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ACUTE EFFECTS OF LONG DISTANCE RUNNING ON C-REACTIVE PROTEIN AND ARTERIAL STIFFNESS

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ACUTE EFFECTS OF LONG DISTANCE RUNNING ON C-REACTIVE PROTEIN
AND ARTERIAL STIFFNESS

By

Bridget E. Durocher

THESIS

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SIGNATURE APPROVAL FORM

ACUTE EFFECTS OF LONG DISTANCE RUNNING ON C-REACTIVE PROTEIN AND ARTERIAL STIFFNESS

This thesis by Bridget Durocher is recommended for approval by the student’s Thesis Committee and Department Head in the School of Health and Human Performance and by the Assistant Provost of Graduate Education and Research.

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Abstract

Central arterial stiffness, indicated by pulse wave velocity (PWV), is increased during the hour post ultramarathon (ULT), but it’s currently unknown if PWV remains elevated 24 hours after ULT or marathon (MAR). **PURPOSE:** To determine the effects of ULT and MAR on C-reactive protein (CRP), aortic blood pressure (BP), aortic augmentation index (AIX), PWV, and LF:HF ratio. We hypothesized that CRP, AIX, PWV and LF:HF ratio would be elevated 24 hours post-race. Our secondary hypothesis was that changes in PWV would be inversely correlated to the percentage of moderate activity (3-6 METs) during competition. **METHODS:** Applanation tonometry was used to measure aortic BP, AIX and PWV in 12 endurance athletes (36±2 yr) approximately 18 hours before and 24 hours after an ULT or MAR. Plasma CRP, resting BP, and LF:HF ratio were also measured. Intensity and pacing were quantified via wrist accelerometry. **RESULTS:** In relation to baseline vs. 24 hour post-race, respectively, CRP was significantly (p<0.05) elevated (0.5±0.1 vs. 7.0±1.0 mg/L), while resting aortic systolic BP (111±3 vs. 101±3 mmHg) and aortic diastolic BP (73±3 vs. 64±3 mmHg) were significantly lower. PWV and LF:HF ratio were similar 24 hours post-race compared to baseline, while AIX was lower (p=0.05) post-race (13±4 vs. 6±3%). Finally, changes in PWV from pre- to post-race were inversely correlated (r=-0.60) to the percentage of moderate activity. **CONCLUSION:** CRP was increased 24 hours post-race, while at the same time aortic BP was decreased. Reduced AIX may contribute to the acute exercise-induced hypotension in long-distance runners 24 hours post-race. Results from our secondary hypothesis indicated that long-distance runners may have better PWV outcomes by pacing with intermittent moderate intensity (i.e., jogging or walking).
Key Words: ACCELEROMETER, ARTERIAL STIFFNESS, AUGMENTATION INDEX, HEART RATE VARIABILITY, POST-EXERCISE HYPOTENSION, PULSE WAVE VELOCITY
DEDICATION

This thesis work is dedicated to my mentor, Dr. Scott Drum and my husband Dr. John Durocher. Dr. Drum was a catalyst in my transformation into an ultramarathon enthusiast. He spoke about the physiology of endurance exercise with such animation that I was finessed into the intricate lifestyle of balancing motherhood, a career, graduate studies and physical training. He spent incalculable hours working to provide me with the background knowledge necessary to begin my research studies. His remarkable aptitude to multifaceted cardiovascular disease processes, mechanisms and research methodology techniques captivated me into the science of studying ultra endurance events.

I created a schedule that many of my peers deemed to be absurd, and would have been, without the sustained encouragement of my family. John played a crucial role in providing an emotional, spiritual and intellectually stable state of well-being. Our multiplex relationship provided me with the mental stability needed to persevere through the exhausting times, while his dexterity created accomplishments within my research study. Together these two professors played an integral role in my achievements over the past two years.
ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. Phil Watts and Dr. Maggy Moore. Their time and dedication to this project was instrumental in my growth as a researcher. Morton Harwood, Jenna Edwards and Chelsea Strong, of Michigan Technological University, endured long hours of collecting and analyzing data as a collaborative effort to complete this work.

This project was funded by a Northern Michigan University Excellence in Education Grant. Additional funding was received from the Michigan Technological University Clinical and Applied Human Physiology Lab who also provided use of their laboratory equipment.
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CHAPTER I – To be submitted to Medicine & Science in Sports & Exercise

Central Blood Pressure, Wave Reflection and Inflammation after a Long-Distance Running Event

INTRODUCTION

**Paragraph 1** Participation in marathon and ultramarathon running events has been on the rise in recent years (18, 51). Despite the increased participation, the acute responses to these long-distance running events are not completely understood. For example, it is presently unclear if long-distance running participants are at an elevated cardiovascular risk or whether they are in a cardioprotective state the day after an event. Examining blood pressures, vascular function, and systemic inflammation before and after such events can help provide important insight into a person’s health when competing in these popular running events.

**Paragraph 2** Routine aerobic exercise is reported to reduce measures of arterial stiffness (39, 47, 48) and to induce post-exercise hypotension (PEH) (7, 29, 40, 41). Recent evidence demonstrated measures of central arterial stiffness were increased within the first hour following an ultramarathon (4), but it is unknown if these measures remain changed 24 hours post-race. Ultra distance triathlons are reported to result in increased sympathetic activation (11), indicated indirectly by the low-frequency to high-frequency (LF:HF) ratio determined from heart rate variability (HRV). In regard to systemic inflammation, C-reactive protein (CRP) is reported to not be elevated immediately post marathon (51, 52), but is elevated immediately after 24-hr (51) and 200 km (25)
ultramarathons and also 24 hours after a marathon (52). Finally, it is unclear if varying intensities may influence acute cardiovascular or inflammatory outcomes.

