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Original Research

Acute Effects of Serial and Integrated Concurrent Exercise on Circulating microRNAs -126 and -222 in Sedentary Adults

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ABSTRACT

International Journal of Exercise Science 17(2): 1444-1460, 2024. The purpose of this study was to compare changes in circulating microRNAs -126 (c-miR-126) and -222 (c-miR-222) following acute serial concurrent exercise (SCE) and integrated concurrent exercise (ICE) sessions among young, sedentary adults. Ten males and 9 females completed the study procedures. For SCE, participants performed resistance exercise (RE) followed by aerobic exercise (AE), without mixing the two. For ICE, participants performed a brief bout of AE before each set of RE. Blood was collected before, immediately after (IP), and 1 h (1HR) after each exercise session. Expression of c-miR-126 significantly increased from baseline at IP (1.6-fold SCE, 2.1-fold ICE; p = .037) and 1HR (1.8-fold SCE, 1.7-fold ICE; p = .034) following both sessions, with no difference between the two sessions. Expression of c-miR-222 significantly increased from baseline at IP (1.7-fold SCE, 1.9-fold ICE; p = .024) and 1HR (2.0-fold SCE, 1.6-fold ICE; p = .038) following both sessions, with no difference between the two sessions. There were no differences in peak heart rate or average heart rate between the two workout sessions. Both SCE and ICE patterns appear equally effective at acutely increasing c-miR-126 and -222.

KEY WORDS: Combined, miR, inactive, aerobic, resistance

INTRODUCTION

MicroRNAs (miR) are ~20-22 nucleotide single-strand non-coding RNAs that regulate gene expression at the post-transcriptional level by inhibiting or degrading mRNA, preventing the translation of target proteins (19). MicroRNAs may be released into the circulation in response to a physiological stimulus and communicate with other cells (9). Circulating microRNAs (c-miR) are emerging as novel, non-invasive measures of physiological responses to physical

exercise (32). An intriguing biomarker and regulator of angiogenesis is microRNA-126 (miR-126) (37). This molecule is stimulated by endothelial shear stress (14, 37) and increases in the circulation following acute exercise (36). Another RNA molecule, miR-222, may be released into the circulation following acute exercise (3) and is essential to exercise-induced cardiac hypertrophy and cardioprotection (23, 35). There is evidence that exercise-induced expression of miR-126 and -222 may protect cardiovascular health (1).

The benefits of performing regular physical exercise are well established. Aerobic exercise (AE) may improve cardiorespiratory endurance (2) and blood pressure (33). Resistance exercise (RE) may improve muscular strength, muscular endurance, and muscle mass (8). Performing a combination of AE and RE may increase muscle capillarity above that observed when the modalities are performed independently (5). There is also evidence that combined training improves markers of cardiovascular health, such as blood pressure, more than either modality independently (5, 11, 12).

A common time-efficient method for training both RE and AE is to perform concurrent exercise (CE), where both AE and RE are performed in a single session. The most widely practiced and researched method of CE, referred to hereafter as serial CE (SCE), is characterized as performing one modality followed by the opposite modality, within the same session, without mixing the two (15). Integrated CE (ICE) is an alternate method of CE that has not been well characterized in the literature. A bout of ICE includes a brief period of AE preceding each set of RE (15). Davis et al. (16) demonstrated that ICE may result in superior cardiovascular adaptations compared to SCE in young athletes, including enhanced skeletal muscle blood flow and angiogenesis (15). Regular periods of AE throughout ICE bouts may also result in an augmented cardiac stimulus (16). Given their respective roles in angiogenesis (37) and exercise-induced cardiac hypertrophy (23), measurement of c-miR-126 and c-miR-222 may be useful for comparing the physiological responses to these different exercise patterns.

It is critical to perform regular AE and RE to optimize health and physical fitness (22). However, more research on varying patterns of CE is needed. To date, no research has been investigated comparing c-miR responses between SCE and ICE. Therefore, the purpose of this study was to compare changes in c-miR-126 and -222 following acute volume- and time-matched SCE and ICE sessions among young, sedentary adults.

