SYNCHRONIZATION OF STEP-PHASE AND CARDIAC CYCLE DURING MAXIMAL TREADMILL TESTING

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In

Kinesiology

by

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CERTIFICATION OF APPROVAL

I certify that I have read Synchronization of Step-Phase and Cardiac Cycle During Maximal Treadmill Testing by Sean Michael Jones, and that in my opinion this work meets the criteria for approving a thesis submitted in partial fulfillment of the requirement for the degree (Example) Master of Science in Kinesiology: Exercise Physiology at San Francisco State University.

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SYNCHRONIZATION OF STEP-PHASE AND CARDIAC CYCLE DURING MAXIMAL TREADMILL TESTING

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Diastolic Cardio-locomotor synchronization (CLS) describes the intentional timing of foot strike to occur precisely during cardiac diastole. Diastolic CLS may maximize the efficiency with which skeletal muscle promotes venous return of blood to the heart, ultimately increasing cardiac output during exercise. A recent study[8] found that diastolic CLS produced lower heart rates during steady-state running compared with systolic CLS. Diastolic CLS has also been shown to improve metabolic efficiency[19] and may prolong aerobic energy production during steady-state running. Currently, there are no studies on the effects of diastolic CLS on maximal oxygen consumption (VO2max). Furthermore, no studies have reported measures of perceived exertion (RPE) during CLS running. Purpose: The aim of this study is to assess metabolic responses, heart rate, and perceived exertion to running when foot strike occurs during CLS or Natural Pace (NP) conditions. Methods: Twenty-two physically active adult participants (15 male; age 18-28 years) performed three separate treadmill VO2max trials, spaced at least 7 days apart. During visit 1, participants completed baseline treadmill VO2max test. During the remaining two visits, participants were blinded and randomized to either CLS or NP condition, both of which guided foot strike to an auditory metronome. During each condition, metabolic variables (VE, VO2, RF, RQ, TV) and heart rate (HR) were recorded continuously and averaged over the last 30 seconds of each two-minute stage. Rating of Perceived exertion (RPE) was recorded during the final 15-sec of each stage. Statistical Analysis: One-way analysis of variance was used to compare VO_{2max} between baseline, CLS, and NP conditions. One-way analysis of variance with repeated measures was used to compare

Results: No significant differences in VO_{2max} were found between conditions for all participants, as well as those who met step criteria. RPE was significantly lower during CLS than NP condition during stages 5 and 6. O_2-Pulse was significantly higher during CLS than NP condition during stage 4. No significant differences were observed in heart rate, VO_2, or RER between conditions at any stage. Conclusion: Synchronization of step phase with cardiac diastole does not improve maximal aerobic capacity during treadmill VO_{2max} test. However, CLS does decrease perceived exertion and increase O_2-Pulse at submaximal intensities, suggesting unique health and performance benefits during exercise.

I certify that the Abstract is a correct representation of the content of this thesis.

Chair, Thesis Committee | 5/20/19
Date

PREFACE

I would like to thank the incredibly supportive faculty, staff and fellow students at San Francisco State University for helping me throughout this entire process. Each of them has offered me inspiration, direction, and support during this project. In particular, Professor Matt Lee has never failed to find humor in nearly every situation. Without him, I would have found the last few years much less enjoyable.

Equally as heartfelt, I would like to thank my wife, Honora. I have yet to meet a more brilliant and inspiring person, who for some reason, considers me a scientist. Without her homemade cookies, I doubt many of my participants (or assistants) would have bothered showing up each week.

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Introduction

Cardio-locomotor synchronization (CLS) is a term used to describe the intentional coordination of skeletal muscle contraction with cardiac phase [1-6], two oscillating bodily rhythms that have been shown to independently influence intra-aortic pressure and subsequent cardiovascular performance [4]. Synergism between skeletal muscle and the heart may potentially hold significant performance and health benefits [4,7,8].

During cardiac systole, the left ventricle rhythmically ejects blood into the systemic circuit to perfuse the body. The movement of blood, termed Hemodynamics, largely contributes to the efficiency with which various tissues, including the myocardium, are perfused. To maximize systemic perfusion, attempts can be made to increase Cardiac Output, a measure of systemic blood flow per minute, by manipulating either heart rate or stroke volume[4]. The combined results from both mathematically-modeled[4,9] and experimental studies[6,8,10] suggest that one or both components of Cardiac Output may be positively influenced using cardio-locomotor synchronization.

During the stance phase of running and walking, rhythmic contractions of skeletal muscle acutely increase local vascular pressure [1,4,11]. At peak intramuscular pressure, capillary perfusion to exercising muscle becomes partially or even fully occluded[10-13]. The venous system, which contains 65-70% of total blood volume, benefits from this pressure increase by redirecting close to one half of its contents back towards the heart[14].

Mechanisms of CLS: O'Rourke and Avolio (1992) proposed a hydraulic mechanism by which vertical movements during running rhythmically generate positive and negative pulse waves, which travel upward toward the ascending aorta as the body moves downward, due to gravity. If such pressure waves occur precisely during cardiac diastole, venous return of blood and subsequent cardiac preload may be enhanced [4]. Meanwhile, by synchronizing muscular relaxation to occur precisely during cardiac systole, cardiac afterload may be decreased, while increasing skeletal muscle perfusion [12]. Using similar principles, clinical counter-pulsation techniques are commonly used to treat symptoms of cardiogenic shock [15,16]. However, such techniques are not practical for non-clinical populations, as they are often invasive, time-consuming and costly. With this in mind, CLS may serve as a more accessible, cost-effective option for the general public.

Benefits of CLS: In the same study by O'Rourke and Avolio (1992), researchers concluded that CLS targeted at 45%-50% of R-R interval (RRI) will likely decrease cardiac afterload and increase ventricular diastolic pressure. From their model, they estimated optimal synchronization would produce ~17% reduction in myocardial oxygen demand, and ~22% increase in myocardial blood flow[4]. Furthermore, when cardiac and step phases are close but not perfectly matching, intermediate effects may be expected [4], specifically a moderately-increased stroke volume and moderately decreased oxygen consumption. A growing body of data from experimental studies support the metabolic benefits of CLS [8,19].

A recent study[8] comparing the effects of diastolic vs systolic CLS on steady-state running found that when stepping occurred at 45% ± 15% of RRI, minute ventilation (VE) and respiratory exchange ratio (RER) were significantly lower, while O_2-Pulse (a surrogate for stroke volume[21]) was significantly greater. In the same study[8], 87% of participants maintained a lower heart rate during diastolic vs systolic CLS, suggesting potential improvements in cardiovascular efficiency.

Considering the effects of CLS on oxygen consumption and heart rate during steady-state exercise, muscle glycogen sparing may be enhanced [22], in turn prolonging aerobic metabolism. By prolonging aerobic energy production during exercise, maximal oxygen consumption (VO_2max) may theoretically be increased. Such results would greatly benefit individuals with low exercise capacity [23], for whom cardiorespiratory fitness may be a limiting factor in exercise progression or activities of daily living. VO_2 max is a widely accepted indicator of cardiorespiratory overall health and fitness [24], with a low score being associated with increasing risk for cardiovascular disease, and high score being associated with a longer lifespan and quality of life [24]. Decreased heart rate, a demonstrated effect of diastolic CLS[8], may also infer significant performance benefits for endurance sports. Relatively small decreases in heart rate maintained over a long period of time can have profound effects on race time[8].

Study Aim and Hypothesis: The aim of the present study was to measure the metabolic, cardiovascular, and subjective responses to CLS during maximal treadmill running. Using a within-subjects design, we compared the effects of guided diastolic CLS to guided Natural Pace (NP) during treadmill VO_2 max testing. The NP condition was individualized for each participant, based off of average step rates (bpm) at each stage, recorded during baseline testing. NP condition acted as the control condition, allowing us to compare effects of diastolic CLS to those which participants naturally display. Doing so also allowed us to measure feasibility in guided CLS. Finally, relationships between the amount of time stepping during diastole and outcome measures were analyzed.