**Paragraph 3** The purpose of this study was to determine how ultramarathon events influenced cardiovascular responses. Our primary hypothesis was that CRP, pulse wave velocity (PWV), aortic augmentation index (AIx), and LF:HF ratio would be elevated 24 hours post-race. Our secondary hypothesis was that intermittent bouts of moderate intensity, quantified as a higher percentage of race time between 3 and 6 METs via wrist accelerometry, would result in lower measures of central arterial stiffness (i.e PWV).

**METHODS**

**Paragraph 4 Subjects.** Data is reported as mean ± SE. Twelve healthy endurance athletes (8 men and 4 women, n = 12), age 36±2 yr with a body mass index (BMI) of 22±1 kg/m², volunteered to participate in this study. Participant characteristics and pre-and post-testing times are shown in Table 1. All volunteers had significant marathon and/or ultramarathon experience. Testing was performed before and after either the Marquette Trail 50 (80 km trail race, n=5; 50 km trail race, n= 3) or Marquette Marathon (42 km road race, n=4) races held in Marquette, MI. All participants were non-smokers, free of cardiovascular disease, and not currently taking any type of medications. They abstained from exercise, caffeine, and alcohol for at least 12 hours before baseline testing. Additionally, all participants fasted for at least 3 hours prior to visiting the lab. Runners provided their written informed consent and all study procedures were approved
by the Institutional Review Boards at Northern Michigan University and Michigan Technological University.

**Paragraph 5 Study design.** All data collection was performed in the Exercise Science Laboratory at Northern Michigan University. First, anthropometric data such as height, weight and body fat were measured. Second, a 50 µL finger poke blood sample was obtained for CRP concentration. Third, the participant rested in a supine position for at least 5 minutes while ECG electrodes and tonometry pulse sites were marked, and then for an additional 5 minutes before any recording. Fourth, heart rate variability was recorded for 5 minutes during supine rest. Fifth, brachial artery blood pressures were measured in triplicate and averaged. Finally, pulse wave analysis was performed at the radial artery near the right hand to obtain two quality recordings. This was immediately followed by performing central pulse wave velocity measurements in duplicate at the carotid and femoral pulse sites.

**Paragraph 6 Anthropometrics.** Height was measured to the nearest cm with a standard stadiometer, while weight and body fat were recorded with a bioimpedance scale (Tanita BC-418). Body mass index was determined from the height and weight measurements above.

**Paragraph 7 Heart rate variability.** Following at least 10 minutes of supine rest, HRV recordings were performed for 5 minutes in a quiet supine position. These recordings utilized a standard 3-lead surface electrode configuration and a SphygmoCor system (CPVH, AtCor Medical, Sydney, Australia). The LF:HF ratio was determined and used as a general indicator of sympathetic vs. parasympathetic activation.
Paragraph 8 Blood pressures and pulse wave analysis. Brachial blood pressures were taken in triplicate after at least 15 minutes of supine rest with an automated cuff (Omron HEM-907XL, Omron Health Care, Vernon Hills, IL) with at least 1 minute between each recording. We reported the averages of these three readings (Table 1) and also used the average systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) to calibrate the SphygmoCor system. Pulse wave analysis was performed in a resting supine position by recording pressure waveforms from the radial artery on the right wrist using a small pressure transducer (Millar Instruments, Houston, TX) in conjunction with the SphygmoCor CPVH system. Pulse wave analysis recordings were completed in duplicate with an operator index of ≥ 80.

Paragraph 9 Pulse wave velocity. Central arterial stiffness was estimated from pulse wave velocity recordings that were obtained in duplicate from the right femoral and right common carotid artery. Proximal distance was calculated in mm from the suprasternal notch to the right common carotid site, and the distal distance was calculated in mm from the suprasternal notch to the right femoral artery. Next, pulse wave velocity was estimated from the foot of the pressure waveforms from the carotid and femoral sites (10 cardiac cycles at each site x 2 measurements at each site). The pressure waveforms were gated to the 3-lead ECG recordings as described by the manufacturer.

Paragraph 10 Accelerometry. Each participant was equipped with an Actical accelerometer (Respiration, Inc.) during baseline testing. It was worn on the wrist throughout racing and until post-race follow-up testing. Accelerometer data was downloaded and summarized according to manufacturer guidelines with markers placed at the start and end of the race. This data was summarized as moderate intensity exercise
between 3 and 6 METs and vigorous intensity exercise as greater than 6 METs. The percentages of race time at moderate and vigorous intensity were used for correlation analyses.

**Paragraph 11 Data analysis.** Height was reported to the closest 0.1 cm, weight to the nearest 0.1 kg, and body fat to the nearest 0.1%. LF:HF ratio or HRV was reported as a 5 min average while starting after at least 10 minutes of supine rest. Resting brachial blood pressures and resting heart rates were presented as the average of 3 supine recordings, with 1 min between each measurement, after at least 15 minutes resting supine. Brachial blood pressures were reported as SAP, DAP, mean arterial blood pressure (MAP), and pulse pressure (PP). Finally, pulse wave analysis and pulse wave velocity were recorded in a supine position following at least 20 minutes supine rest. From the pulse wave analysis recordings we reported aortic systolic arterial pressure (aSAP), aortic diastolic arterial pressure (aDAP), aortic mean arterial pressure (aMAP) and aortic pulse pressure (aPP). We also reported aortic augmentation index (AIx) from the radial pressure waveform recordings. Pulse wave velocity (PWV) was calculated from the average of two consecutive recordings from the carotid and femoral sites.