METHODS

Participants

Participants were recruited from Texas Woman's University, The University of North Texas, and the surrounding vicinity via emails, flyers, classroom announcements, and social media. Healthy male and female participants: 1) were 21 to 34 yrs of age, 2) were not underweight or obese according to body mass index (BMI), 3) possessed a waist circumference of ≤ 102 cm (for men) or ≤ 88 cm (for women), and 4) were currently engaged in sedentary behavior (i.e., less than 1 h of structured exercise per week for the previous year). All participants were without: 1) a history, or current diagnosis of, cardiovascular, pulmonary, or metabolic disease, 2) current

or previous musculoskeletal injuries that limited lower body exercise or range-of-motion at the hip or knee, 3) current pregnancy, lactation, or a plan to become pregnant, 4) an irregular menstrual cycle within the previous 3 months (e.g., oligomenorrhea or amenorrhea), 5) current consumption of contraceptives (e.g., oral, injections, patches, intravaginal), nicotine, alcohol, medications that may have affected exercise responses (e.g., androgenic-anabolic steroids, statins, growth hormone, or glucocorticoids), nutritional supplements, or an atypical diet (e.g., ketogenic, intermittent fasting, vegetarian), and 6) an allergy to soy, wheat, almonds, or other tree nuts. The study was approved by the Institutional Review Board at Texas Woman's University and aligned with the ethical policies of the International Journal of Exercise Science (28). All participants were made aware of the risks and benefits associated with the study and signed an institutionally approved informed consent.

Nineteen participants (10 males, 9 females) completed the study procedures. Characteristics of the participants may be viewed in Table 1. Results of the participants' preliminary tests are presented in Table 2. All participants met the criteria for maximal exertion on the VO_{2max} test (22).

Table 1. Descriptive Characteristics of the Participants

	Males (n = 10)	Females (n = 9)
Age (years)	27.0 ± 4.4	24.0 ± 2.4
Height (cm)	179.6 ± 6.5	158.0 ± 5.0
Weight (kg)	81.6 ± 13.0	57.1 ± 7.0
BMI (kg/m^2)	25.2 ± 3.2	22.9 ± 2.4
Waist Circumference (cm)	91.6 ± 11.2	75.7 ± 6.8

Results are presented as mean ± SD; BMI = Body Mass Index, calculated as: Body Mass (kg) / Height (m)^2.

Table 2. Muscular and Cardiorespiratory Fitness Measures

	Males (n = 10)	Females (n = 9)
1RM Leg Press (kg)	225.9 ± 52.2	149.3 ± 18.6
1RM Seated Leg Curl (kg)	58.4 ± 8.8	34.7 ± 5.5
1RM Seated Leg Extension (kg)	81.1 ± 15.5	46.1 ± 6.2
VO_{2max} (L/min)	2.5 ± 0.4	1.6 ± 0.2
VO_{2max} (ml/kg/min)	31.0 ± 2.8	29.0 ± 3.9
HR_{max} (bpm)	182 ± 7	190 ± 12
Peak Power (W)	1013.5 ± 149.7	757.3 ± 140.4

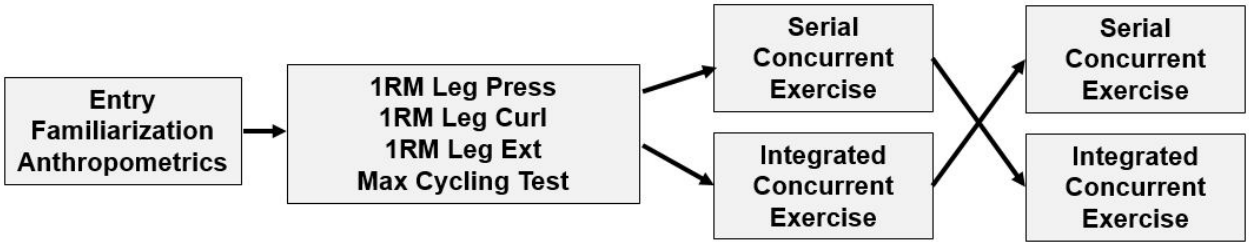
Results are presented as mean ± SD; 1RM = one-repetition maximum; VO_{2max} = maximal oxygen consumption; HR_{max} = peak heart rate achieved on a cycling VO_{2max} test, in beats per minute; W = peak power in watts achieved on a cycling VO_{2max} test.

Protocol

This study was structured as a randomized, counterbalanced, crossover design with repeated measures. Participants were recruited and screened for eligibility, performed baseline testing, and then underwent randomized assignment to either SCE or ICE. The experimental design is pictured in Figure 1. The participants performed the crossover condition approximately 4 to 8

weeks after completion of the first condition to allow for washout and standardization of the menstrual phase for females.

Figure 1. Experimental Design



During visit 1, participants were screened for eligibility and asked to complete a university-approved informed consent, medical questionnaire, and the Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) before pre-testing. Following satisfactory completion of study forms, the investigator familiarized the participant with the equipment and protocols to be used in the study. The participant performed several practice repetitions on the equipment during this time to assess the ability to perform the appropriate range-of-motion without musculoskeletal pain or complications.

