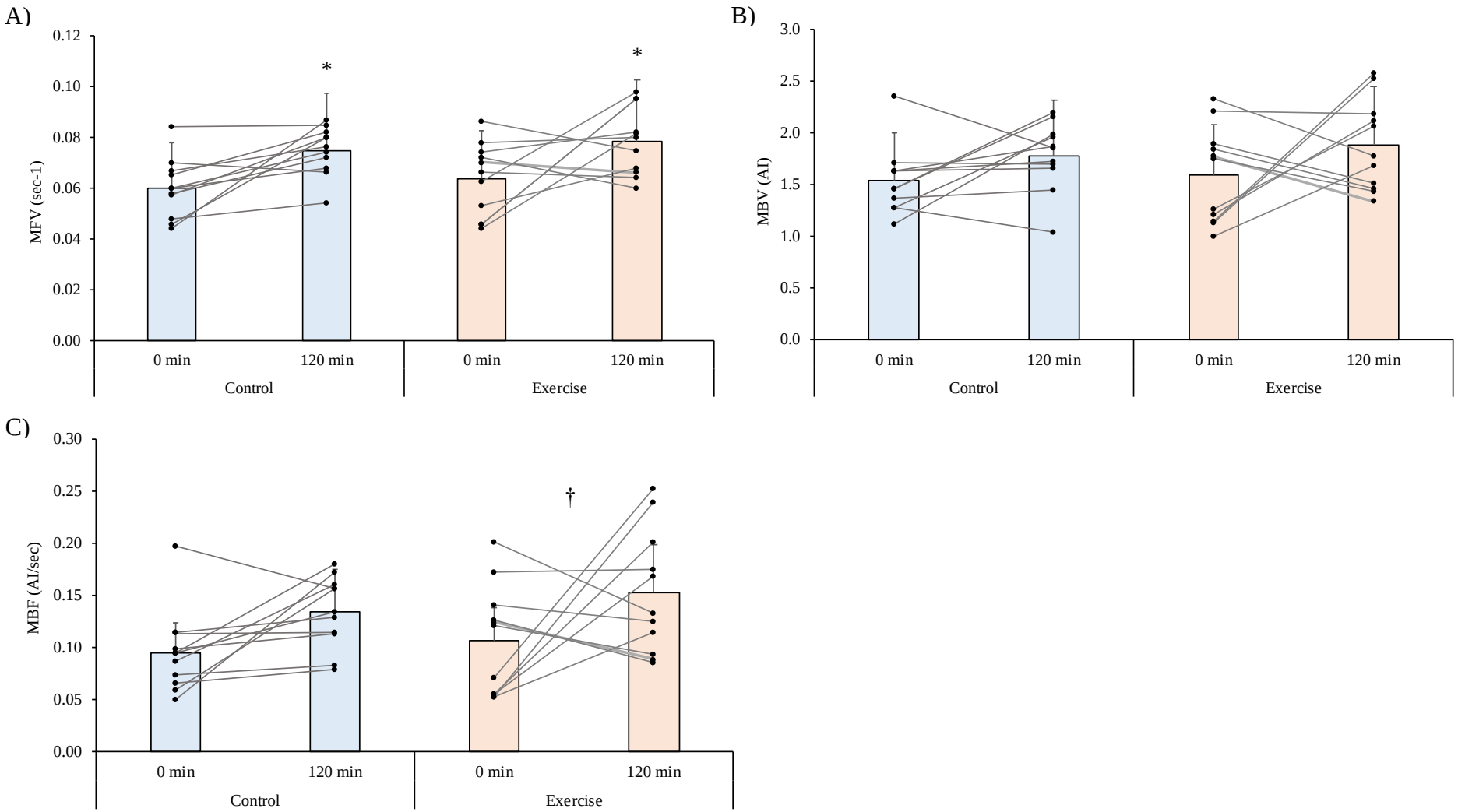


Figure 3: Effect of control or acute exercise on arterial stiffness (AIx_{75}). n=10. *P=0.065

