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Undergraduate Student Award Competition – Oral Presentation

- 1. Daily heat treatment maintains mitochondrial function and attenuates atrophy in human skeletal muscle subjected to immobilization**

*Kaitlin Abbott, Paul S. Hafen, Jennifer Bowden, Ryan Lopiano, Chad R. Hancock, and Robert D. Hyldahl
Brigham Young University, Utah*

- 2. 6th Vital Sign App: Testing Validity and Reliability**

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- 3. Comparison of an iPad Application and 3D Body Scanner to the Bod Pod for Measurement of Body Fat Percentage**

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Utah State University, Utah*

- 4. Differing Impact of Weight Cycling on Ambulatory Blood Pressure versus Conventional Blood Pressure Assessment: A Possible Explanation to Controversy**

*Mallory Durnbaugh and Zachary Zeigler, Ph.D.
Grand Canyon University, Arizona*

- 5. Downhill Running: An Effective Countermeasure to Limitations of Exercise in Acute Hypoxia?**

*Felipe Gorini Pereira and Trevor Gillum, Ph.D.
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- 6. Effect of Fed State on Affective Response to Exercise Following Public Health Guidelines**

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1. Daily heat treatment maintains mitochondrial function and attenuates atrophy in human skeletal muscle subjected to immobilization

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Background:

The loss of skeletal muscle mass (atrophy) that accompanies disuse can lead to the development of several chronic diseases (e.g. type II diabetes and obesity) and contributes to the loss in work capacity throughout the lifespan (Wolfe, 2006; Russell, 2014). Evidence suggests that the maintenance of mitochondria during periods of disuse protects skeletal muscle against atrophy (Romanello, 2010). Along these lines, rodents with denervated muscle are protected against muscle atrophy following whole-body heat stress. The mechanism of protection appears to be tied to the observed increases in heat shock proteins (HSPs) and PGC-1 α , following heat stress (Tamura, 2015). These potential mechanisms of protection are supported by transgenic animal research that has shown significant reductions in muscle atrophy with the overexpression of PGC-1 α (Sandri, 2006), HSP27 (Dodd, 2009), or HSP70 (Senf, 2008).

Heat-induced protection against muscle atrophy has not yet been shown in human skeletal muscle. It has been shown previously that pulsed shortwave diathermy results in significant increases in intramuscular temperature (~3-4 °C), increasing both HSP and PGC-1 α expression in human skeletal muscle (Hafen, 2018). Thus, the aim of this study was to test whether daily heat stress could maintain mitochondrial function and provide protection against muscle atrophy in human skeletal muscle subjected to 10 days of single-leg immobilization.

Methods

Subjects and Ethical Approval: Twenty-four young adults (12 male, 12 female; 18-39 years) were recruited for this study. Inclusion criteria included regular exercise (≥ 3 hrs/wk) and passing a screening questionnaire. Exclusion criteria for subjects included: known cardiac or peripheral vascular disease and/or a high body mass index (BMI > 25). The study protocol was approved by the Brigham Young University Institutional Review Board and conformed to the standards set by the *Declaration of Helsinki*.

Study Design: This study was a single-blinded, sham-controlled study. After providing written informed consent, participants were fitted for a therapeutic knee brace (Bledsoe Revolution 3). On the first day of the study, the left quadriceps muscle group of each participant was scanned using MRI, after which muscle biopsies were collected. Immobilization and heating protocol began 5 days following the first muscle biopsy. The left leg of all participants was immobilized, and each participant was randomly assigned, in a counterbalanced fashion, to either the control (Imm; diathermy placed over the muscle but not emitting waves) or treatment (Imm + H; diathermy placed over muscle and emitting) groups. All participants reported to the lab for 2 hrs per day over the 10 consecutive days of immobilization to receive their heating or sham treatment. Subjects were not told which group they were a part of. For confirmation of intramuscular temperature during treatments, both a temperature probe and visual analog scale of warmth were used to quantify perceived and actual heat changes in the muscle.

Participants reported to the laboratory 24 hrs after their last heating session for the final MRI scan and muscle biopsies, according to the procedures used for the baseline measures.

Mitochondrial Respiration: Measurements of skeletal muscle mitochondrial respiration were performed on permeabilized fibers using a Clarke oxygen electrode high-resolution respirometer (Oxygraph O2k, Oroboros Instruments). To assess mitochondrial respiratory capacity, we followed a standard substrate-uncoupler-inhibition-titration (SUIT) protocol, allowing us to assess individual components of the respiratory chain (Gnaiger, 2009).