We hypothesized that participants would reach a higher VO2max when performing CLS compared to NP condition. We also hypothesized that heart rate and RPE would be lower during diastolic CLS compared to subjects' natural pace. Finally, we expected intermediate effects when CLS is performed yet imperfectly timed, such as when foot-strike occurs during ± 15% of the optimal 45% R-R interval. Such effects may include moderate decreases in oxygen utilization, heart rate, and perceived exertion.

Methods

Population and Study design: Eighteen undergraduate students at San Francisco State University participated in this study. The participants were college-aged, recreationally-active individuals. All participants gave written informed consent to a protocol approved by the Institutional Review Board of San Francisco State University.

Study design graphically presented in Figure 1. Each participant visited the Exercise Physiology Laboratory at San Francisco State University for threes testing sessions, each spaced one week apart a part. Exercise testing was performed using a motor-driven treadmill (Model T-150; COSMED, Inc., Rome, Italy). Subjects were instructed to refrain from caffeine, alcohol and strenuous physical activity for 24 h before testing.

Figure 1. Study design.

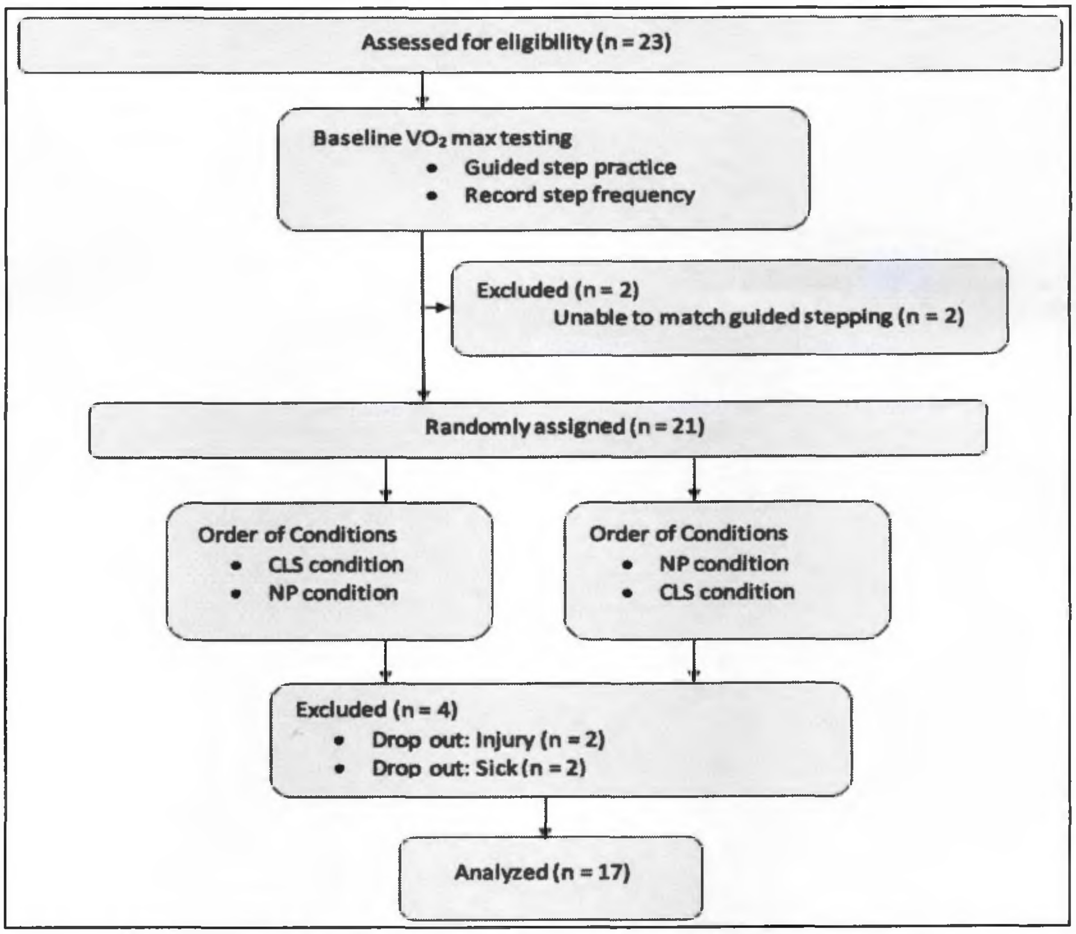


Fig. 1

Exercise Protocol: Table 1 outlines VO_2 max protocol. All participants were blinded to treatment conditions, and the order of conditions during Visits 1 and 2 were randomized. During visit 1, participants were equipped with chest-strap [Counterpace Sensor; Pulson, Palo Alto, CA], followed by baseline treadmill VO_2 max using incremental test protocol (Figure 2). In short, participants performed a 4-min warm-up, walking at 3.5 mph for 2 min, followed by jogging at 5.0 mph for 2 min. During the last 90 sec of warm-up, participants practiced matching their foot-ground contact to an auditory tone sounding at

165, 175 and 185 bpm, each for 30 sec, which served as a screening tool for participant inclusion. These paces were selected based on the average range of step rates observed during VO_2 max pilot testing. At minute 4, the metronome was silenced, and participants completed remaining 2-min stages of treadmill VO_2 max test until exhaustion. Cardiovascular and metabolic variables were continually monitored and recorded at each stage, and rating of perceived exertion (RPE) was recorded during the last 15 sec of each stage.

CLS session: During the CLS session, participants were equipped with chest strap and then performed incremental treadmill VO_2 max using test protocol. From minute 4 until the completion of test, participants were asked to match their steps to an auditory tone that repeated at their real-time measured HR, guiding them to constant diastolic stepping. The target for diastolic phase is an estimate of the 45% of the RRI at which diastole begins, which coincides with aortic valve closure during ventricular relaxation, as well as end T-wave timing. If participants could no longer match foot-strike with metronome due to a heart rate greater than ~190 bpm, participants were instructed to disregard metronome and complete test without metronome guidance. Cardiovascular and metabolic variables were continually monitored and recorded at each stage, and RPE was recorded during the last 15 sec of each stage.

Table 1. Treadmill VO_{2max} test protocol. Following stage 3, treadmill speed was held constant at 6.0 mph while grade was increased by 2.0 % every two minutes, until failure. Stages 1 and 2 were considered "warm-up" stages, during which times no dependent variables were collected, nor were participants provided metronome guidance.

Stage	Time (min)	Speed (mph)	Grade (%)
1	0	3.5	0
2	2	5.0	0
3	4	6.0	0
4	6	6.0	2
5	8	6.0	4
6	10	6.0	6

Table 1

NP session: During the Natural Pace (control) session, participants were equipped with chest strap and then performed incremental treadmill VO_2 max using test protocol. From minute 4 until the completion of test, participants were asked to match their steps to an auditory tone that repeated at the average step rate (bpm) for each stage that the individual performed during baseline testing. If participants could no longer match foot-strike with metronome due to a heart rate greater than ~190 bpm, participants were instructed to disregard metronome and complete test without metronome guidance. Cardiovascular and metabolic variables were continually monitored and recorded at each stage, and RPE was recorded during the last 15 sec of each stage.

Gait cycle timing guidance: All participants were fitted with a chest strap mounted sensor that captured both accelerometer and ECG R-wave signals (Counterpace sensor;

Pulson, Inc., Palo Alto, CA). An onboard ECG ASIC identified the onset of each R-wave, which was timestamped by the processor on the sensor. Additionally, a foot strike was identified and timestamped by the processor each time that the 3-axis accelerometer signal crossed 1g in a positive direction during each gait cycle. These timing signals were streamed wirelessly in real-time, via Bluetooth, to a tablet computer (iPad 9.7in; Apple, Cupertino, CA) running proprietary step timing guidance software (Counterpace software application running in iOS, Pulson, Inc., Palo Alto, CA). The software processed incoming step and ECG R-wave timestamps in order to identify the timing relationship between each foot strike (approximate timing of maximal skeletal muscle contraction) and each R-wave in order to compute foot strike phase timing in the cardiac cycle (%RRI).

$$\%RRI = 100\,(tstep - tR\text{-}wave1)/(tR\text{-}wave2 - tR\text{-}wave1)$$

where tR-wave1 and tR-wave2 refer to the times of two consecutive R-waves, and tstep refers to the time of the step that occurred between them. Heart rate (HR) was computed from the R-R interval (RRI) in the conventional manner. The software further generated a beep tone to audibly guide the user's step timing, which repeated nominally at the subject's HR.