After removal of footwear, body mass (kg) was assessed using a digital scale (Tanita BWB-800, Arlington Heights, IL, USA), height (m) was assessed using a stadiometer (Perspective Enterprises Model PE-AIM-101, Kalamazoo, MI, USA), and body mass index (kg/m^2) was calculated. Waist circumference was measured with a standard tape measure at the level of the iliac crest while the participant was relaxed and respirating normally. The measure was taken twice and an average was recorded, as long as the two measurements were within 5 mm.

For visits 2-4, participants: 1) arrived at the lab between 0700-1000 following a 10 h fast (water only, no caffeine), 2) slept their typical amount the previous night, 3) performed no strenuous physical activity for the previous 96 h, 4) and consumed no alcohol during the preceding 48 h. Participants recorded all food and fluid intake 24 h prior to this visit, which they were instructed to match for Visits 3 and 4. Euhydration was defined as urine specific gravity <1.020 which was assessed using a handheld refractometer (Schuco, Williston Park, NY, USA). If dehydrated, the participant was given 16 oz of water to drink. Participants consumed a light breakfast individualized as 4 kcal/kg of body mass (Clif Bar & Company, Emeryville, CA, USA; 250 kcal per 68 g serving; 18% fat, 68% carbohydrate, 14% protein). Water was allowed ad libitum throughout each session.

Visit 2 occurred 1 to 4 weeks following Visit 1. Thirty minutes after consuming breakfast, participants performed a standardized warm-up consisting of 5 min of free-cycling on a stationary bike (Star Trac SRBx, Vancouver, WA, USA) followed by light dynamic stretches (e.g., heel kicks, lunges, high knees, and high kicks). The participant then performed the 1RM protocol for the Leg Press exercise (Cybex Squat Press, Rosemont, IL, USA). The technique for each lift and procedures for 1RM testing followed the guidelines established by the National Strength and Conditioning Association (20). After the participant performed 5 to 10 repetitions with no

weight and proper form, participants were familiarized with the 40 beats/min cadence to be used in the workout protocols. To control for time during the workout sessions, all RE was performed at a pace of 20 reps/min (40 beats/min using a metronome app (Metronome Beats, Stonekick, London, UK); 1:1 concentric to eccentric ratio). Thereafter, approximately 50% of the participant's estimated 1RM was loaded. The participant then attempted 3 to 5 repetitions. Following a 2 min rest period, 10 to 20% more resistance was added, and the participant performed 2 to 3 additional repetitions. Following a 2 to 4 min rest, a similar percent resistance was added. The participant then attempted a 1RM. This pattern of conservatively adding resistance and resting between attempts continued until the weight could not successfully be lifted with proper form. A 1RM was obtained within 3 to 5 sets to prevent fatigue. A 5-min rest period followed the Leg Press exercise. Similar procedures were then followed with the Leg Curl (Cybex VR2 Seated Leg Curl, Rosemont, IL, USA) and Leg Extension (Cybex VR2 Leg Extension, Rosemont, IL, USA) exercises.

Following 20 min of rest, a maximal incremental test on a friction-braked cycle ergometer (Ergomedic 828E; Monark Exercise AB, Vansbro, Sweden) was performed. This study utilized a similar protocol from other research in which CE was used as an intervention (7). The seat was adjusted to the height of the participant's greater trochanter, so the extended leg while seated was approximately straight (i.e., 5-10° bend). The participant was affixed with a chest-worn monitor (Polar Electro T31, Lake Success, NY), nose clip, and one-way rebreathing valve connected to an automated gas mixer and analyzer (Parvomedics, Sandy, UT, USA). The gas analyzer was calibrated prior to each participant's test with room air and a known gas mixture (16% O_2, 4% CO_2), and the flowmeter was calibrated with a 3 L syringe. Heart rate (HR) and expired respiratory gasses were continually assessed. After 5 min of free cycling with no resistance, the participant began cycling at a 300 kgm/min (50 W) workload for 2 min while maintaining a cadence of 50 rpm. The workload increased by 150 kgm/min (25 W) every 2 min. The test ceased when the participant reached volitional exhaustion or was unable to maintain a cadence of 50 rpm. The peak power (PP, in kgm/min) was calculated using the following formula, where Wcom is the workload (kgm/min) of the last completed stage, t is the time (s) completed during the participant's final stage, and ΔW is the workload increment each stage (150 kgm/min) (7).

$$PP = Wcom + ((t/120) \times \Delta W)$$

At 2 min post-test, blood lactate was assessed from a fingertip capillary using a single-use lancet and automated analyzer (Lactate Scout, EKF Diagnostics, Penarth, UK). Criteria from the American College of Sports Medicine were used to determine if the participant reached maximal exertion (22). Maximal aerobic fitness was determined as the highest VO_2 value recorded during 30 sec intervals.