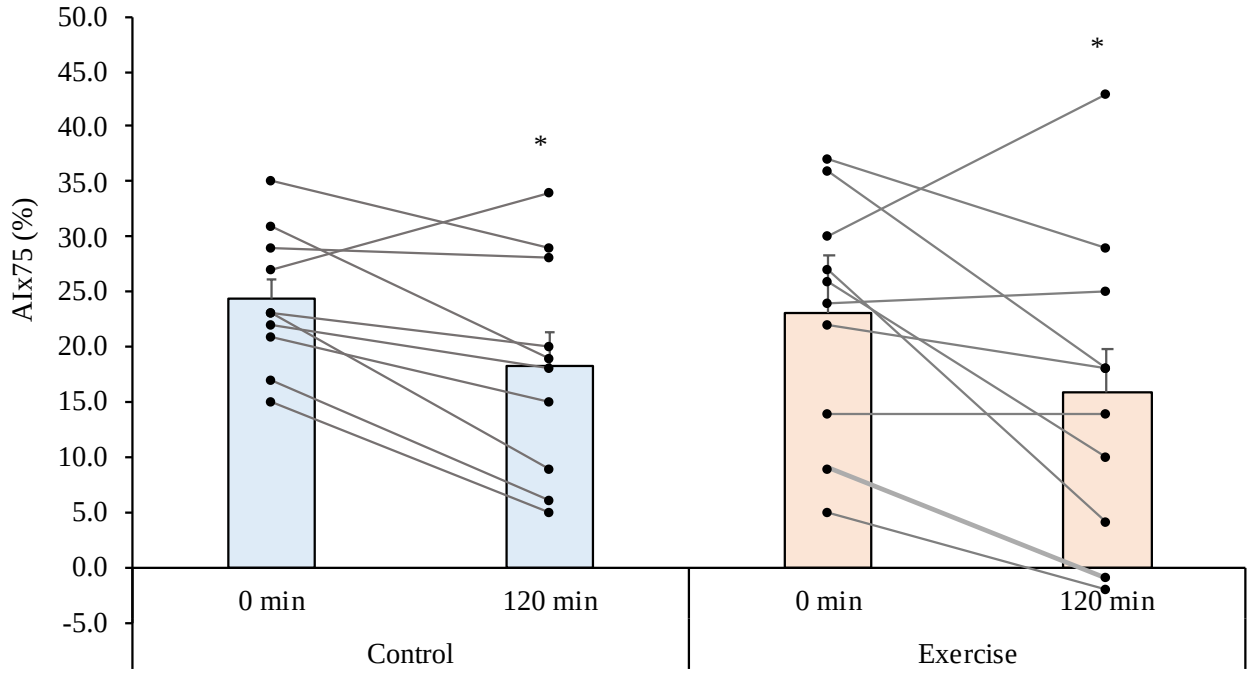


Figure 4: Effect of control and acute exercise metabolic insulin sensitivity. Data are mean ± SEM. *P=0.065