**Paragraph 12 Statistical analyses.** Repeated measures analysis of variance was utilized to compare major variables such as CRP, blood pressures, LF:HF ratio, AIx, and PWV from pre vs. post (condition x 2) all entered as within subjects factors with race distance entered as a between-subjects factor (i.e., 42 km 50 km and 80 km). No condition x distance interactions were detected (P > 0.05 for all), thus major variables were compared using 2-tailed paired t-tests. All P-values reported represent results from the paired t-tests. An *a priori* alpha was set at 0.05. All data are reported as means ± SE.
RESULTS

**Paragraph 13** Per Table 1, our results indicated body mass, SAP, DAP, and MAP were significantly decreased 24 hours post-race when compared to baseline values. In contrast, resting HR and HRV (LF:HF ratio) were similar when comparing the pre-race baseline values to 24 hour post-race values. General systemic inflammation was significantly (P < 0.01) increased from pre- to post-race as indicated by changes (i.e., elevated) in CRP, shown in Figure 1.

**Paragraph 14** Comparisons of pre-race baseline and 24 hour post-race aortic blood pressures are shown in Figure 2. Figure 3 demonstrates that while PWV was unchanged, AIx was significantly (P = 0.05) reduced 24 hours post-race. Even though mean PWV was unchanged, changes (Δ) or fluctuations in PWV were observed and associated with percentage of race time at a specific intensity. Figure 4a demonstrates that ΔPWV was inversely correlated with the percent of race time performing moderate activity, while Figure 4b shows that ΔPWV was directly correlated with the percentage of race time performing vigorous activity.

DISCUSSION

**Paragraph 15** This study is the first to examine inflammation, blood pressure, wave reflection, and sympathetic vs. parasympathetic tone 24 hours after a long-distance running event. We present one confirmatory and four new findings. Results of the present study confirmed that CRP is significantly elevated 24 hours after a long-distance running event. In addition, there are 4 novel findings: 1) brachial blood pressure is reduced 24
hours post-race, 2) aortic blood pressure is reduced 24 hours post-race, 3) AIx is reduced 24 hours post-race, and 4) PWV, heart rate and HRV were similar to pre-race values 24 hours post-race. Finally, our correlation data suggested that central arterial stiffness (i.e., PWV) may be lower 24 hours post-race when a participant performs intermittent moderate intensity bouts throughout the race even though these fluctuations may be incidental to stopping and starting, such as around an aid station.

**Paragraph 16** Two previous studies indicated that CRP was not elevated immediately after a marathon (51, 52), but became elevated 24 hours post-marathon (52) and at 12 hours and 24 hours of a 24 hour race (51). One other ultramarathon study reported a 3 fold increase in CRP after 100 km and 23 fold increase after 200 km (25). CRP was significantly elevated 24 hours post-race which was consistent with the findings of previous studies. Elevated CRP is reported to potentially stiffen arterial walls and prevent vasodilation (27) which may contribute to increases in blood pressure. Likewise one recent study reported simultaneous increases in peripheral arterial stiffness and CRP following a 3.5 day military drill that required participants to travel 135 km on foot (23). However, one previous study, after short duration maximal exercise, reported an inverse relationship between systolic arterial blood pressure and baseline CRP (7). Thus the effect of increased CRP post-race on PEH and arterial stiffness remains unclear. Results of our study are in contrast to Kampus et al. (23), as CRP was elevated 24 hours post-race, while brachial and central PEH persisted along with AIx being reduced. Our results are in agreement with a previous study where genetically induced increases in CRP did not result in an elevated risk for ischemic vascular disease (54).
**Paragraph 17** One previous ultramarathon study has demonstrated brachial PEH 5 minutes post-race (19) while other studies have reported brachial PEH up to 60 minutes post-race (4, 42). One of the aforementioned studies indicated that central arterial stiffness is increased within the first hour post-race (4), while another similar study reported that peripheral arterial stiffness was decreased at the same time (42). Results of the current study reveal that brachial and aortic PEH persist for 24 hours post-race. The duration of this PEH may be related to the duration and intensity during the long-distance running events (29, 40, 41). Although central arterial stiffness was unchanged 24 hours post-race in the present study, AIx was significantly reduced. It is possible that the reduction of AIx contributed to PEH 24 hours after a long-distance running competition.

**Paragraph 18** One previous study indicated an increased LF:HF ratio 24 hours after an Ironman triathlon (11), which represented an increase in sympathetic activation. Our results are in contrast to Gratze et al. (11), as the LF:HF ratio was unchanged by marathon or ultramarathon participation in the present study. The lack of change in LF:HF ratio in the current study may be due to the marathon or ultramarathon in the present investigation inducing a lower ‘dose’ of training. One previous study indicated that changes in LF:HF ratio are dose dependent (31).

**Paragraph 19** One recognized limitation in the present study was the relatively small number of participants. We coordinated recruitment with the race director for the Marquette Trail 50 race for several weeks in advance, but it was difficult to coordinate participation. Many participants traveled from a long distance, while others did not meet all of the study inclusion criteria. Because of the small number of participants in the ultramarathon, we examined 4 participants before and after the Marquette Marathon. We
do not view this as a major limitation because we did not detect any interactions of distance on our primary independent variables (i.e., blood pressure, AIx, and CRP).

**Paragraph 20** In summary, we determined that CRP was elevated 24 hours post long-distance running race, while at the same time brachial and aortic PEH persisted. At the same time point, AIx was significantly reduced which may contribute to the PEH observed. Heart rate, LF:HF ratio, and central PWV were not different than pre-race values at 24 hours post-race. Finally, the percentage of moderate intensity activity during the race appears to result in more favorable central PWV responses as demonstrated by the significant inverse correlation. In conclusion, metabolic inflammatory markers are increased 24 hours post-race, but brachial blood pressure, central blood pressure, and AIx were all significantly reduced one day after a long-distance running event.