Protein Immunoblotting: Muscle tissue was homogenized in chilled homogenization buffer, with added protease inhibitors. Homogenates were centrifuged and stored at -80°C. Protein concentration was determined using a Pierce™ Bicinchoninic Acid (BCA) kit (ThermoFisher) and spectrophotometer (Victor3™, Perkin Elmer), according to the manufacturer's specifications.

Both samples from each subject were run on the same gel to avoid variability. Following electrophoresis, standard Western Blotting procedures were followed. The primary antibodies used in this study were for HSP70, HSP90, PGC-1α, MAFbx, Murf1, and Total Oxphos Human Cocktail. The Total Oxphos Human Cocktail consisted of 5 antibodies, one each against Complex I subunit NDUF8, Complex II subunit 30 kDa, Complex III subunit Core 2, Complex IV subunit II and ATP synthase subunit alpha. All data were normalized to total protein (Ponceau) to account for potential variations in loading.

Myofiber CSA: Tissue sections were immunostained for dystrophin, myosin heavy chain I, and DAPI. Dystrophin allowed visualization of the muscle fiber membranes, while myosin heavy chain staining was used to differentiate between Type I and Type II fibers. Sections were then subjected to a blocking, primary and secondary incubation protocol. Imaging was done using an Olympus IX73 microscope and Olympus XM10 camera. Images were taken at 10 and 20X magnifications.

Statistics: A mixed model analysis of variance (ANOVA) was used to examine the effects of time (pre vs. post) and group (Imm vs. Imm + H) on our dependent variables (mitochondrial respiration, protein content, CSA). In the case of a significant [time x group] interaction, individual comparisons were made using student t-tests with Bonferroni corrections for multiple comparisons. JMP® Pro 13.0.0 (©SAS Institute, 2016) statistical software was used for all statistical analyses with alpha set *a priori* at 0.05.

Results

Intramuscular temperature and HSP expression: Diathermy treatment resulted in a significant increase ($4.2 \pm 0.29^\circ\text{C}$, $p < 0.0001$) in intramuscular temperature, while there was no change observed in the sham group during the 2-hr sham heating period (Figure 1A). Reported sensations of warmth from the 10-cm visual analog scale

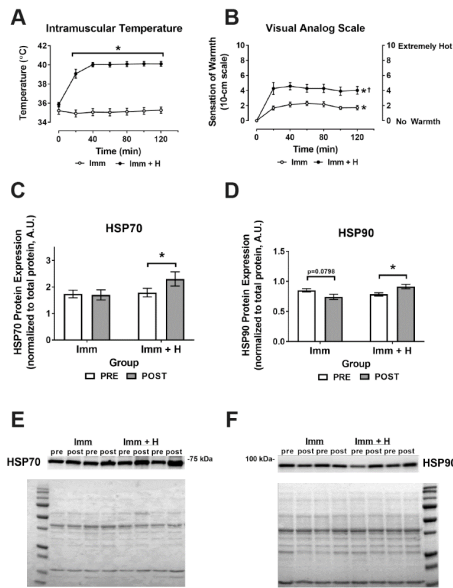


Figure 1. Effect of diathermy heating. A single, 2-hr diathermy treatment (Imm + H) resulted in elevated (A) intramuscular temperature, and (B) sensations of warmth compared to control (Imm). Following the 10-day immobilization period, (C) HSP70 and (D) HSP90 were significantly increased with Imm + H. Representative blots and ponceau red stains for (E) HSP70 and (F) HSP90. * $p < 0.05$, significant increase from baseline (pre). † $p < 0.05$, significantly different sensation of warmth between groups. Data are mean \pm SEM, $n = 23$.

measured as CI-mediated, maximal coupled, maximal uncoupled, and CII-mediated uncoupled respiration. However, with daily heat therapy (Imm + H), mitochondrial respiratory dynamics were maintained following the immobilization protocol, as there were no changes in GM ($p = 0.5184$), GMp ($p = 0.5116$), GMSp ($p = 0.6553$), GMSe ($p = 0.0884$), or S[Rot]e ($p = 0.0655$). Furthermore, with Imm only, these alterations resulted in decreased OXPHOS coupling efficiency (0.88 ± 0.017 vs. 0.77 ± 0.038 , $p = 0.0003$) and ETS coupling efficiency (0.91 ± 0.016 vs. 0.83 ± 0.024 , $p < 0.0001$), while neither OXPHOS or ETS coupling efficiency were altered with Imm + H ($p = 0.8723$ and $p = 0.7407$, respectively; Figures 2C-F). We observed no changes in the OXPHOS control ratio (P/E coupling control ratio) within either group. (Figure 2G).