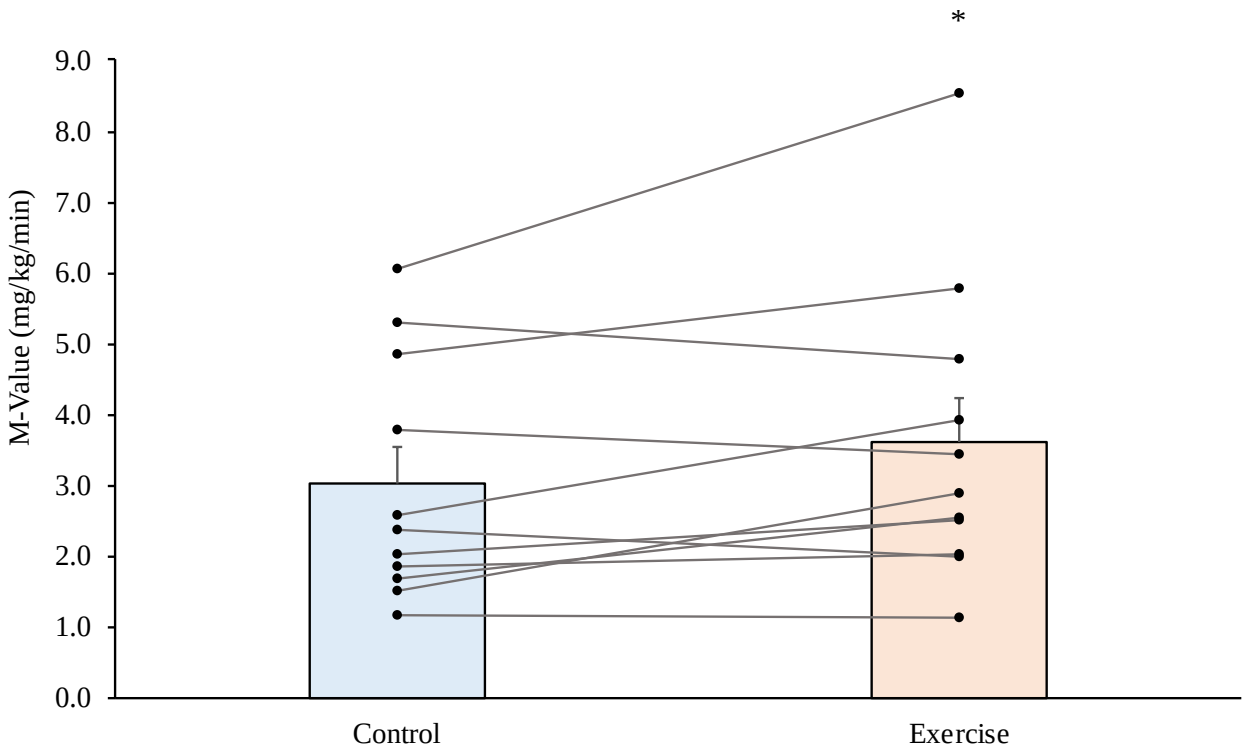


Figure 5: Effect of control and acute exercise on fasting fat oxidation. Data are mean ± SEM. n=10. *P=0.03.