**Paragraph 21 Acknowledgements.** This project was funded by an Excellence in Education Research Grant from Northern Michigan University to Bridget Durocher. The coauthors of the manuscript to be submitted to Medicine and Science in Sports and Exercise will include: Scott N. Drum, Phillip B. Watts, Marguerite T. Moore, Morton H. Harwood, John J. Durocher. We appreciate our volunteer participants and research assistance from Jenna Edwards, Courtney DeCramer, and Chelsea Strong.
Table 1. Participant testing time, weight, brachial blood pressures and heart rate.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Race</th>
<th>Post-Race</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing time (hrs)</td>
<td>18±3</td>
<td>24±1</td>
<td>= 0.061</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65±3</td>
<td>65±3</td>
<td>= 0.046</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>129±4</td>
<td>121±4</td>
<td>= 0.001</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>73±3</td>
<td>65±3</td>
<td>= 0.003</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>92±3</td>
<td>83±3</td>
<td>= 0.001</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>55±3</td>
<td>56±3</td>
<td>= 0.773</td>
</tr>
<tr>
<td>HRV (LF:HF ratio)</td>
<td>2±1</td>
<td>1±0</td>
<td>= 0.304</td>
</tr>
</tbody>
</table>

SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure; MAP, mean arterial blood pressure; HR, heart rate; HRV, heart rate variability in the frequency domain.
Figure Legends

**Figure 1.** Plasma concentration of C-reactive protein (CRP) is shown for pre- and post-race. Plasma CRP was significantly higher post-race than pre-race. *P < 0.01.

**Figure 2.** Aortic blood pressures are shown for pre- and post-race. Aortic systolic arterial pressure (aSAP), aortic diastolic arterial pressure (aDAP), and aortic mean arterial pressure (aMAP) were all significantly lower post-race, while aortic pulse pressure (aPP) was similar at pre- and post-race. *P < 0.01.

**Figure 3.** Measures of arterial stiffness are shown for pre- and post-race. Central pulse wave velocity (PWV; on the left) was similar at pre- and post-race, but aortic augmentation index (AIx; on the right) was significantly lower post-race. *P = 0.05.

**Figure 4.** Changes (Δ) in PWV versus the percentage of race time performing moderate activity (i.e. 3 to 6 METs) was inversely correlated as shown on the top. The bottom shows that ΔPWV was directly correlated to the percentage of race time performing vigorous activity (i.e. > 6 METs).
Figure 1.
Figure 2.
Figure 3.
Figure 4.

a) 

\[ r = -0.596 \]

b) 

\[ r = 0.609 \]
CHAPTER II – LITERATURE REVIEW

Overview of Distance Running

Ultramarathon running has grown in popularity since the first premier 100 mile running event in 1977 (18). That particular ultramarathon event reported 14 participants and 3 finishers, and currently there are 400 awarded slots for the race and about 300 finishers (18). An ultramarathon is classified as any running event over 26.2 miles (42km) (33). Some of the most common lengths in the United States are 50 km, 50 miles, 100 miles, timed 24 and 48 hour events, and multi-day events. Many of these races travel over mountainous terrain somewhat similar to the local Marquette Trail 50 (Marquette, MI) that is held annually in August.

Physiological Characteristics

The most common age range of ultramarathon participants is 40-44 years old (18) and the average body mass index (BMI) is 22-24 (kg/m$^2$) (26, 42). Knetchle et al. (26) report most ultramarathon runners train 11±6 hours/week during training. The percentage of male and female ultramarathon participants is not entirely clear, but one recent ultramarathon study included about 33% females (42). Ultra-endurance runners tend to have a high percentage of type I muscle fibers which aid in their ability to utilize aerobic metabolism and to prevent fatigue (34, 35). Well developed, but flexible, muscles of the lower body such as those of the quadriceps, hamstrings, and calves are also important to ultra-runners (35). The upper body, especially the upper limbs, should be slender and less
developed to improve performance (26). Finally, several other performance characteristics may be important to ultra-endurance runners, such as relative VO$_2$max, lactate threshold (LT), and running velocity at LT (51).

**Impact of Regular Aerobic Exercise on Metabolic and Cardiovascular Health**

Regular aerobic endurance exercise has numerous health benefits such as reducing: resting blood pressure (12), body fat (53), general inflammation (2), sympathetic autonomic tone (21), and arterial stiffness (21, 48). However excessive doses of aerobic exercise may potentially be harmful on these same measures of health. It is currently unclear how marathon running or ultra-marathon running impact cardiovascular health in regard to the relationship between inflammation (i.e., C-reactive protein) and arterial stiffness. Additionally, there is no evidence of how pacing strategies might impact short-term cardiovascular health. For instance, is acute cardiovascular health impacted negatively if someone has an undulating pace versus someone who runs at a steady pace throughout the race? Previous studies have shown that the arterial stiffness of marathon runners is higher than those of age matched physically active control groups (32). Yet the lower intensity and longer duration of ultramarathon runners have been controversial in this area. Therefore, the goal of the present review is to investigate how an extreme dose of aerobic exercise such as an ultramarathon influence traditional (i.e. CRP and brachial blood pressure) and non-traditional (i.e., arterial stiffness, aortic blood pressure and autonomic tone) cardiovascular risk factors (21).
C - Reactive Protein and Long-Distance Running

CRP is an acute phase protein that is an indicator of acute, general inflammation. Micro damage to the skeletal muscle created by exercise can induce and inflammatory response. This inflammation in conjunction with glycogen depletion can signal the production of cytokines, such as interleukin-6 (IL-6), which stimulates the liver to increase production of CRP (24). Excess plasma concentration of CRP may play a direct role in the development of atherosclerosis (51), and is associated with increases in endothelium stiffness and poor vasodilation (27). In addition, increases in CRP appear to be associated with increased sympathetic and decreased parasympathetic activation (14). Based on previous studies CRP appears to have a multifactorial influence on increasing cardiovascular risk.