Mitochondrial protein expression: In conjunction with decreased respiratory capacity, Imm resulted in decreases in the expression of proteins for all 5 of

increased during the 2-hr diathermy treatment session in both groups ($p < 0.0001$ and $p = 0.004$, respectively). Additionally, the heating group (Imm + H) reported higher levels of warmth than the sham (Imm) group (Figure 1B). Following the 10 day treatment period, the Imm + H group displayed increases in HSP70 and HSP90 protein expression ($p = 0.0002$ and $p = 0.0059$, respectively). With Imm only, HSP70 and HSP90 were unchanged following the 10-day immobilization period ($p = 0.5024$ and $p = 0.0798$, respectively; Figure 1 C-F).

Mitochondrial respiratory capacity: In response to immobilization, mitochondrial respiratory dynamics were significantly altered. The Imm group displayed increased leak (GM; 5.39 ± 0.49 vs. 7.69 ± 0.97 $\text{pmolO}_2 \cdot \text{sec}^{-1} \cdot \text{mg}^{-1}$, $p = 0.0136$). In the absence of ADP, this increased leak with Imm suggests greater intrinsic uncoupled respiration when oxygen flux is elevated to compensate for the proton leak as opposed to active phosphorylation of ADP to ATP (Figure 2A). In addition, Imm resulted in decreased respiratory capacity when

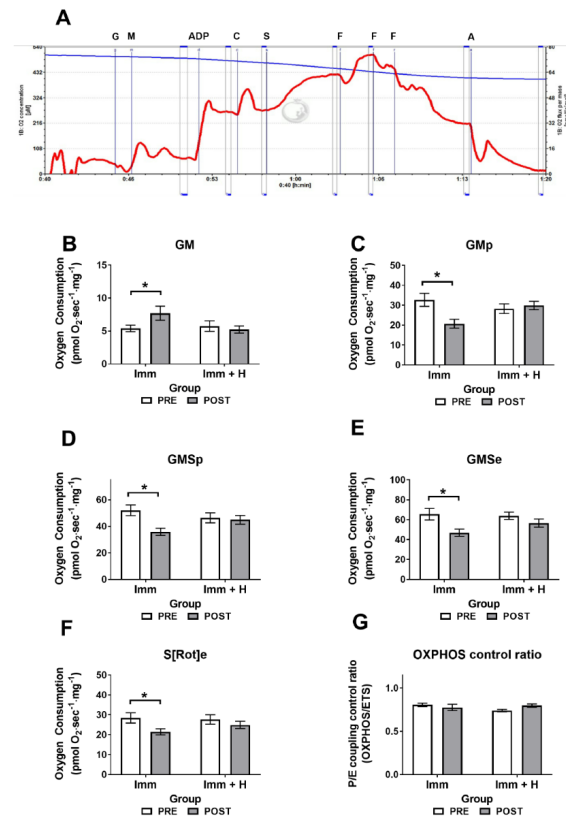


Figure 2. Mitochondrial respiratory capacity was altered after 10 days of immobilization (Imm), but was rescued with daily heating (Imm + H). (A) Representative respiratory run following the standard SUIT protocol with stepwise additions of Glutamate (G), Malate (M), ADP, Succinate (S), FCCP (F) uncoupler, and Antimycin A (A). Individual graphs for (B) Leak; GM, (C) Complex I-mediated; GMp (D) Maximal-coupled; GMSp, (E) Maximal-uncoupled; GMSe, and (F) Complex II-uncoupled; S[Rot]e respiratory capacity. (G) OXPHOS control ratio (GMSp/GMSe) was not changed following immobilization. * $p < 0.05$, significant change from baseline (pre). Data are mean \pm SEM, $n = 19-21$.