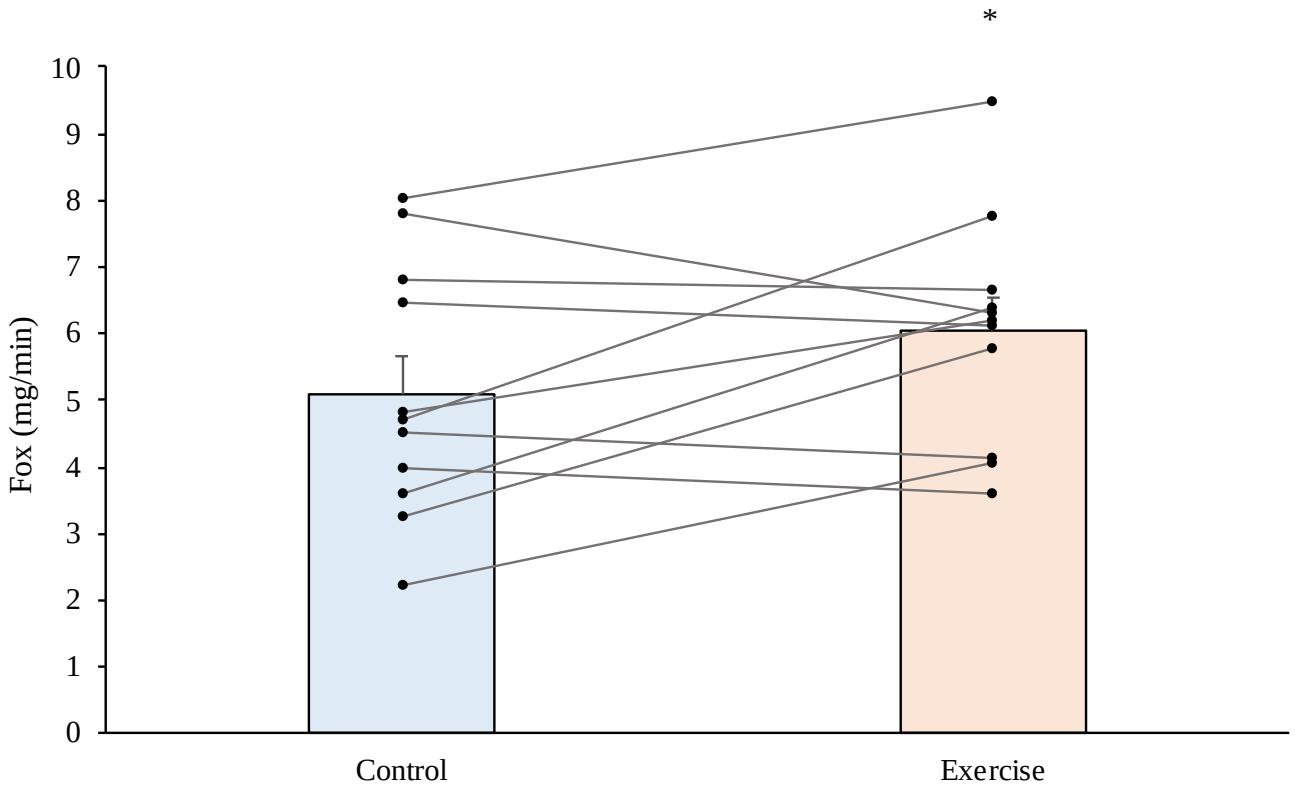


Figure 6: Effect of control and acute exercise on fasting and insulin stimulated carbohydrate oxidation. Data are mean ± SEM. n=10. Interaction: ‡*P*=0.02.