At least one week following the second study visit, the participant returned to the lab for the first exercise session. All females performed the exercise sessions during the early follicular phase (days 2-7 following the onset of menses) to standardize physiological responses. Following the preliminary procedures, the participant rested in a reclined position for 10 min.

A blood sample was then collected. Participants then consumed the same breakfast as consumed during the previous visits. Thirty min after consuming breakfast, the participant began the warm-up phase of the exercise session.

The exercise sessions are detailed in Table 3. The exercise modes and intensities were determined based on previous research, with primary goals of improving muscular strength, muscular hypertrophy, and aerobic fitness (20, 22). Both exercise sessions were matched for time and volume and differed only in the order of the exercises. During rest periods, participants sat on the RE apparatus or on the ergometer seat. If a participant was unable to lift the load for the desired number of repetitions during RE, the weight was reduced immediately by 10 to 20 lbs so that the set could be completed. This was recorded so that the same volume and intensity of exercise could be performed in the subsequent session.

Table 3. Concurrent Exercise Sessions

Serial Concurrent Exercise	Integrated Concurrent Exercise
Warm-Up -Cycle 5 min @ 40% PP -1 min rest -Leg Press 1 set of 5 reps @ 40% 1RM -1 min rest -Leg Press 1 set of 5 reps @ 60% 1RM -1 min rest	*Warm-Up* -Cycle 5 min @ 40% PP -1 min rest -Leg Press 1 set of 5 reps @ 40% 1RM -1 min rest -Leg Press 1 set of 5 reps @ 60% 1RM -1 min rest
Leg Press 3 sets of 10 reps @ 70% 1RM -2 min rest after each set	3 Rounds: -Cycle 2.25 min @ 60% PP -0.25 min rest -Leg Press 1 set of 10 reps @ 70% 1RM -2.25 min rest
Leg Curl 3 sets of 10 reps @ 70% 1RM -2 min rest after each set	3 Rounds: -Cycle 2.25 min @ 60% PP -0.25 min rest -Leg Curl 1 set of 10 reps @ 70% 1RM -2.25 min rest
Leg Extension 3 sets of 10 reps @ 70% 1RM -2 min rest after each set	3 Rounds: -Cycle 2.25 min @ 60% PP -0.25 min rest -Leg Extension 1 set of 10 reps @ 70% 1RM -2.25 min rest
-2.25 min additional rest	
Cycle 20.25 min @ 60% PP	

PP = peak power achieved during an incremental exercise test to exhaustion on a cycle ergometer; all resistance exercises were performed at a 40 bpm cadence (1:1 concentric to eccentric ratio); rep = repetition; cycling exercise was performed at a cadence of 45-55 rpm

Blood was collected during exercise sessions (Visits 3 and 4) 35 min before exercise (PRE), immediately after the exercise protocol (IP), and at 1 h (1HR) post-exercise into 10 mL EDTA vacutainers. These collection timepoints are similar to previous research on exercise and c-miRs (3, 36). For each blood collection time point, a small amount of blood (10 mL) was collected from

an antecubital vein with a sterile needle. The samples were centrifuged immediately (1500 g, 10 min, 4° C) and the plasma was stored at -80° C until analysis.

A small portion of whole blood for each collection time point was used to measure hematocrit and hemoglobin concentrations, and subsequently to correct data for plasma volume shifts that occur with exercise (17). Two hundred µl of plasma from each blood collection time point was used for miR extraction using a miRNeasy Serum/Plasma Advanced Kit (Qiagen, Germantown, MD, USA). As a method to normalize sample-to-sample variation during miR extraction, a synthetic C. elegans miR, cel-miR-39 spike-in control (Qiagen, Germantown, MD, USA) was added to each plasma sample. The procedures followed the manufacturer's guidelines. Extracted miRs were stored at -80°C until enough samples were available for analysis. Expression of miRs were determined using Taqman microRNA assays (ThermoFisher Scientific, Waltham, MA, USA) and quantitative polymerase chain reaction analysis (qPCR; (PCR System: QuantStudio 3, Applied Biosystems, San Francisco, CA, USA). The primer sequences were as follows: hsa-miR-126 was 002228 UCGUACCGUGAGUAAUAAUGCG; hsa-miR-222 was 002276 AGCUACAUCUGGCUACUGGGU; cel-miR-39 was 000200 UCACCGGGUGUAAAUCAGCUUG. The procedures followed the manufacturer's guidelines. All measures were performed in duplicate and coefficients of variation (CV) were calculated. Relative changes in c-miR-126 and -222 expression were calculated as fold-change from baseline using the comparative Ct (delta-delta-Ct) method (29), and using cel-miR-39 as the normalization control. The procedures were similar to previous studies on exercise and c-miRs (3, 4, 34, 36).