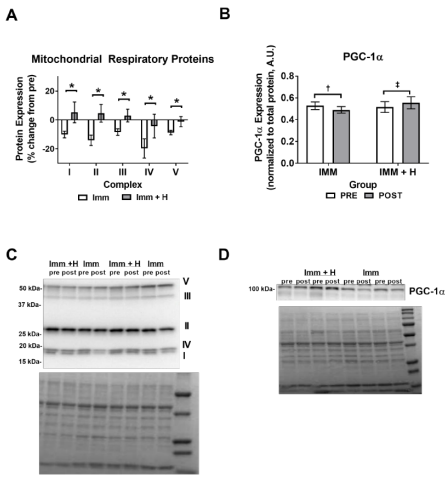


Figure 3. Mitochondrial respiratory complex and PGC-1 α expression decreased with immobilization only (Imm), but not after immobilization and daily heat therapy (Imm + H). **(A)** Expression of proteins for each of the 5 respiratory complexes decreased with Imm, but not Imm + H. **(B)** PGC-1 α expression decreased with Imm, but increased with Imm + H. Representative blots and ponceau red stains for **(C)** total OXPHOS and **(D)** PGC-1 α . * $p < 0.05$, significant difference between groups. † $p < 0.05$, significant decrease with Imm. ‡ $p < 0.05$, significant increase for Imm + H. Data are mean \pm SEM, $n = 18-23$.

the respiratory protein complexes. Specifically, we observed decreases in Complex I subunit NDUFB8 ($-10 \pm 2.3\%$, $p = 0.0056$), Complex II subunit 30 kDa ($-15 \pm 3.5\%$, $p = 0.0043$), Complex III subunit Core 2 ($-8 \pm 2.3\%$, $p = 0.0133$), Complex IV subunit II ($-22 \pm 8.6\%$, $p = 0.0106$), and ATP synthase subunit alpha ($-9 \pm 1.5\%$, $p = 0.0020$). However, Imm + H prevented the decrease in protein expression (Figure 3 A, C). Moreover, we observed decreases in the expression of PGC-1 α with Imm (0.54 ± 0.039 vs. 0.50 ± 0.037 AU, $p = 0.0284$). Conversely, with Imm + H, PGC-1 α expression was increased following the 10-day immobilization period (0.49 ± 0.056 vs. 0.54 ± 0.064 AU, $p = 0.0337$; Figure 3 B, D).

Muscle cross-sectional area (CSA): Whole-muscle CSA decreased 7.3% with Imm. The decrease in *vastus lateralis* CSA was significantly larger ($p = 0.0256$) than the 4.5% decrease observed with Imm + H (Figure 4A-B, I). Similarly, myofiber CSA decreased by 10.8% with Imm. This decrease was significantly larger ($p = 0.0359$) than the 5.8% decrease following Imm + H. In addition, while type I fibers were significantly smaller than type II fibers ($p = 0.0051$), fiber type did not demonstrate an interactive ($p = 0.9340$) effect on changes in CSA, between groups, over time. Thus, the degree of atrophy appeared to be independent of fiber type (Figure 4C-H, J).