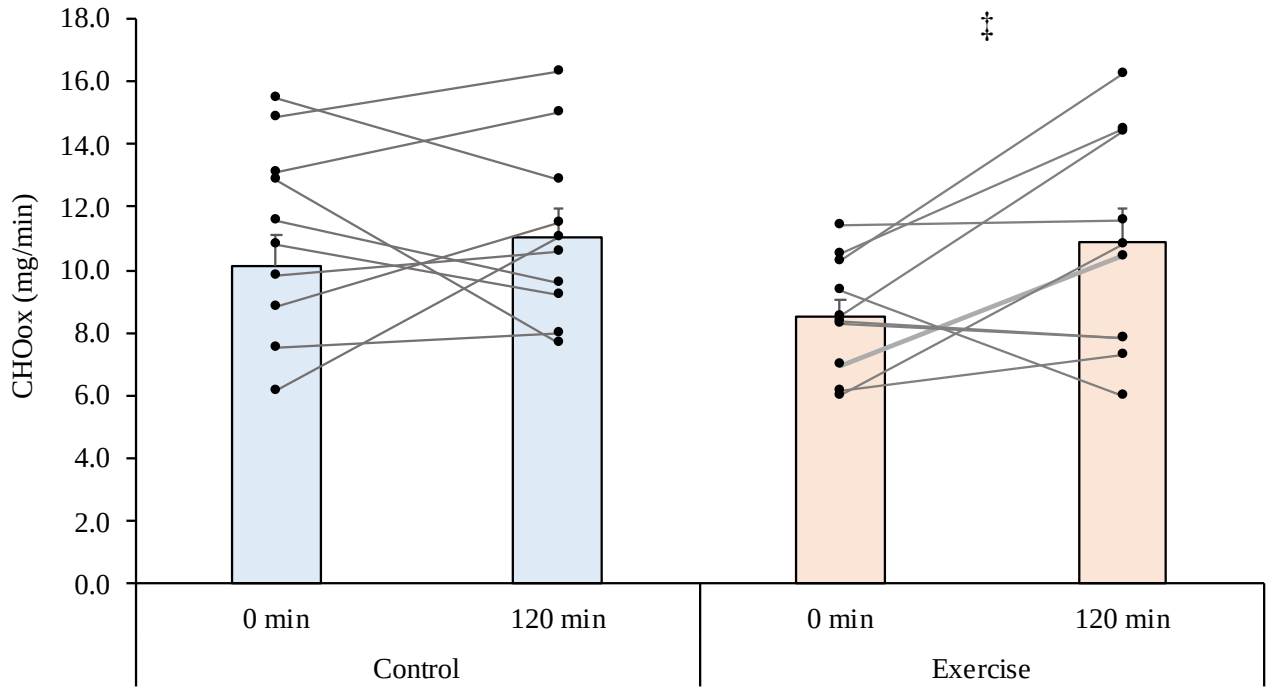


Figure 7: The association between the change (Δ) in metabolic insulin sensitivity and change (Δ) 120 min $FMD_{allometric}$ following exercise.

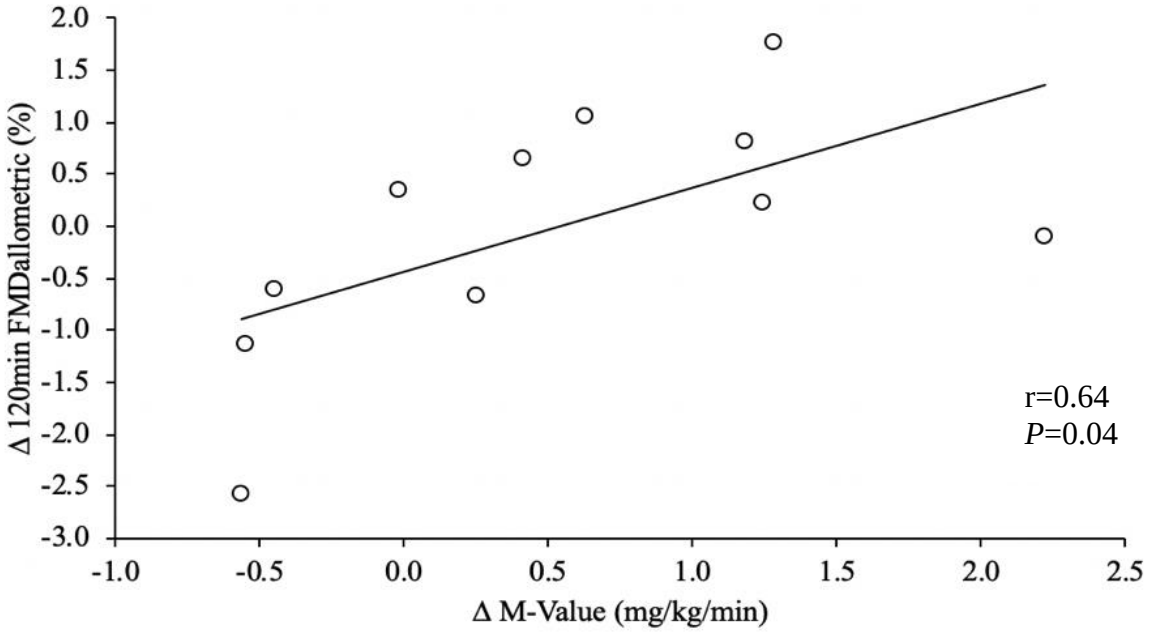
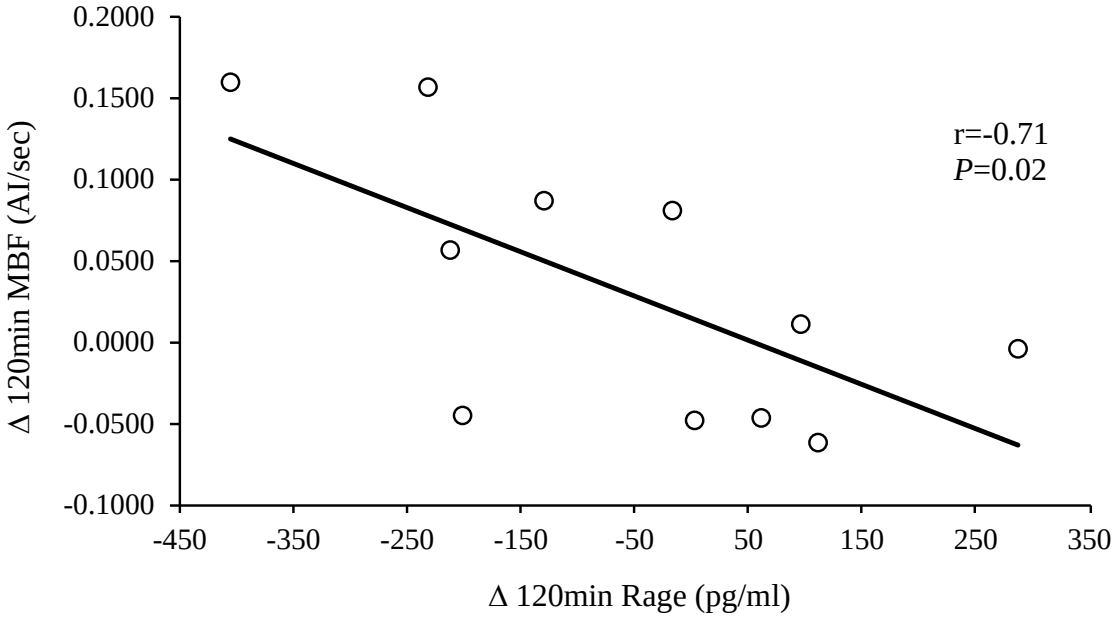