Statistical Analysis

An a priori power analysis was conducted using statistical software (G*power 3.1.9.2, Dusseldorf, Germany). With a desired level of power at .80, an alpha (α) level at .05, and a moderate effect size of .25 (f) (36), it was determined that 12 participants would be required. Data were analyzed using statistics software (IBM SPSS Statistics v.24, Armonk, NY, USA). Data were checked for normality and outliers. Statistical significance was set at $p \leq .05$. Circulating miRs -126 and -222 were analyzed using two-way (2 [treatment] x 3 [time]) ANOVAs with repeated measures. If significant interactions were found, a post-hoc Bonferroni test was used. Dependent t-tests were used to compare heart rate responses and time to completion between both workout sessions. Data were reported as means ± standard deviation. Males and females were analyzed together.

RESULTS

Descriptive characteristics of each exercise session are presented in Table 4. Heart rate data for two workouts sessions in the males and one workout session in the females were not included due to equipment malfunction. Time to completion data were not normally distributed; thus, a Wilcoxin Signed-Rank test was used. There were no differences in average HR ($p = .0590$), peak HR ($p = .2697$), or time to completion ($p = .8809$) between the exercise sessions.

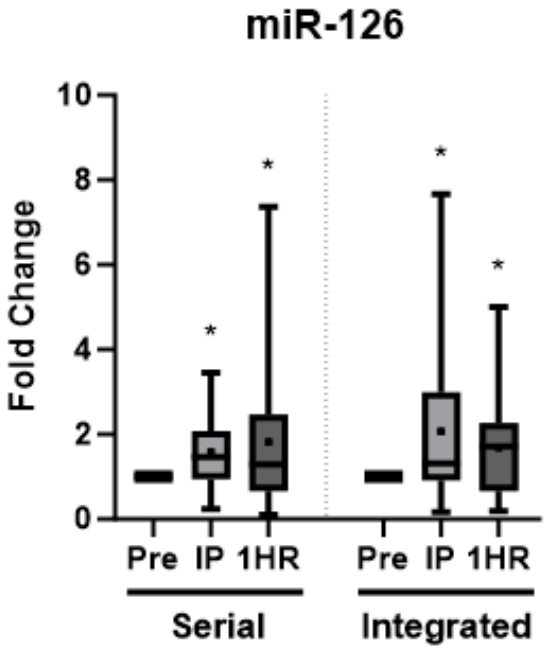
Table 4. Heart Rate and Time to Completion During Exercise Sessions

	Males (n = 10)				
	Serial		Integrated		
	$\bar{X}$ ± SD	CI	$\bar{X}$ ± SD	CI	
Average HR (bpm)	128 ± 14	120 – 137	133 ± 16	123 – 144	
Peak HR (bpm)	161 ± 13	152 – 170	164 ± 13	155 – 173	
Time to Complete (min)	52.9 ± 3.4	50.7 – 55.1	53.0 ± 3.0	51.1 – 54.8	
	Females (n = 9)				
	Serial		Integrated		
	$\bar{X}$ ± SD	CI	$\bar{X}$ ± SD	CI	
Average HR (bpm)	143 ± 10	136 – 149	140 ± 13	131 – 148	
Peak HR (bpm)	183 ± 10	176 – 190	180 ± 12	171 – 189	
Time to Complete (min)	53.7 ± 1.3	52.9 – 54.6	57.3 ± 1.8	54.1 – 60.4	
	Combined (n = 19)				
	Serial		Integrated		
	$\bar{X}$ ± SD	CI	$\bar{X}$ ± SD	CI	p-value
Average HR (bpm)	136 ± 14	129 – 142	136 ± 14	130 – 143	.0590
Peak HR (bpm)	172 ± 16	164 – 180	172 ± 15	165 – 179	.2697
Time to Complete (min)	53.3 ± 2.5	52.2 – 54.5	55.1 ± 4.8	53.1 – 57.1	.8809

$\bar{X}$ = mean; SD = standard deviation; CI = 95% confidence interval; bpm = beats per minute; min = minutes