A standard aerobic endurance exercise program can help reduce inflammatory markers such as C-reactive protein (46), but more extreme bouts of aerobic exercise such as a mountain marathon (52) or ultramarathon (25, 51) can lead to significant increases in plasma CRP. While CRP is not increased immediately at marathon distance (51, 52), it is greatly increased at 24 hours post marathon (51) and by 12 hours and 24 hours of a 24-hr race (51). Another study reports that CRP is significantly increased at 100 km and 200 km of a 200 km race (25). Wilhelm et al. (52) determined that CRP returned to baseline within 5 days after a mountain marathon, but they did not test between days 1 and 5 after the race. Another study by Irving et al. (20) specifies that CRP remained elevated for 3 days following a 56 km running race. Thus, it appears that ultramarathons induce a significant inflammatory response that persists for at least 3 days. It is possible that the extent of increased CRP following an ultramarathon is related to the eccentric muscle
contractions required when running downhill. One recent study reports that muscle damage and exercise-induced arterial stiffness appears to be related to eccentric contractions during downhill running (6).

**Heart Rate Variability and Ultra-Endurance**

Heart rate variability, or spontaneous changes in the R-R interval on an electrocardiogram, can be used to indicate sympathetic vs. parasympathetic autonomic nervous system tone. The frequency of changes in the R-R interval are specifically useful when converted from the time domain to the frequency domain with Fourier transform algorithm, with low frequency (LF) primarily representing sympathetic activation and high frequency (HF) indicating parasympathetic activation (30, 37). The LF: HF is often utilized to indicate the balance between sympathetic and parasympathetic activation, with an increase in the ratio indicating increased sympathetic autonomic tone (9, 22, 37).

Aerobic endurance training generally leads to a decrease in the LF: HF ratio; however, a recent study indicated that this response was dose-dependent and followed a U-shaped curve (31). In fact, another study by Gratze et al. (11) determined that sympathetic activation is increased (indicated by an increase in the LF:HF ratio) following an Ironman Triathlon, and that the LF:HF ratio remains elevated for at least 24 hours post-race. Therefore, it is likely that high-dose endurance events such as marathon or ultramarathon would also lead to increased sympathetic activation. Increased sympathetic activation, indirectly indicated by elevated LF:HF ratio, is associated with increased risk for hypertension, myocardial ischemia and myocardial infarction (30).
Blood Pressure and Ultramarathon Running

Two previous articles summarize the influence of aerobic endurance exercise on blood pressure and report significant post-exercise hypotension (PEH) within the hour following most aerobic exercise bouts (29, 40). The extent and duration of PEH appears to be influenced by intensity (7, 41) and increases in exercise duration up to 90 minutes (40). One early study on ultramarathon runners indicated that PEH was present 5 minutes after an 80 km running event (19). Two more recent studies on ultramarathoners indicate that PEH persists for at least 40 to 60 minutes after a 120 km or 195 km event (4, 42). It is presently unclear if PEH persists into the next day following completion of an ultramarathon running event. It is likely that PEH may persist for at least 24 hours, as one early case-study reported significant PEH for up to 4 to 10 hours after jogging (8). If PEH lasts into the next day following an ultramarathon, perhaps this could induce a significant cardioprotective effect (40).

Potential mechanisms for PEH include may include factors that reduce cardiac output (heart rate x stroke volume) and / or reductions in total peripheral resistance (TPR) (the overall resistance to blood flow in systemic vessels). Changes in TPR are mostly due to changes in the diameter of blood vessels (40). The specific mechanisms for PEH include reduced TPR (40), reduced HR (10), or reductions in plasma volume that could reduce stroke volume (19, 20). The primary mechanism for PEH appears to be the reduction in TPR through either a reduction in sympathetic activation and / or release of localized substances that can induce vasodilation (ex. nitric oxide) (40).
Central Blood Pressure and Arterial Stiffness

Normal aortic blood pressure (45) and reduction in reflected waves back towards the heart during systole are extremely important factors for cardiovascular health (3, 38). To perform aortic wave reflection analysis, a small surface tonometer is utilized to record a forward traveling wave generated during ventricular systole and to estimate reflected waves from the peripheral circulation, mostly from small vessels such as arterioles (38). The most important aortic wave reflection characteristic is the aortic augmentation index (AIx). AIx is used to non-invasively estimate systemic arterial stiffness and is calculated as: [peak aortic systolic pressure - inflection point of the reflected pressure wave] / [peak aortic systolic pressure - minimum aortic diastolic pressure] as shown below in Figure 5 (38). Nichols et al. (38) defined central arterial stiffness to be a key indicator of cardiovascular health. The central arterial pressure is derived from a forward travelling pulse wave that travels down the artery to the peripheral arterioles, or until it hits an area too stiff to continue. The wave from the periphery is then projected back to the central aorta. If the wave reflects back before the end of systole the augmentation index is then elevated, indicating arterial stiffness is increased and there is an elevated risk of cardiovascular disease (3, 38).

Although AIx is a strong indicator of vascular health by measuring aortic wave reflection and providing a general indicator of arterial stiffness (3, 38, 45), assessment of central (i.e. carotid to femoral) pulse wave velocity (PWV) is reported to be the ‘gold standard’ assessment for arterial stiffness (13). The pulse wave analysis technique is a desired method to AIx and trusted estimates of aortic blood pressures, while PWV is preferred for the most accurate assessments of arterial stiffness (13).
Arterial stiffness, as indicated by AIX, can fluctuate based on levels of estradiol in female participants. For instance, AIX is lowest during the late follicular phase of the menstrual cycle when estradiol is highest (1). Absence of the menstrual cycle, or amenorrhea, may also lead to poor vascular function due to low levels of estradiol (15, 28). Two recent studies have shown that supplementation with folic acid can improve endothelial function in amenorrheic athletes (16, 17). Therefore when assessing AIX and other wave reflection measures, controlling for the potential confounding effect of female sex hormones is advisable.