To lead the participant to the targeted diastolic step timing (CLS condition), the software made small continuous adjustments to the beep prompt period, based on the difference between the sensed step timing and the cardiac cycle timing target during that stage. The tone prompt was played on a speaker loud enough to be easily heard by the participants running on the treadmill. Provided participants maintained their general running rhythm with the recurring beep prompt, the software adjustments aligned their measured %RRI phase to the target value. Data from the software program (R-wave and step timing) were logged for later analysis.

Optimal diastolic step timing in a rhythmic gait cycle is hypothesized to occur when the subject's foot strike consistently occurs immediately after the aortic valve closure, at the onset of cardiac diastole. The fraction of the cardiac cycle required for systole relative to the fraction required for diastole increases as the heart rate increases. At low heart rates, systole is normally well less than half of the cardiac cycle, whereas at high heart rates, systole persists beyond half of the cardiac cycle, with a relatively smaller percentage of the cycle time allowed for diastole. The relative durations of systole vs. diastole as a function of heart rate, which is fairly consistent across subjects with normal cardiac function, has been described in various studies under resting and exercise conditions[25,26]. Because subjects in this study exercised at various heart rates, the following algorithm was used to calculate target timing in the heart cycle:

$$\text{Target Phase} = 17.5 \times \ln(\text{HR}) - 37$$

This algorithm was empirically derived during unpublished walking and running treadmill calibration studies performed with the Counterpace guidance system. These calibration studies used measured heart rate response to carefully controlled step timing targets across the cardiac cycle in a wide range of individuals with normal cardiac function. At constant work output (e.g. fixed treadmill speed and incline), heart rate is very responsive to step timing within the cardiac cycle, with early diastolic phase timing consistently corresponding to the lowest relative heart rate in an exercise study. Because most studies showed a few percent of inter-individual variation in calculated aortic valve closure timing at physiological walking and running heart rates, the step timing algorithm employed in this study is a conservative estimate of the onset of diastole, calculating a target timing that is consistently approximately 4% later than the time of average aortic valve closure as a % of the RRI. As a conservative estimate of optimal step phase timing, the target phase algorithm derived in these calibration exercises, as described above, is consistent with algorithms in a variety of previous peer reviewed publications reporting the ratio of systolic to diastolic duration as a function of heart rate during exercise [25,26].

Metabolic parameters. Ventilatory and metabolic variables were continuously measured and monitored during exercise using a computer-interfaced, open-circuit, indirect calorimetry system (Quark CPET: COSMED, Inc., Rome, Italy). Participants wore a facemask and breathed through a low-resistance, two-way valve (7450, Hans Rudolph). A 5-L mixing chamber was used for the collection of expired gases. Analyzers were calibrated each day before testing using commercially available gas mixtures within a physiological range. The dependent variables of VO_2 and CO_2 production were averaged over 5-second intervals during exercise and converted to minute values, with VO_2 and V CO_2 corrected to STPD.

Data processing. For all dependent variables, averages were taken from the last minute of exercise at each stage. Data were considered to be valid if mean within-subject diastolic stepping occurred 10% before and 15% after target, at least 60% of a given stage, during two or more stages. The early diastolic target timing of 45% of the RRI is consistent with the timing of mechanical counterpulsation technologies used regularly in the clinical setting, including intra-aortic balloon counter- pulsation, left ventricular assist device counterpulsation, and external counterpulsation [16,17]. Optimal timing of the musculoskeletal pump during the gait cycle, similar to the preferred timing of clinical counterpulsation therapies, is immediately after aortic valve closure during the heart's pump cycle, when intra-aortic blood pressure first exceeds intra ventricular pressure [8].

Data Analysis. Data were analyzed using SPSS 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). Pearson Correlation was used to examine relationships between dependent variables. One-way analysis of variance was performed to assess differences in VO_{2max} between conditions. Multivariate analysis of variance with repeated measures was performed to analyze mean differences in dependent variables between conditions during sub-maximal stages. Multiple one-way analysis of variance with repeated measures were used to further analyze the subset of participants who met step criteria during sub-maximal stages. For all tests, statistical significance was set at $P \leq 0.05$.

Results

Participant characteristics. Participant characteristics are presented in Table 2. Nine female and eight male participants completed all testing. Mean age was 22.9 (± 2.2) years. Eight participants met step criteria, each of whom accurately timed their steps with the auditory tones at least 60% of a given stage, during ≥2 stages. * indicates participants who met step criteria.

*Table 2. Participant characteristics. * indicates participants who met step criteria.*

Participant	*Age (yr)*	*Sex*	*Height (cm)*	*Mass (kg)*
*1	23	M	185	76
*2	22	F	157.5	62.5
3	22	M	167.5	70.5
*4	28	M	164	76
5	22	F	159.5	66
6	23	M	170	78.5
7	23	F	160	54
8	27	M	182	86
*9	21	F	159	57
*10	22	M	170	69.5
11	21	F	155	55.5
12	23	F	165	61.5
*13	27	F	150.5	58.5
14	21	F	158	48.5
*15	22	M	172	86.5
16	21	M	161	54
*17	22	F	159	56.5
Mean	22.9	9F/8M	164.4	65.7
SD	2.2		8.9	11.3

Table 2

VO_{2max}. Of the entire study sample (N=17), average VO_{2max} was not significantly different between baseline, CLS, and NP conditions (47.5 ± 7.6 vs 48.1 ± 5.6 vs 48.1 ± 6.4 ml/kg/min) (Figures 2a, 2b). Of the subset of participants who met step criteria (n=8), average VO_{2max} was not significantly different between baseline, CLS, and NP conditions (49.5 ± 5.1 vs 49.3 ± 5.3 vs 47.7 ± 5.3 ml/kg/min).

Figure 2. Average VO_{2max} between baseline, CLS, and NP conditions for all participants (N=17).

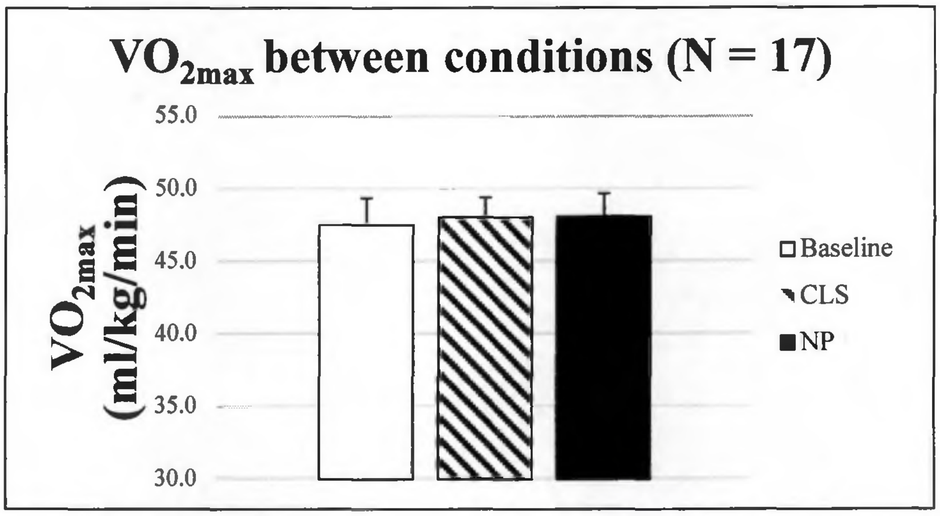


Figure 2

Figure 3. Average VO_{2max} between baseline, CLS, and NP conditions for all participants (n=8)