Figure 8: The association between the change (Δ) in 120 min RAGE and change (Δ) in 120 min MBF following exercise.



ABBREVIATIONS

AI: Acoustic intensity

AIx_{75}: Augmentation index standardized to 75 bpm

ANOVA: Analysis of variance

AU: Arbitrary unit

BMI: Body mass index

CEU: Contrast-enhanced ultrasound

cfPWV: Carotid-femoral pulse wave velocity

CHO: Carbohydrate

CHOox: Carbohydrate oxidation

CRP: C-reactive protein

CRU: Clinical Research Unit

CVD. Cardiovascular disease

DBP: Diastolic blood pressure

dl: Deciliter

DXA: Dual-energy X-ray absorptiometry

ECM: Extracellular matrix

ET-1: Endothelin-1

FMD: Flow-mediated dilation

Fox: Fat oxidation

FPG: Fasting plasma glucose

HbA1c: Hemoglobin A1c

hCG: Human chorionic gonadotropin

HDL: High-density lipoprotein

Hr: Hour

Kcal: Calorie

Kg: Kilogram

L: Liter

LDL: Low-density lipoprotein

M-value: Glucose metabolized

MBF: Microvascular blood flow

MBV: Microvascular blood volume

MFV: Microvascular flow velocity

mg: Milligram

Min: Minute

ml: Milliliter

mm: Millimeter

mmHg: Millimeters of mercury

MMP: Matrix metalloproteinase

mU: Milliunit

PRO: Protein

RAGE: Receptor for advanced glycation end productions

REE: Resting energy expenditure

RER: Respiratory exchange ratio

RMR: Resting metabolic rate

RPE: Rating of perceived exertion

RQ: Respiratory quotient

SBP: Systolic blood pressure

SEM: Standard error of the mean

T2D. Type 2 diabetes

TG: Triglycerides

VAT: Visceral adipose tissue

VO_2max: Maximal oxygen

WC: Waist Circumference