Numerous studies have indicated an association between increased physical activity / aerobic exercise and reduced arterial stiffness (39, 47, 48). Aerobic exercise can effectively decrease arterial stiffness in those that appear healthy (48) or clinical populations such as obese individuals (36). More recent studies have indicated that training for marathons (49) and ultramarathons (5, 43) does not appear to heighten aortic blood pressure or arterial stiffness. Two recent studies following ultramarathon indicate that central arterial stiffness is increased (4), but peripheral arterial stiffness is decreased (42) during the first hour post-race. It is somewhat unclear if measures of arterial stiffness are correlated to inflammatory markers post-ultramarathon, but a study on military cadets traveling 135 km over 3.5 days suggests that long distance exercise-induced increases in CRP may contribute to increased peripheral arterial stiffness (23).

**Potential Role of Intensity Varations on Arterial Stiffness**

It appears that reductions in arterial stiffness may depend on significant intensity (44) and duration (50). Some recent evidence suggests that intermittent exercise may be
more advantageous than continuous exercise for helping to induce the vascular adaptations necessary to reduce arterial stiffness. (50). While intermittent short-duration cycling exercise appears to induce a more favorable acute response in arterial stiffness (50), the influence of varying intensities on measures of arterial stiffness following an ultramarathon remain unknown.

Summary

Long-distance running events like ultramarathons or marathons appear to increase systemic inflammation, which is often quantified as plasma concentration of CRP. Long-distance running events appear to induce significant PEH for at least 1 hour post-event, but it is unclear how long PEH persists. It is likely that aortic blood pressure is also reduced post long-distance running event, but it is unclear how long aortic PEH may persist. Based on Ironman triathlon data, it appears that sympathetic activation, indicated by an increase in the LF:HF ratio, would be increased for at least 24 hours post-ultramarathon. Finally, previous literature demonstrates central arterial stiffness is increased during the first hour post-ultramarathon, while peripheral arterial stiffness is decreased at the same time. However, it appears that peripheral arterial stiffness may increase within the first day post-race in conjunction with increases in plasma CRP.
**Figure 5.** Demonstration of the aortic augmentation index (AIx). [Produced with Morton Harwood]

\[
\text{Aortic augmentation Index (AIx)} = \frac{AP}{PP}
\]
CHAPTER III – CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The present study had one verified one previous finding and presents four novel findings. Results of the present work confirm that long-distance running (i.e. ultramarathon or marathon) induces a significant elevation in plasma CRP that persists for at least 24 hours post-event. In addition, there are four new findings from the present study: 1) brachial PEH persists for at least 24 hours post-race, 2) aortic PEH persists for at least 24 hours post-race, 3) AIx is reduced at 24 hours post-race, and 4) 24 hour post-race PWV, heart rate and HRV are similar to pre-race values. Finally, our correlation data suggests that if a participant performs intermittent moderate intensity bouts throughout the race, central arterial stiffness (i.e. PWV) may be lower 24 hours after the race compared to those who maintained primarily a constant vigorous pace throughout the race.

Recommendations

It is recommended that a future study examines the role of exercise intensity on non-traditional cardiovascular outcomes such as central blood pressure and arterial stiffness. The present study explored the potential influence of exercise intensity on central arterial stiffness (i.e. PWV) responses to ultramarathon or marathon events. Figure 6A below demonstrates a consistent vigorous intensity (> 6 METs) during a 50 mile run for one participant, while Figure 6B demonstrates an intermittent intensities...
strategy with a higher percentage of race time performing moderate intensity activity (i.e. 3 to 6 METs). Our preliminary results suggest that a higher percentage of time at moderate intensity may result in more favorable (i.e. reduced) PWV outcomes. Thus, runners might choose to go out and enjoy an ultramarathon with some intermittent moderate intensity for health benefits, rather than having a performance outcome and performing consistent vigorous activity. The influencing of intensity on markers of acute cardiovascular deserves further research on a larger number of participants.
Figure 6. Continuous (A) and intermittent (B) intensity variations (blue line = 3 METs and pink line = 6 METs).

A)

B)
References


APPENDIX A – Informed Consent Form

Acute Effects of the Marquette 50 and Marquette Marathon on Cardiovascular Health

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Phone: (906) 227-2195

²Department of Biological Sciences
Michigan Technological University
Houghton, MI 49931
Phone: (906) 487-1659
Fax: (906) 487-3167
Email: bduroche@nmu.edu, jjduroch@mtu.edu or sdrum@nmu.edu

Introduction

We are writing to invite you to participate in a research study starting one or two days before the Marquette Trail Ultra-Marathon Race on August 17, 2013 in Marquette, MI and / or one or two days before the Marquette Marathon on August 31, 2013. The purpose of this study is to analyze your activity / sleep patterns starting 24 to 48 hours preceding the race and up to 15 days post-race with an ActiCal accelerometer (worn on the wrist), and arterial stiffness and blood C-reactive protein concentration once about 24 to 48 hours preceding the race and again 24 hours post-race.

M.S. student Bridget Durocher, Dr. John Durocher, Dr. Scott Drum and graduate/undergraduate research students will undertake this research study. Dr. Durocher and Dr. Drum are assistant professors in their respective departments listed above. Neither are medical doctors, nor will the experiment be supervised by a medical doctor. The purpose of this study is to determine the effect of marathon and ultra-marathon running on measures of arterial stiffness (i.e. a non-invasive estimate of how well your arteries stretch) and on C-reactive protein concentration (i.e. estimate of general inflammation via small finger poke blood sample). This study will also quantify your activity levels during the race and your sleep habits on the days surrounding the race with a very light (16 gram) wrist accelerometer.