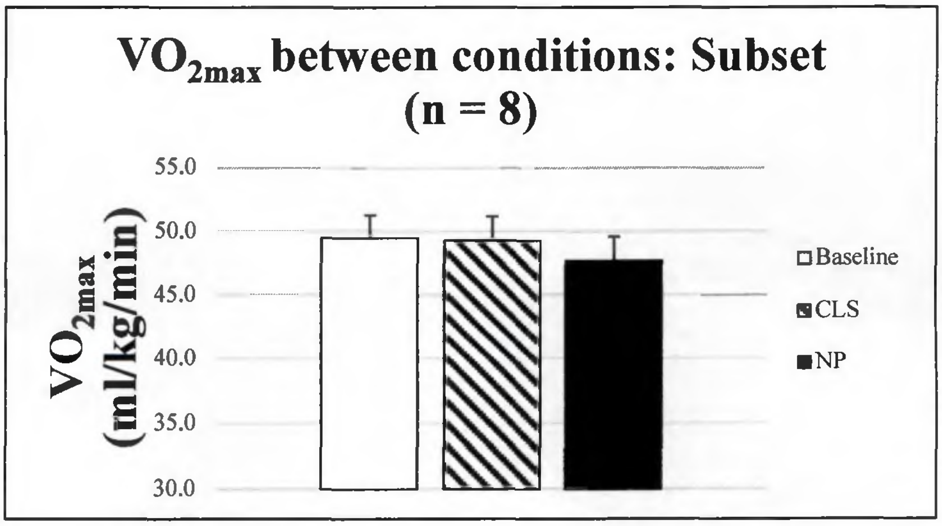


Figure 3

Sub-maximal dependent variables. None of the dependent variables were significantly correlated. Analysis of dependent variables included stages 3-6, as significantly fewer participants continued into stage 7 (n=5) and stage 8 (n=3) of the protocol. Subset analysis revealed statistically significant differences for O_2-Pulse ($p = 0.018$) and RPE ($p = 0.001$) between baseline, CLS, and NP conditions at sub-maximal stages (Figures 3a, 3b). During stage 4, CLS showed significantly greater O_2-pulse (15.15 ± 2.6 vs 14.04 ± 2.09 ml/beat; $p = 0.009$) compared to NP. CLS revealed significantly lower RPE at stage 5 (12 ± 1.5 vs 13 ± 1.7; $p = 0.02$) and stage 6 (14 ± 2.3 vs 15 ± 2.2; $p = 0.003$) compared to NP. Average VO_2, heart rate, and RER were not significantly different between baseline, CLS, and NP conditions during any sub-maximal stage (see Table 3).

*Table 3. Statistical differences between baseline, CLS, and NP conditions for each dependent variable at sub-maximal stages using one-way analysis of variances with repeated measures (n=8). * indicates statistical significance (p ≤ 0.05).*

Stage	VO_2	Heart Rate	RPE	RER	O_2-Pulse
3	0.916	0.911	0.422	0.552	0.361
4	0.99	0.504	0.183	0.198	*0.018
5	0.193	0.480	*0.001	0.240	0.522
6	0.189	0.467	*0.007	0.570	0.095

Table 3

Tables 4a and 4b. Dependent variables at sub-max stages. Shown as mean (SD).

Stage	Heart Rate (bpm)			VO_2 (ml/kg/min)			O2-Pulse (ml/beat)		
	Baseline	CLS	NP	Baseline	CLS	NP	Baseline	CLS	NP
3	160.8 (12.7)	159.8 (12.7)	159.8 (13.4)	31.13 (4.52)	29.7 (2.9)	29.5 (3.2)	12.3 (2.9)	12.4 (2.7)	12.2 (2.6)
4	172.0 (11.7)	170.0 (12.6)	170.8 (12.6)	35.13 (4.9)	36.1 (2.7)	34.0 (3.06)	13.3 (3.1)	14.2 (2.9)	13.2 (2.6)
5	181.5 (9.7)	177.0 (10.7)	180.9 (12)	38.9 (4.9)	38.7 (2.3)	37.4 (3.3)	13.3 (4.6)	14.7 (2.7)	12.9 (4.2)
6	185.5 (6.8)	182.6 (7.5)	185.7 (10.1)	42.5 (6.5)	41.7 (2.6)	40.4 (3.4)	15.5 (3.2)	16.0 (2.8)	14.7 (3.0)

Table 4a

Stage	RPE			RER		
	Baseline	CLS	NP	Baseline	CLS	NP
3	9.1 (1.3)	9.4 (1.2)	0.82 (0.08)	0.82 (0.08)	0.85 (0.09)	0.82 (0.06)
4	10.7 (1.7)	11.1 (1.7)	0.89 (0.07)	0.89 (0.07)	0.91 (0.06)	0.89 (0.06)
5	13.1 (2.4)	14 (2.3)	0.92 (0.08)	0.92 (0.08)	0.95 (0.07)	0.93 (0.08)
6	14.4 (2.6)	15.5 (2.5)	0.95 (0.07)	0.95 (0.07)	0.90 (0.24)	0.97 (0.07)

Table 4b

Figure 4. Average between baseline, CLS, and NP conditions for participants who met step criteria at sub-maximal stages (n=8). RPE was significantly lower during CLS than NP and baseline conditions at stages 5 and 6.

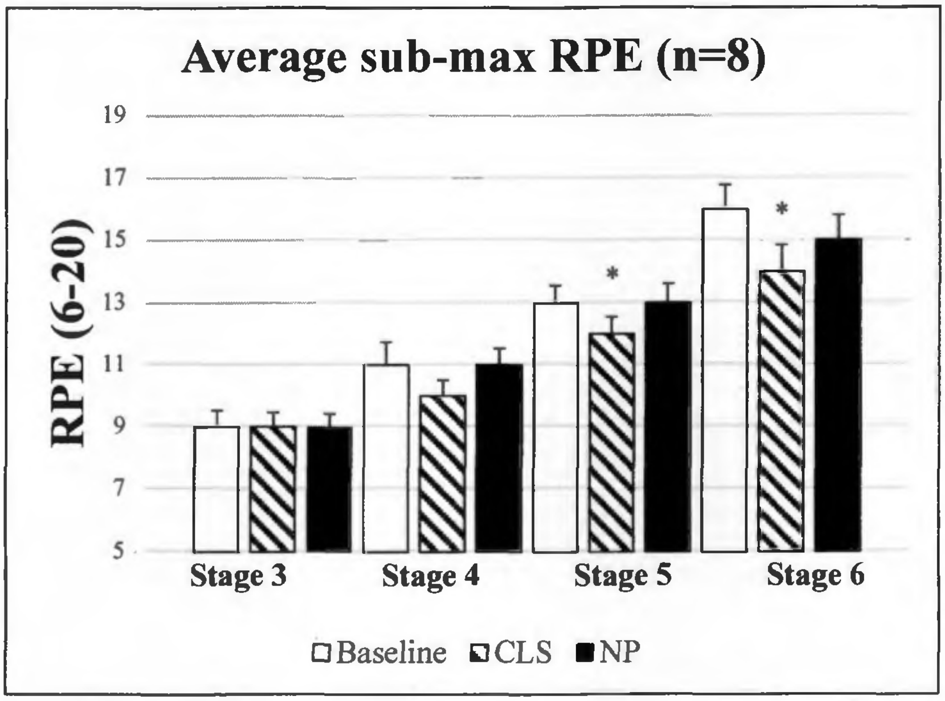


Fig. 4

Figure 5. Average O_2-Pulse between baseline, CLS, and NP conditions for participants who met step criteria at sub-maximal stages (n=8). O_2-Pulse was significantly lower during CLS than NP and baseline conditions at stage 4.