Data for the miR-cel spike-in control were missing from two of the males' timepoints, so the data was replaced with the average of the other miR-cel values at those timepoints. The CV of all the miR-cel spike-in controls was 9.2%. The adjustments made for plasma volume shifts were overall minimal (0.99 ± 0.07). Circulating miR-126 responses are depicted in Figure 2. The CV of the miR-126 duplicate assays was 0.14 ± 0.20%. There were no significant effects of workout ($F_{1,16}$ = 0.399, p = .091, η^2p = 0.024) or workout x time interaction ($F_{2,32}$ = 0.701, p = .512, η^2p = 0.085). There was a significant effect of time ($F_{2,32}$ = 4.726, p = .026, η^2p = 0.387). Expression of c-miR-126 was significantly increased from baseline to immediately post-exercise (IP; p = .037) following both SCE (1.58 ± 0.84-fold change, 95% confidence interval [CI] 1.19 – 1.98-fold change) and ICE (2.07 ± 1.90-fold change, 95% CI 1.16 – 2.97-fold change). Expression also increased from baseline to 1 h post-exercise (1HR; p = .034) following both SCE (1.82 ± 1.86-fold change, 95% CI 0.94 – 2.70-fold change) and ICE (1.69 ± 1.30-fold change, 95% CI 1.07 – 2.30-fold change). There was no difference between the IP and 1HR timepoints.

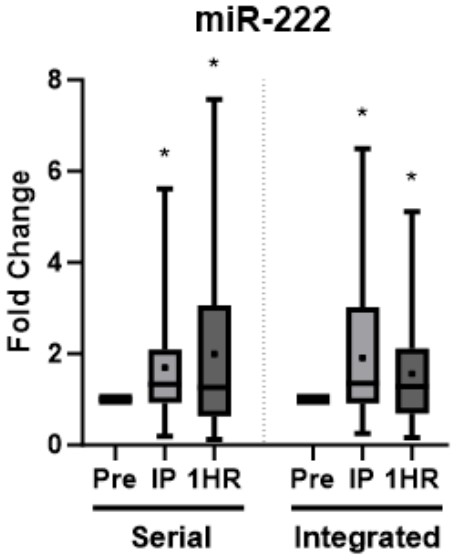
Figure 2. Circulating miR-126 Responses



Pre = Before exercise; IP = Immediately post-exercise; 1HR = 1-hour post-exercise; Values are presented as fold-change from the PRE timepoint; *indicates statistically significant increase ($p \leq .05$) from the PRE timepoint; statistical analyses were performed on males and females combined; The gray box denotes 25% to 75% percentile confidence intervals, error bars reflect max and min values, the horizontal bars denote statistical median, and the dots in the bars denote statistical mean.

Circulating miR-222 responses are depicted in Figure 3. The CV of the miR-222 duplicate assays was 0.15 ± 0.30%. There were no significant effects of workout ($F_{1,16} = 0.9$, $p = .768$, $\eta^2p = 0.006$) or workout x time interaction ($F_{2,32} = 0.435$, $p = .655$, $\eta^2p = 0.055$). There was a significant effect of time ($F_{2,32} = 6.007$, $p = .012$, $\eta^2p = 0.445$). Expression of c-miR-222 was significantly increased from baseline to IP ($p = .024$) following both SCE (1.70 ± 1.23-fold change, 95% CI 1.12 – 2.28-fold change) and ICE (1.91 ± 1.56-fold change, 95% CI 1.16 – 2.65-fold change). Expression also increased from baseline to 1HR ($p = .038$) following both SCE (2.00 ± 1.97-fold change, 95% CI 1.06 – 2.93-fold change) and ICE (1.56 ± 1.20-fold change, 95% CI 0.99 – 2.13-fold change). There was no difference between the IP and 1HR timepoints.

Figure 3. Circulating miR-222 Responses



Pre = Before exercise; IP = Immediately post-exercise; 1HR = 1-hour post-exercise; Values are presented as fold-change from the PRE timepoint; *indicates statistically significant increase ($p \leq .05$) from the PRE timepoint; statistical analyses were performed on males and females combined; The gray box denotes 25% to 75% percentile confidence intervals, error bars reflect max and min values, the horizontal bars denote statistical median, and the dots in the bars denote statistical mean.

In an unstructured interview after completion of the study, participants were asked which workout session they preferred. Six participants (32%) preferred the SCE pattern, ten participants (53%) preferred the ICE pattern, and three participants (16%) had no preference. The participants who preferred the SCE pattern cited the simplicity and lower perceived effort compared to the ICE pattern as reasons for their preference. The participants who preferred the ICE pattern cited the higher perceived challenge and aversion to the 20.25 min cycling protocol in the SCE pattern as reasons for their preference.