You will be excluded from the proposed research study if you:
- Smoke
- Are diabetic or have ever suffered from a stroke or heart attack
- Take any heart/blood pressure medications or have a pacemaker
- Are pregnant or on hormone replacement therapy
- Weigh less than 110 pounds
- Have had caffeine, alcohol, or regular exercise in the 12 hours preceding laboratory testing
- Have not fasted for at least 3 hours prior to laboratory testing
- Have a resting blood pressure >140/90 mmHg and / or a central pulse pressure > 50 mmHg during the pre-race screening

**Experimental Protocol:**

If you agree to participate, we would like to collect information on you before, during, and after the Marquette Trail Ultra-Marathon Race and / or the Marquette Marathon Race, this data will include:

1) **Activity / sleep patterns** starting the day before the race until at least 24 hours post-race via ActiCal accelerometer. The small wrist watch-like devices can indicate energy expenditure, exercise intensity and sleep time.

2) **Participant data** including height, weight, body fat (via impedance scale), resting blood pressure, C-reactive protein concentration (you will have an ~50 µL finger poke blood sample taken by a trained investigator for this measurement), brief cardiovascular history for men and women, and menstrual cycle information from women.

3) **Heart rate variability testing** for a 5 minute resting supine baseline with a 3-lead electrocardiogram. Two surface electrodes will be placed near the sternum and one near the lower rib cage to record electrical activity of the heart.

4) **Arterial stiffness** will be recorded 24-48 hours before the race as a baseline measurement and then about 24 hours post-race with a non-invasive tonometer. This is a very small probe that simply needs to be held over the desired pulse site for 10 seconds. Two consecutive recordings of 10 seconds will be taken at each pulse site (i.e. radial, carotid and femoral).

5) You are required to report to the Exercise Science Laboratory on the first floor of the Physical Education Instructional Facility at Northern Michigan University for an orientation session to become familiar with the experimental protocol and equipment 24-48 hours before the scheduled race begins. We will answer any questions that you have at this meeting, and then proceed with pre-race measurements. You will also be required to report back to the same laboratory 24 hours after you complete the race for post-race measurements and at one more scheduled time to return your ActiCal accelerometer.

6) The following summarizes the experimental protocol to be performed at each laboratory visit.
<table>
<thead>
<tr>
<th>Activity</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set-up / Download ActiCal Accelerometer</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Record Descriptive Data (height, weight, bodyfat, etc.)</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Finger Poke Blood Sample</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Placing of Surface Electrodes and Marking Pulse Sites</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Heart Rate Variability Testing</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Tonometry Testing</td>
<td>10 minutes</td>
</tr>
</tbody>
</table>

- Heart rate variability testing and tonometry testing requires you to lay relaxed in a supine position.

**Risk-Benefits**

We do not guarantee that you will derive personal benefits from this study. However, the pre-race laboratory test results (i.e. resting blood pressure >140/90 mmHg when averaged from two automated cuff readings and / or a central pulse pressure > 50 mmHg when averaged from two tonometry readings) could indicate you have an elevated risk for adverse cardiovascular events, such as a heart attack. If you have an abnormal reading on either of the two variables mentioned above, we will verbalize our results to you and provide written information explaining the increased health risks, including the suggestion that you should discuss the results with your physician; however, because we are not medical doctors and unable to diagnose adverse cardiovascular events, we cannot mandate that you don’t run the race. However, you would be excluded from the current study if your resting blood pressure or estimated central pulse pressure exceed the limits above. We recognize that you understand the inherent risks of running an endurance event (based on you signing a race waiver form explaining said risks), including the possibility of inducing a heart attack or other adverse event, such as severe dehydration or muscle cramps. Finally, your participation may benefit others by enabling scientists to learn more about the effects of long-distance running races on the cardiovascular system, including how blood pressure and/or arterial stiffness change from pre- to post-race.

**Compensation**

You will be provided a $20 gift card to Doncker’s in Marquette, MI for your participation.

**Risks, Inconveniences, Discomforts**

*Electrocardiogram*: Local skin reactions may occur in susceptible individuals. This is extremely unusual, but we can stop the experiment if this irritation occurs. To date, we have never had a problem with our electrodes. There is no other known risk.
Actigraphy: There are no known risks associated with actigraphy. Skin irritation could potentially arise around the wrist band used to secure the accelerometer to the participant.

Finger Poke Blood Sample: There is a very small risk of infection. To combat this, sterile techniques will be used, and an experienced investigator will perform the procedure in the Exercise Science Laboratory. All of the supplies used for this procedure are for single use only, thus further preventing the risk of infection. With only a 50 µL sample required this should produce minimal discomfort.

Applanation Tonometry: This is a non-invasive procedure that is comparable to someone checking your radial, carotid or femoral pulse via finger palpation. There is no known risk of undergoing the procedure; however, as stated prior, you may learn of an abnormal reading and how this may increase the chance of a cardiovascular event during exercise.

Impedance Analysis of Body Fat (Tanita BC-418): A very low voltage, high frequency, electrical current will pass through your body from the hand and feet electrodes, but you will not be able to detect the current. This current passes very easily through water-rich muscle, but is resistant to flow through water-poor fat. Thus the extent of resistance to this high frequency current is used to indicate body fat (i.e. high resistance indicating higher fat). There are no known risks; the manufacturer only recommends that those with pacemakers should not use the device and that testing should not occur during menstruation (for accuracy) in women.

Cost of Participation

There will be no cost to you for participating in this research.

Research Related Injury

In the event of physical and/or mental injury resulting from participation in this research project, Northern Michigan University and Michigan Technological University do not provide any medical, hospitalization or other insurance for participants in this research study, nor will Northern Michigan University or Michigan Technological University provide any medical treatment or compensation for any injury sustained as a result of participation in this research study, except as required by law. If you are taking medications, it is your responsibility to consult with your physician regarding your participation in this research study. Do not volunteer for this study if you have been instructed to abstain from this research study by a physician. Any problems you experience throughout this study should be discussed immediately with your physician.