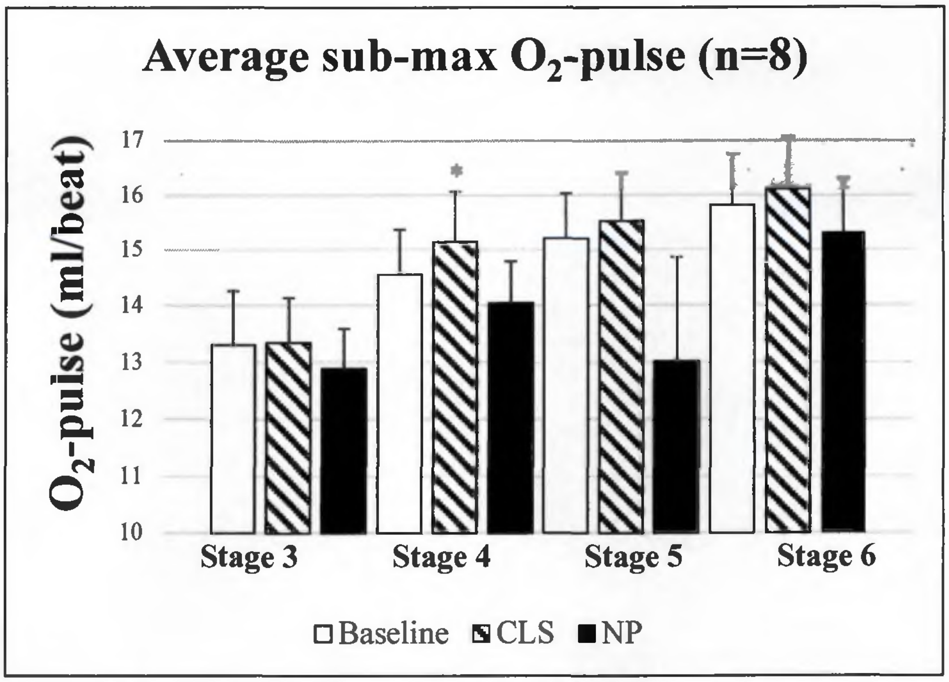


Fig. 5

Figures 6-8. No significant differences were found between conditions at any stage for average VO_2 (6), RER (7), and HR (8) between baseline, CLS, and NP conditions.

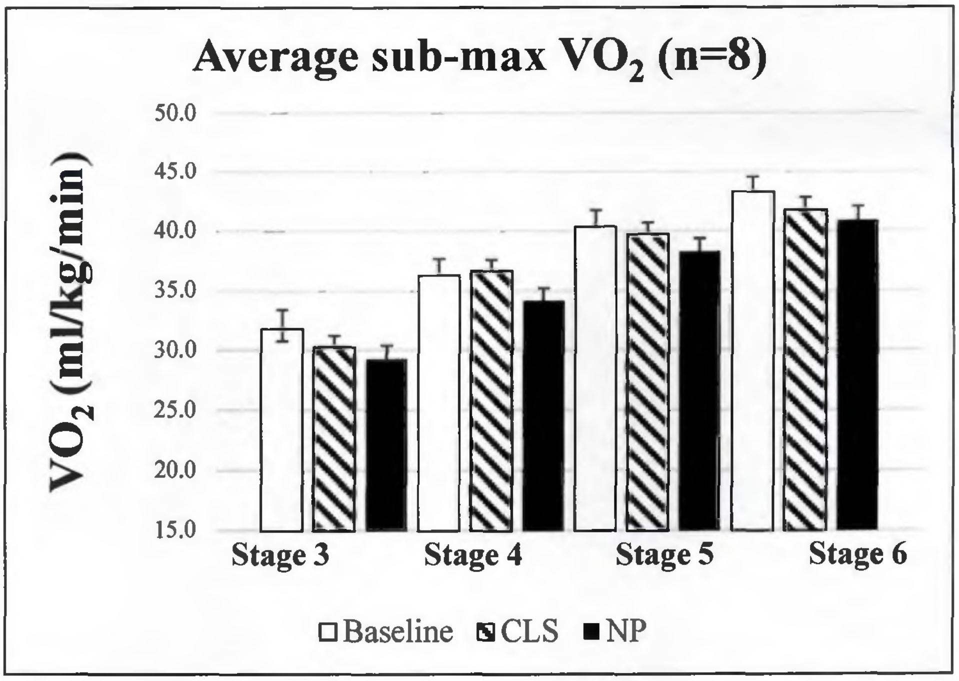
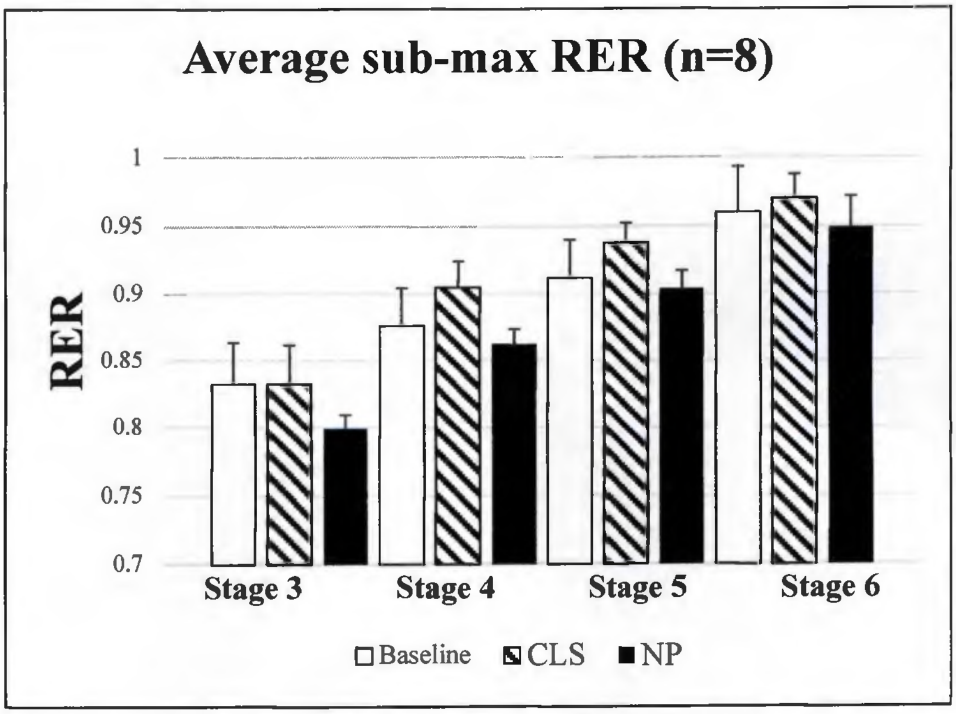
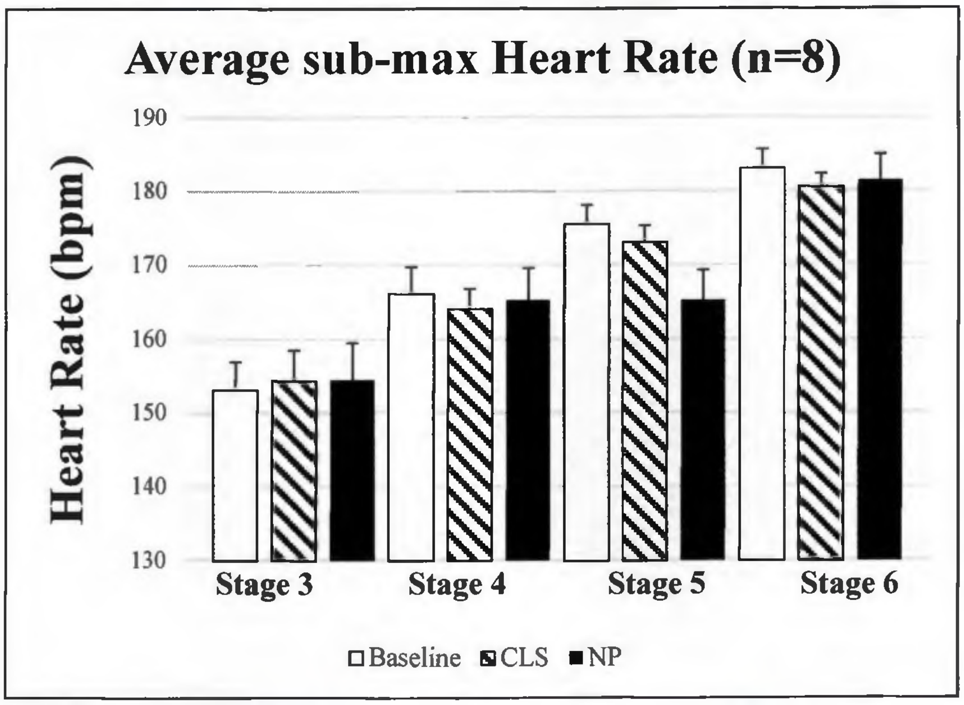


Fig. 6



Average sub-max RER (n=8)
RER
1
0.95
0.9
0.85
0.8
0.75
0.7
Stage 3
Stage 4
Stage 5
Stage 6
Baseline
CLS
NP

Fig. 7



Average sub-max Heart Rate (n=8)
Heart Rate (bpm)
190
180
170
160
150
140
130
Stage 3
Stage 4
Stage 5
Stage 6
Baseline
CLS
NP

Fig. 8

Discussion

Primary findings. The primary finding of this study was that synchronizing foot strike with diastolic phase of cardiac cycle does not improve maximal aerobic capacity during treadmill VO_{2max} test. However, CLS significantly improved both RPE and O_2-Pulse at submaximal stages, indicating that diastolic step timing provides unique subjective and physiological advantages during exercise.