DISCUSSION

Physical exercise is one of the most effective strategies for combating adverse cardiovascular conditions (11). However, the most efficient exercise pattern to improve cardiovascular health continues to be investigated. While many researchers have examined the SCE pattern, ICE has not been extensively studied. Circulating miRs are emerging as novel, non-invasive measures of physiological responses to physical exercise (32), and exercise-induced expression of miR-126 and -222 may protect cardiovascular health in individuals with chronic disease (1). The primary finding of this study was that acute volume- and time-matched SCE and ICE sessions elicited similar increases in c-miRs -126 and -222 in young, sedentary adults.

No statistically significant differences in HR were observed between the exercise patterns. The average HR during both the SCE and ICE sessions was 136 bpm. Using the peak heart rates from the cycling test, the values corresponded to an average intensity of 73% HR_{max}. Therefore, the

workouts were of moderate to vigorous intensity (22). Comparable intensities have been reported in another study with similar protocols (21). The peak HR achieved during both the SCE and ICE sessions was 172 bpm, which corresponds to an intensity of 92% HR_{max}. Therefore, near maximal intensity was reached at least briefly in both exercise sessions (22). This may be noteworthy for clinical populations for which vigorous-intensity exercise is contraindicated (22). There were no significant differences in total exercise time between the workout sessions (53 min for SCE, 55 min for ICE). Overall, both exercise sessions met professional recommendations for cardiorespiratory exercise volume and intensity (22).

MicroRNA-126 expression increases in human endothelial cells exposed to shear stress (27), which may result from increased blood flow during exercise (29). MicroRNA-126 promotes angiogenesis by blocking sprout-related protein 1 and phospho-inositol-3 kinase regulatory subunit 2, downstream inhibitors of vascular endothelial growth factor signaling (18). In the present study, both SCE and ICE led to a significant in-crease in c-miR-126 expression post-exercise, potentially due to exercise-induced hyperemia. Wahl et al. (36) reported that maximal-effort sprint interval training (4 x 30 sec) led to an approximate 2.2-fold increase in c-miR-126 immediately post-exercise in trained males. Also, Uhlemann et al. (34) reported a 2.1-fold increase in c-miR-126 after a maximal incremental cycling test. In the same study, a 4.6-fold increase in c-miR-126 after 4 h of cycling and a 3.4-fold increase after a marathon run was observed, but these values may be inflated, as they were not adjusted for plasma volume shift (34). Changes in c-miR-126 appeared slightly less in the present study, which may be due to the sedentary status of the participants. There was a 1.6-fold increase in c-miR-126 immediately post-exercise and a 1.8-fold increase 1-hour post-exercise after SCE, and a 2.1-fold increase immediately post-exercise and 1.7-fold increase 1-hour post-exercise after ICE. Zhou et al. (40) reported no significant changes in c-miR-126 after 60 min of cycling at 70% VO_{2max}. In another study, cycling for 2 h at 55% PP (a similar intensity used in the present study) led to a 1.9-fold increase in c-miR-126 in trained males, which was not statistically significant (36). Sapp et al. (31) also reported no significant change in c-miR-126 after moder-ate-intensity (60% PP) continuous cycling, but c-miR-126 significantly increased approximately 2-fold after high-intensity interval cycling (3-min intervals at 85% PP interspersed with 4-min intervals at 40% PP), which matched the continuous cycling for time and workload. Exercise intensity may therefore be the most significant contributor to changes in c-miR-126. However, contrary to findings from Wahl et al. (36) and Sapp et al. (31) after performing moderate-intensity aerobic exercise, the increase in c-miR-126 reached statistical significance in the present study. It may be that combining RE and AE elicits a more consistent increase in c-miR-126 compared to AE alone. Uhlemann et al. (34) reported no change in c-miR-126 following only eccentric RE, so RE alone may not significantly influence c-miR-126. There were no significant differences in c-miR-126 between SCE and ICE, so exercise pattern may not have an effect on this biomarker. Although the study was not powered to do so, an exploratory statistical analysis was performed comparing the c-miR-126 responses of males and females, and no significant differences were observed.