Confidentiality of Records

We will treat your identity with professional standards of confidentiality. The information obtained in this study may be published, but your identity will not be revealed. Paper files containing your subject information sheets and corresponding six digit code will be
stored in a secured file cabinet in Dr. Drum’s office or Dr. Durocher’s research laboratory (Dow 521). Northern Michigan University and Michigan Technological University reserve the right to inspect both the research data collected and your experimental records.

Withdrawal

Participation in this study is voluntary. Bridget Durocher, Dr. Drum or Dr. Durocher will answer any questions you may have about the study. Any significant new findings which develop during the course of the research study which in our opinion may affect your willingness to continue to participate will be provided to you as soon as possible. You are free to withdraw your consent and discontinue participation at any time and for any reason. This includes the right to withdraw during the actual test. If you withdraw yourself during the actual testing, you will still be compensated for that session.

Subject's Rights Information

The NMU and MTU Institutional Review Boards have reviewed my request to conduct this project. If you have any further questions regarding your rights as a participant in a research project you may contact Dr. Brian Cherry of the Human Subjects Research Review Committee of Northern Michigan University (906-227-2300) bcherry@nmu.edu. Any questions you have regarding the nature of this research project will be answered by the principal researcher who can be contacted as follows: Dr. Scott Drum (906-227-2195 office or 970-371-2620 cell) sdrum@nmu.edu. You may also contact the MTU-IRB at 906-487-2902 or email irb@mtu.edu.

Review Board Approval Number:_______

I, _______________________, have read through this consent form. The investigator provided me an opportunity to ask questions and I wish to voluntarily participate in this study.

__________________________________________         ______
Signature of Subject                           Date

__________________________________________         ______
Signature of Investigator                      Date
Hello,

You are receiving this e-mail because you are registered for the Marquette Ultramarathon and have expressed interest in participating in a research study about the event. In response to your inquiry, you should have received from us an informed consent document describing the study procedures, benefits, and potential risks in detail. We are contacting you to confirm your interest in the study, and also to schedule pre and post-race testing times with you.

Pre-race and post-race testing will each take between 45 minutes and one hour to complete. Pre-race testing may be completed at your convenience on Thursday, August 15th or Friday, August 16th. Post-race testing will be completed on Sunday, August 18th, and should be completed as near as possible to 24-hours after you finish the race. If you are still interested in participating, please respond with the following information to aid us in scheduling pre- and post-race testing times:

1. Your desired pre-race testing date (August 15th or 16th) and time.
2. Your expected race completion time. We will use this to help build a tentative post-race testing schedule, and will confirm an actual post-race testing appointment with you based on your actual race completion time.

Please note that although we prefer to conduct your post-race test as near as possible to 24-hours after you complete the event, your appointment can be adjusted slightly to accommodate travel or other scheduling conflicts.

Thank you for your interest, and we hope to see you this weekend!

Name
e-mail
Phone
Marquette Ultramarathon / Marathon Study  
Pre & Post Testing Procedure Outline

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<th>Supine Resting BP &amp; HR</th>
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<td>Setup Actical (pre-test) or download Actical data (post-test)</td>
<td>Descriptives</td>
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<tr>
<td>Finger poke blood sample</td>
<td>Place electrodes⁴; monitor/identify pulse sites²</td>
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1. Notes:

1. All resting BP & HR measurements will be recorded as the average of two measurements separated by one minute. Each of these two measurements must differ by ≤ 10.0 mm/Hg for DBP and SBP, and by ≤ 10.0 bpm for HR. If this is not the case, additional measurements will be taken (each separated by one minute) until two consecutive measurements meet these criteria.

2. Pulse sites that need to be marked: radial, femoral, carotid.

3. Criteria for central pulse pressure: must be ≤ 50 mm/Hg. Greater than 50 mm/Hg for two consecutive readings will exclude participants from the study.

4. Electrode placement sites: White – inferior to suprasternal notch; Red – xyphoid process; Black – just below 10th rib @ left midaxillary line.
APPENDIX D – Participant Descriptive Data

Pre-Race Data:

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APPENDIX E – Resting Cardiovascular and Metabolic Data

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<th>RHR (bpm)</th>
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<th>DAP</th>
<th>MAP</th>
<th>[CRP] mg/L</th>
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</tbody>
</table>
APPENDIX F – Accelerometry Data

<table>
<thead>
<tr>
<th>Distance</th>
<th>Time (hr:min:sec)</th>
<th>EE (kcal)</th>
<th>% Light</th>
<th>% Mod</th>
<th>% Vig</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 km</td>
<td>12:48:16</td>
<td>7522</td>
<td>4.56</td>
<td>29.43</td>
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<tr>
<td>80 km</td>
<td>12:44:43</td>
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<td>11:04:26</td>
<td>5543</td>
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<td>38.55</td>
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<tr>
<td>80 km</td>
<td>10:50:32</td>
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<td>7573</td>
<td>4.69</td>
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<td>50 km</td>
<td>6:28:24</td>
<td>3740</td>
<td>0.26</td>
<td>31.70</td>
<td>68.04</td>
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<td>50 km</td>
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<td>0.27</td>
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<td>50 km</td>
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<td>3143</td>
<td>0.28</td>
<td>11.11</td>
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<tr>
<td>42 km</td>
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<td>0.81</td>
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* Participant number 1 was the only participant that recorded sedentary activity (i.e. 1 to 1.1 METs) during the race which was 0.13 % of total race time.
### APPENDIX G – Means, Standard Deviations and Standard Errors for Major Variables

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<tr>
<th>Variable</th>
<th>Race</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
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<td>Weight (kg)</td>
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<tr>
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<td>Post</td>
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<td>HR (BPM)</td>
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