Physiological response to CLS. Instances of both intentional and unintentional cardio-locomotor synchronization have been well documented in adult humans [5,8,10,17,18], with significant benefits seen during running [1,4,8]. While literature does exist supporting physiological and performance benefits of CLS [8,19], this was the first experimental study to measure metabolic, cardiovascular, and subjective measures of exertion during maximal exercise.

The lack of significant differences in VO_{2max} may have been attributed to the small subset of participants who met step criteria (n=8), accounting for approximately 47% of the entire sample. Upon review of the subset, VO_{2max} was greater during CLS than NP condition, but not when compared to baseline (Fig. 2b). One interpretation of these results is that metronome guidance by either condition was actually detrimental to performance.

Our hypothesis that CLS would decrease oxygen consumption (VO_2) at sub-maximal stages was not supported by these results. These findings may be attributed to enhanced biomechanical costs associated with matching step rate with heart rate. Most subset participants were able to correctly match foot strike with heart rate at speeds of $\leq$ 185 bpm. At speeds greater than 185 bpm, participants visually fatigued as they attempted to match foot strike with metronome guidance, likely increasing their energy expenditure and subsequent oxygen consumption.

The increased O_2-Pulse during CLS at a sub-maximal intensity may be explained by central and peripheral mechanisms. During CLS running, foot strike occurs synchronously with cardiac diastole, which may improve cardiovascular efficiency by increasing cardiac preload and decreasing cardiac afterload [4,10]. Though heart rate was not significantly lower during CLS than NP, O_2-Pulse was significantly higher, suggesting an increased cardiac stroke volume (Fig. 3b). Theoretically, increasing stroke volume without similarly decreasing heart rate would likely result in an increase in cardiac output. By decreasing cardiac output, it is reasonable to expect perceived exertion to decrease, which was seen during stages 5 and 6 (Fig. 3a). These results support an earlier study by Udo et al (1990) which found CLS significantly decreased 3-mile run in endurance trained adults, while making running seem subjectively easier.

The precise timing of skeletal muscle contraction during cardiac phase has been shown to modulate skeletal muscle blood flow 10,12,40, suggesting a peripheral mechanism must also be considered. At peak intramuscular pressure, capillary perfusion to exercising muscle may be decreased or fully prevented [10-13]. Subsequently, blood is redirected back toward the heart [14] and enhancing cardiac preload. Furthermore, vertical movements during running may generate positive and negative pulse waves, resulting in enhanced venous return and subsequent cardiac preload [4].

Limitations. We did not directly measure cardiovascular blood volumes and arterial pressures, and instead relied on O_2-Pulse as an indicator of stroke volume. Doing so may have helped clarify if the proposed central and/or peripheral mechanisms contributed to increases in both O_2-Pulse. Study participants were young, college-age adults. Accordingly, the range for age predicted maximum heart rate was between 185-200 bpm. At peak heart rates, step rate was seemingly very difficult to maintain, especially for prolonged periods of time. Furthermore, all but two participants (1 & 9) were recreationally active with limited running experience. Future studies should look at the effects of CLS on groups of older individuals, whose age predicted maximum heart rate may be lower and more easily matched for longer durations, as well as well-trained endurance athletes who might more easily maintain a faster step pace. Finally, future studies should include the clinical populations effected by insufficient cardiac output, for whom traditional external counterpulsation methods have been shown to reduce angina and increase cardiac output. In conjunction with such physiological improvements, they Results from the current study suggest that CLS may reduce perceived exertion at sub-maximal intensities, which may help

Conclusion. In conclusion, maximal aerobic capacity was not significantly improved through CLS during treadmill VO_{2max}. During sub-maximal intensities, CLS decreased perceived exertion and increased O_2-Pulse. Heart rate, VO_2, and RER were not significantly enhanced using CLS. In summary, enhanced cardiac function and reduced perceived exertion were observed during treadmill VO_{2max} testing.

References

1. Kirby R, Nugent S, Marlow R, MacLeod D, Marble A. Coupling of cardiac and locomotor rhythms. *J Appl Physiol.* 1989;66(43):323-329.

2. Niizeki K, Kawahara K, Miyamoto Y. Interaction among cardiac, respiratory, and locomotor rhythms during cardiolocomotor synchronization. *J Appl Physiol.* 1993;75(4):1815-1821.

3. Nomura K, Takei Y, Yoshida M, Yanagida Y. Phase-dependent chronotropic response of the heart during running in humans. *Eur J Appl Physiol.* 2006;97(2):240-247.

4. O'Rourke, Avolio. Improved cardiovascular performance with optimal entrainment between heart rate and step rate during running in humans. *Coron Artery Dis.* 1992;(1992):863-869.

5. Zhang D, Celler BG, Lovell NH. The effect of heartbeat-synchronised running on the cardiovascular system. *Proc Second Jt 24th Annu Conf Annu Fall Meet Biomed Eng Soc [Engineering Med Biol].* 2002;2(10):1295-1296.

6. Phillips B, Jin Y. Effect of adaptive paced cardiolocomotor synchronization during running: A preliminary study. *J Sport Sci Med.* 2013;12(3):381-387.

7. Donville J, Doherty T, Kirby R, Gupta S, Eastwood J, MacLeod D. Effect of cardio-locomotor coupling on the metabolic efficiency of pedaling. *Can J Appl Physiol.* 1993;18(4):379-391.

8. Constantini K, Stickford ASL, Bleich JL, Mannheimer PD, Levine BD, Chapman RF. Synchronizing Gait with Cardiac Cycle Phase Alters Heart Rate Response during Running. *Med Sci Sports Exerc.* 2018;50(5):1046-1053.

9. Zhang D, Celler B. Possible mechanism for modulating cardiovascular system during runnig in humans. *Proc 23rd Annu EMBS Int Conf Oct 25-28*, Istanbul, Turkey. 2001:173-176.

10. O'Rourke, Avolio A, Stelliou V, Young J, Gallagher D. The rhythm of running: Can the heart join in? *Aust N Z J Med.* 1993;23(6):708-710.

11. Kirby R, Marlow R, Kriellaars D, MacLeod D, Marlow R. The effect of locomotion speed on the anterior tibial intramuscular pressure of normal humans. *J Biomech.* 1988;21:357-360.

12. Radegran G. Ultrasound doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *J Appl Physiol.* 1997;83(4):1383-1388.

13. Walloe L, Wesche J. Time course and magnitude of blood flow changes in the human quadriceps muscles during and following rhythmics exercise. *J Physiol.* 1988;(405):257-273.

14. Astrand, P., & Rodahl, D. *Textbook of Work Physiology: Physiological Bases of Exercise* (3rd ed.). 1986. New York: McGraw-Hill, inc. doi:10.2310/6640.2004.0003015.

15. Capoccia M, Bowles CT, Pepper JR, Banner NR, Simon AR. Evidence of clinical efficacy of counterpulsation therapy methods. *Heart Fail Rev.* 2015;20(3):323-335.

16. Tagouchi I, Ogawa K, Kanya T, Matsuda R, Kuga H, Nakatugawa M. Effects of enhanced external counterpulsation on hemodynamics and its mechanism: relation to neurohumoral factors. *Circ J.* 2004;68(2):1030-1034.

17. Cerqueira LS, D'Affonsêca Netto A, Mello RGT, Nadal J. Crossover assessment of cardiolocomotor synchronization during running. *Eur J Appl Physiol.* 2017;117(2):315-322.

18. Niizeki K, Saitoh T. Cardiolocomotor phase synchronization during rhythmic exercise. *J Phys Fit Sport Med.* 2014;3(1):11-20.