The precise mechanisms behind miR-222 expression are still under investigation; however, it is upregulated in response to physical exercise, and increases cardiomyocyte growth and myosin

heavy chain α/β ratio by targeting cell-cycle inhibitor p27, homeodomain interacting protein kinases-1 and -2, and transcriptional repressor HMBOX1 (8). MicroRNA-222 may be critical to exercise-induced cardiac growth (23), protection of the heart from ischemia-reperfusion injury (8), and protection of pressure-overloaded hearts from fibrosis (35). In the present study, a significant increase in c-miR-222 was observed following both SCE and ICE, potentially due to exercise-induced cardiac stimulation. There was a 1.7-fold increase in c-miR-222 immediately post-exercise and a 2-fold in-crease 1-hour post-exercise after SCE, and a 1.9-fold increase immediately post-exercise and 1.6-fold increase 1-hour post-exercise after ICE. Baggish et al. (3) reported that c-miR-222 increased 2.46-fold immediately after a maximal incremental exercise test on a cycle ergometer in young male athletes. Ramos et al. (30) reported a 2 to 3-fold increase in c-miR-222 following treadmill runs between 6 mph and maximum speed in healthy males, with a greater increase following 60 minutes of treadmill running com-pared to 30 minutes of running. D'Souza et al. (13) reported a 3-fold increase in c-miR-222 following ten 60-sec intervals of cycling at 100% PP interspersed with 75-sec intervals of rest. The authors of these studies did not mention control for plasma volume shifts in the protocols, so the reported concentrations may be inflated. It is also possible that sedentary individuals, such as in the present study, experience less of an increase in this miR. Zhou et al. (40) reported no significant changes in c-miR-222 after a maximal cycling test or 60 min of cycling at 70% VO_{2max} in male college students. Liu et al. (23) reported a 1.8-fold in-crease in c-miR-222, similar to the present study, after a maximal incremental exercise test on a cycle ergometer in patients with heart failure. To the authors' knowledge, c-miR-222 does not increase following acute RE. It is currently unknown how the interaction of RE and AE may impact this miR. There were no significant differences in c-miR-222 between SCE and ICE, indicating exercise pattern may not have an effect on this biomarker. Although the study was not powered to do so, an exploratory statistical analysis was performed comparing the c-miR-222 responses of males and females, and no significant differences were observed.

There was variability in c-miR-126 and -222 responses between the participants, which may be due to individual genetic differences. High variability in individual c-miR responses to exercise has been observed in other similar studies (3, 4). The issue of responders vs. non-responders in exercise research has long been a topic of interest (25). There is a strong link between individual DNA sequences and responsiveness to physical exercise (39). Furthermore, some genotypes allow superior adaptations to specific forms of exercise (39). These concepts may eventually allow for individualized exercise prescriptions based on genetic profile (6).

Exercise modality preference results were mixed. Some participants preferred the ICE pattern, some preferred the SCE pattern, and others had no preference. The largest proportion of participants preferred the ICE pattern and cited the 20.25 min of cycling in SCE as being unfavorable. Wilke et al. (38) reported that healthy, inactivate adults experienced greater exercise enjoyment and motivation when exercise was performed in a circuit compared to moderate-intensity continuous exercise. Performing exercise modalities in an alternating fashion, such as with ICE, may result in greater sense of enjoyment in inactive adults.

There are several limitations in this study. First, the sample size of males and females was small. To ensure adequate statistical power, males and females were analyzed together. Exploratory statistical analyses were performed to compare males and females, but no differences were observed. To the authors' knowledge, there is no research that suggests c-miR-126 or -222 responses to exercise differ between males and females. Additionally, while the analyses in this study included the evaluation of two specific biomarkers related to cardioprotection and angiogenesis, there are many other complementary biomarkers and variables related to these physiologic mechanisms. Thus, it is difficult to generalize the results in this manuscript to the effects of these processes. Another potential limitation is that when miRs are measured in circulation, tissues of origin and potential target tissues are unknown. Furthermore, since the protocol was acute in nature, results cannot be extrapolated to characterize long-term adaptations. However, the present study is also marked by several notable strengths. Participants maintained strict adherence to multiple potential confounding factors (i.e., menstrual cycle phase, contraceptive use, physical activity status, hydration, correction for plasma volume shifts). The present study was also the first to examine the changes in circulating biomarkers following SCE and ICE patterns in healthy, sedentary males and females. To date, c-miR responses have been poorly characterized in females and sedentary adults.

In summary, both SCE and ICE patterns appear equally effective at acutely increasing c-miR-126 and -222 post-exercise in young, sedentary adults. Heart rate responses to both exercise patterns were also similar. Healthcare and exercise practitioners seeking to improve the cardiovascular health of their previously sedentary clients may prescribe either pattern of CE, although lower intensities than prescribed in the present study may be warranted for individuals with cardiovascular impairments (22).

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