19. Udo M, Yoshida T, Nakazumi T, Kinugawa K, Watanabe A. Occurrence of entrainment of heart beat into endurance running pith and its significance in energy consumption. In: *Fitness for the aged, disabled, and industrial worker.* 1990. Champaign, Ill: Human Kinetics Books; 79-83

20. Simmons A, Carrier D, Farmer C, Gregersen C. Lack of locomotor coupling in trotting dogs. *Am J Physiol.* 1997;(273):1352-1360.

21. Whipp B, Higgenbotham MB, Cobb F. Estimating exercise stroke volume from asymptotic oxygen pulse in humans. *J Appl Physiol.* 1996;81(6): 2674-2679.

22. Gollnick P, Piehl K, Saltin B. (1974). Selective glcogen depletion patterns in human muscle fibers after exercise of varying intensity and at varying pedalling rates. *J Physiol.* 1974;241(1): 45–57.

23. Laukkanen J, Kurl S, Salonen R, Rauramaa R, Salonen J. The predictive value of cardiorespiratory fitness for cardiovascular events in men with various risk profiles: a prospective population-based cohort study. *Eur. Heart J.* 2004;25(16): 1428–1437.

24. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood J. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med.* 2002;14(11): 793–801.

25. Ferro G, Guinta A, Maione S, Carella G, Chiariello M. Diastolic time during exercise in normal subjects and in patients with cardiovascular disease. *Cardiology.* 1984;71(5): 266-272

26. Chung CS, Karamanoglu M, Kovacs SJ. Duration of diastole and its phases as a function of heart rate during supine bicycle exercise. *Am J Physiol Heart Circ Physiol.* 2004;287(5): H2003-8.

27. Bramble D, Carrier D. Running and breathing in mammals. *Science.* 1983;219: 251-258

28. Eldridge F, Millerhorn D, Kiley J, Waldrop T. Stimulation by central command of locomotion, respiration and circulation during exercise. *Respiration Physiology.* 1985;59(3): 313-337

29. Kenner T, Pessenhofer H, Schwaberger G. Method for the analysis of the entrainment between heart rate and ventilation. *Eur J Phy.* 1976; 363: 263-265

30. Schafer C, Rosenblum M, Kurths J, Abel H. Heartbeat synchronization with ventilation. *Nature.* 1998;392(6673): 239-240

31. Coleman WM. The psychological significance of bodily rhythms. *J. Comp. Psychol.* 1921a; 1(3): 213-220

32. Nomura K, Takei Y, Yanagida Y. Comparison of cardio-locomotor synchronization during running and cycling. *Eur. J. Appl Physiol.* 2003;89(3–4): 221–229.

33. Coleman, WM. On the correlation of the rate of heart beat, breathing, bodily movement and sensory stimuli. *J. Physiol.* 1921b; 54: 213-217

34. Aurie A. Coordination between the activity of the heart and the flight muscls during flight in small birds. *Comp. Biochem. Physiol. B.* 1971; 38: 91-97

35. Dawson T, Taylor C. Energetic cost of locomotion in Kangaroos. *Nature.* 1973; 246: 313-314

36. Edwards R, McCutcheon E, Knapp C. Cardiovascular changes produced by brief whole-body vibration of animals. *Journal of Applied Physiology.* 1972;32(3): 386-390

37. Hornicke H, Meixner R, Pullman, U. Respiration in exercising horses. *Proceedings from the first international conference of equine exercise physiology.* September, 1983. Pgs 7-16

38. Franz M, Cima R, Wang D, Profitt D, Kurz R. Electrophysiological effects of myocardial stretch and mechanical determinants of stretch-activated arrhythmias. *Circulation.* 1992; 86(3): 968-978

39. Kohl P, Hunter P, Noble D. Stretch-induced changes in heart rate and rhythm: clinical observations, experiments and mathematical models. *Prog Biophys Mol Biol.* 1999;71: 91-138

40. Nomura K, Takei Y, Yanagida Y. Analysing entrainment of cardiac and locomotor rhythms in humans using the surrogate data technique. *Eur. J Appl Physiol.* 2001;84:373-378

41. Eckberg D, Kifle Y, Roberts V. Phase relationship between normal human respiration and baroreflex responsiveness. *J. Physiol.* 1980;304: 489–502.

42. Legramante J, Raimondi G, Sacco C, Iellamo F, Peruzzi G, Kaufman M. Group III muscle afferents evoke reflex depressor responses to repetitive muscle contractions in rabbits. *Am J Physiol.* 2000;278: H871-877.

43. Niizeki K. Intramuscular pressure-induced inhibition of cardiac contraction: implications for cardiac-locomotor synchronization. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2004; 288: R645-R650

44. Bernasconi P, Kohl J. Analysis of co-ordination between breathing and exercise rhythms in man. *Eur. J. Appl. Physiol.* 1993;471: 693–706.

Appendix

APPENDIX I: Literature Review

Introduction: Synchronization, defined as the spontaneous or purposeful coupling of two or more oscillating bodily rhythms, has been well documented when respiration [2,27-31]or heart rate [1-5,8,10,32] are coupled with locomotion. The latter was first described by Coleman [33], who noticed a 1:1 ratio of heart rate to step cadence among different animals at the London Zoological Gardens. Subsequent research has shown dogs, birds, horses, and other animals display evidence of synchronization [34-37]. The first documentation of synchronization in humans also came from Coleman [33] who observed that uphill walking became easier when one subject synchronized breathing and stepping with heart rate. One of the most studied forms of synchronization is cardio-locomotor synchronization.

Cardio-locomotor synchronization (CLS) describes the intentional contraction of skeletal muscle to coincide precisely during cardiac contraction [1-6,18]. CLS has been shown to happen spontaneously in untrained adults [10,17], elderly individuals [18], and elite endurance athletes [5,8,10]. Synchronization has been reported during running when the frequency ratio of heart rate to step rate reaches 1:1 [8] with combined frequencies often reported between 151-180 beats per minute [1,6,32]. Conversely, Niizeki et al. (1993) reported synchronization to be independent of absolute heart rate and treadmill speed. Studies suggest that coordination of locomotion and cardiac timing may be biologically advantageous by improving cardiac efficiency and optimizing systemic blood flow [1,19].

Spontaneous coupling of heart rate with locomotion has been well documented during rhythmic exercise, including cycling [1,7,32], walking [1,2,18] and running [1,8,18,32]. Kirby and colleagues (1989) documented the occurrence and resilience of CLS (spontaneous re-synchronization after interruption) during running. As well as spontaneous occurrence of CLS, intentional coupling of locomotion with heart rate has been documented. Physiological and/or performance benefits of CLS, typically achieved by guiding step frequency through audible beep or metronome, have been theorized [1,4,10,5] and experimentally found [6,8]. During rhythmic exercises, synchronizing muscular contractions with cardiac cycle has been shown to minimize cardiac afterload, while maximizing cardiac preload [4,5,910,12]. By doing so, improvements in cardiovascular and metabolic indices of both health and athletic performance have been reported [4,6,8,10]. By creating guided CLS programs, we may be able to extend the health and performance benefits associated with CLS past elite athletes, and into the lives of recreational runners and unhealthy populations.

Mechanisms of CLS: Researchers have proposed two primary mechanisms to explain the occurrence of CLS, focusing specifically on how heart rate is modulated towards entrainment. The first mechanism relies on an intrinsic property of the heart [38,39], while the second relies on a complex synergy between the first mechanism and peripheral neural circuitry [18,32].

First, Kohl et al. (1999) showed that cardiac R-R interval is independently modulated by mechanical stretch of myocardium within the atrial wall. To induce a higher degree of stretch, an attempt could be made to increased venous return of blood and subsequent cardiac preload [20], both of which may be increased by CLS [4,9,10]. During the stance phase of running and walking, rhythmic contractions of skeletal muscle promotes acute increases in local vascular pressure [1,4,11]. At peak intramuscular pressure, capillary perfusion to exercising muscle may be decreased or fully prevented [10-13]. Meanwhile, the venous system, which contains 65-70% of total blood volume, benefits from this pressure increase by redirecting close to one half of its contents upward to the heart [14].

To amplify this effect, O'Rourke and Avolio (1992) showed how vertical movements during running generate positive and negative pulse waves, which travel upward toward the ascending aorta as the body moves downward, due to gravity. As a result, venous return and subsequent cardiac preload is enhanced [4]. A remarkable example of this mechanism has been documented in Kangaroos, whose tendons store large amounts of elastic energy during hopping, which lead to a significant upward pulse wave and increased stroke volume via cardiac preload [35]. These findings suggest rhythmic exercises such as running may exert significant modulatory effects on heart rate by providing constant arterial stretch. Based on this mechanism, mature persons may have some advantage over younger, more fit individuals who exhibit greater central arterial compliance. Theoretically, less compliant vessels would allow for quicker vertical movement of pressure waves, compared to more compliant vessels which could hinder such waves through lateral expansion [4].

The occurrence and efficacy of cardiac entrainment based on this mechanism may depend on the phase relationship between step cycle and cardiac cycle [3,5,6,9,18,19]. When step frequency occurs approximately 180 degrees out of phase with cardiac cycle, oscillating downward and upward pressure waves enhanced skeletal muscle perfusion during cardiac systole, and increase ventricular filling during cardiac diastole, respectively [4,10]. Furthermore, Zhang et al. (2002) found phase relationship to independently modulate stroke volume during exercise simulation. While constant phase delay between heart rate and step phase allowed for constant stroke volume, deviations from optimal phase delay led to decreased stroke volume [5]. Interestingly, Kirby et al. (1989) reported the spontaneous occurrence of CLS during cycling, which lacks vertical movement of the trunk, suggesting entrainment may not be directly caused by the movement of pressure waves.

A second mechanism has been proposed, which builds on the notion that pressure waves benefit the heart and skeletal muscle. Since vertical movements of the torso were not required to produce CLS during cycling [5], this mechanism is likely more complex. In response to increasing arterial pressure, arterial baroreceptors increase their rate of discharge during cardiac systole [41]. Upon reaching the cardiovascular medullary center, these afferent signals effectively decrease sympathetic and increase parasympathetic outflow to the heart, with the latter resulting in a prolonging of R-R interval [41], potentially leading to entrainment [18,42]. This mechanism supports the findings of Niizeki et al. [43] who produced entrainment using only pneumatic pressure cuffs, in a seated position, demonstrating that upward and downward body movements are not necessary to promote entrainment. While Niizeki et al. (2004) did not evaluate possible metabolic or cardiovascular benefits, synchronizing cardiac cycle with mechanically produced pressure waves has become a common strategy for increasing cardiac output in clinical settings [15,16].

Clinical External Counterpulsation: Similar to CLS, the primary objective of mechanical counterpulsation is to force blood towards the heart immediately after the aortic valve closes during early diastole [15,16]. During early diastole, intra-aortic pressure exceeds intra-ventricular pressure [7], occurring at approximately 45% of R-R interval [8] . Whereas running accomplishes this through vertical movements [4], external pneumatic cuffs and intra-aortic balloon pumps generate pressure waves through cyclic inflation/deflation [15,16]. Both external pneumatic cuffs and intra-aortic balloon pumps are currently being used to treat patients with heart failure, myocardial infarction or other conditions that limit cardiac output, as these tools have been shown to increase venous return and cardiac output [15,16]. However, these clinical alternatives are not practical for most people, since clinical alternatives are expensive, invasive, and generally reserved for otherwise unhealthy populations. This places CLS through running in a unique position. Research into the potential benefit of CLS is promising, with studies showing outcomes that could significantly improve performance in athletes and health outcomes in clinical populations.

Benefits of CLS: With optimal synchronization of step phase and cardiac diastole, cardiac output [1,4,5,10] and skeletal muscle blood flow [12] may be enhanced. As a result of reduced left ventricular pressure and augmented diastolic pressure, cardiac perfusion may also be increased [4,5,8,10]. In a model by O'Rourke and Avolio (1992), 180-degree synchronization of step rate and heart rate reduced peak pressure during systole by 30 mmHg and diastole by 19 mmHg. From this model, researchers also reported ~17% reduction in myocardial oxygen demand, and ~22% increase in myocardial blood flow. Furthermore, when cardiac and step phases are close but not perfectly matching, intermediate effects may be expected [4], specifically a moderately-increased stroke volume and moderately decreased oxygen utilization.

A recent study on elite endurance runners compared guided diastolic CLS occurring at 45% ± 15% of R-R interval to systolic CLS occurring at 100% R-R interval during 30 minutes of steady-state treadmill running [8]. In their study, diastolic CLS produced significantly lower expired minute ventilation (V_E) and respiratory exchange ratio (RER), along with significantly higher O_2 Pulse (a surrogate for stroke volume), compared to systolic CLS. Udo et al. (1990) reported significantly lower oxygen consumption when step rate and heart rate were close, suggesting metabolic benefits. Cardio-respiratory entrainment during running has also been shown to decrease oxygen consumption [44]. In the study by Constantini et al. (2018), 87% of participants maintained a lower heart rate during diastolic CLS compared to systolic CLS, suggesting cardiovascular benefits. This study was the first to measure the metabolic and cardiovascular effects of guided diastolic vs. systolic CLS running during prolonged running. Their findings highlight potential performance and health benefits associated with guided CLS. Such favorable effects on heart rate and stroke volume seem to support the concept of oppositional pumps working in synchrony to maximize cardiovascular efficiency [4]. When modeled, CLS at 45%-50% of R-R interval will likely decrease cardiac afterload and increase ventricular diastolic pressure [4,10]. These outcomes are comparable with clinical counter pulsation devices, yet potentially more accessible.

In the study by Constantini et al. (2018), diastolic CLS significantly enhanced metabolic variables (V_E and RER), highlighting important health benefits associated with CLS. If metabolic efficiency is enhanced, muscle glycogen may be spared during exercise, perhaps prolonging aerobic metabolism [22]. By prolonging aerobic metabolism during incremental exercise, maximal oxygen consumption (VO_2max) may be increased, which would greatly benefit individuals with limited exercise capacity [23]. Maximal oxygen consumption (VO_2 max) has been widely used as an indicator of cardiorespiratory fitness and overall health [24]. Low levels of maximal oxygen consumption have been associated with high risk for cardiovascular disease, while high levels are associated with a longer lifespan and quality of life [24]. Decreased heart rate, associated with diastolic CLS, may also infer significant performance benefits for endurance sports. Relatively small decreases in heart rate maintained over a long period of time can have profound effects on race time. Constantini et al. (2018) calculated that a 2.6 bpm decrease in heart rate during sub-maximal running can decrease race time during a marathon (42.2 km) by 172 seconds.

Study Aim and Hypothesis: The aim of the present study is to measure the metabolic, cardiovascular, and subjective responses to CLS during maximal treadmill running. To our knowledge, no studies have specifically measured the effects of cardio-locomotor synchronization on VO_2 max, nor has any study measured perceived exertion (RPE) during guided CLS. Only one study by Phillips and Jin (2013) assessed a subjective response, where participants reported running to be easier during guided CLS. Though CLS did not increase running speed for all participants, a few mentioned that CLS running gave them "more energy" [6].

To explore the aforementioned outcomes, we will use a within-subjects design to measure the effects of CLS. In contrast to Constantini et al. (2018) who compared diastolic CLS with systolic CLS, the current study will compare diastolic CLS with subjects' natural pace (NP), both of which will be guided through audible metronome. NP condition will act as the control condition, allowing us to compare effects of diastolic CLS to those which participants naturally display. Doing so will also allow us to measure feasibility in guided CLS as we document how often participants obtain and maintain CLS. This will also allow us to measure relationships between the amount of time stepping during diastole and outcome measures.

We hypothesize that participants will reach a greater VO_2max when performing CLS during treadmill running, compared to NP condition. We expect outcome measures during baseline and NP conditions to be identical, attributing metronome pacing to any differences we may see. In accordance with Constantini et al. (2018), we hypothesize that heart rate will be lower during diastolic CLS compared to subjects' natural pace. If metabolic and cardiovascular efficiency is indeed increased through diastolic CLS, we also hypothesize decreases in perceived exertion (RPE) during exercise in the diastolic CLS condition. Based on the model by O'Rourke et al. (1992) we expect intermediate effects when CLS is performed yet imperfectly timed, such as when foot strike occurs during ± 15% of the optimal 45% R-R interval. Such effects may include moderate decreases in oxygen utilization, heart rate, and perceived exertion.