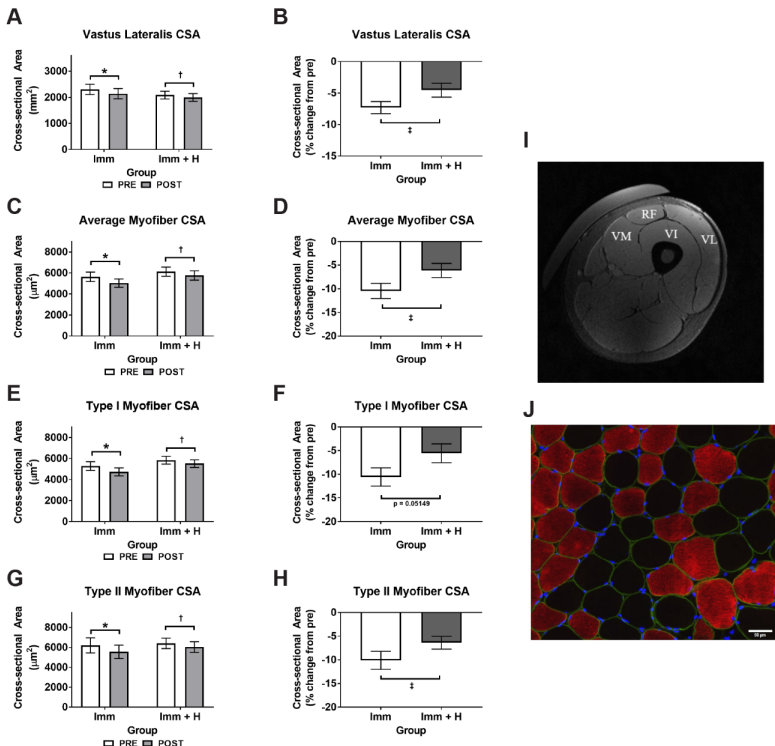


Figure 4. Muscle cross-sectional area (CSA) was decreased to a lesser extent after 10 days of immobilization with heating (Imm + H), compared to control (Imm). Decreases in both **(A & B)** whole-muscle CSA of the *vastus lateralis*, and **(C-H)** myofiber CSA were attenuated with heating. **(I)** Representative MRI image with all 4 quadriceps muscles labeled (VM- vastus medialis, RF- rectus femoris, VI- vastus intermedius, VL- vastus lateralis). Note the silicone mold on the top of the quadriceps to mark the plane of heating. **(J)** Representative image of myofiber staining (blue- DAPI, green- dystrophin, red- MHC I). * $p < 0.05$, significant decrease with Imm. † $p < 0.05$, significant decrease with Imm + H. ‡ $p < 0.05$, significant difference between groups. Data are mean \pm SEM, $n = 17-20$.

Discussion

This study provides the first evidence that deep-tissue heating in human skeletal muscle subjected to immobilization is capable of: I) maintaining mitochondrial function and II) attenuating muscle atrophy. These findings are consistent with previous explorations using whole-body heat stress as a means to combat disuse atrophy in rodents. (Naito, 2000; Tamura, 2015). Our findings in humans add to a growing body of literature that suggests heat treatment as a potential countermeasure for the metabolic and functional consequences that accompany muscle disuse.

We observed significant alterations in mitochondrial respiratory capacity with Imm. These findings reinforce previous observations that two-weeks of leg immobilization results in substantial losses of respiratory proteins and mitochondrial respiratory capacity in both the young and elderly (Gram, 2014). Other studies corroborate the profound decreases in metabolic and mitochondrial-related proteins, which precede human muscle disuse atrophy (Brocca, 2012). Thus, the preservation of mitochondrial function observed here appears to be an important mechanism for heat-induced muscle sparing. Our data suggest that daily muscle heating is capable of preventing the loss of the respiratory proteins and preserving mitochondrial respiratory capacity.

In addition to maintaining mitochondrial function, muscle atrophy was reduced by 37% with daily heat therapy. It is likely that this muscle sparing effect of heat therapy can be attributed not only to the beneficial metabolic adaptations observed (increased PGC-1 α and preserved mitochondrial function), but also to increased expression of the heat shock proteins (HSPs). In this study, both HSP70 and HSP90 were significantly increased following Imm + H. When overexpressed in rodent skeletal muscle, HSP70 has been shown to completely abolish skeletal muscle atrophy caused by immobilization (Senf, 2008).

In conclusion, we show that heat therapy, applied for 2 hrs daily during 10 days of immobilization, prevents losses of mitochondrial function and attenuates atrophy in human skeletal muscle. The results of the study have clear and potentially far reaching clinical implications. While exercise interventions remain the most effective strategy to maintain, or even increase, respiratory capacity and/or muscle size, many patients for whom exercise would be most beneficial are either unable to exercise (i.e. immobilization post-surgery, on bed rest) or have very low tolerance for exercise (i.e. aged, obese, diseased populations). In these populations, heat therapy may serve as an alternative or adjunct therapy to maintain skeletal muscle metabolic function and size.

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2. 6th Vital Sign App: Testing Validity and Reliability

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Abstract

Purpose: Walking tests are simple easy tests to examine health. These tests are useful indicators to foretell different health outcomes in people. With technology continuing to advance, these tests can be administered by the convenience of one's smartphone. In order to give legitimate results, the device or application must demonstrate valid and reliable results.

Methods: This study used the 6th Vital Sign app to have 15 CSUMB students walk at their normal pace for two minutes to test the app's validity and reliability. Distance and gait speed were recorded in each trial. Speed was recorded by the 6th Vital Sign app, Brower timing gates, and by hand measurement of distance walked divided by 2 minutes. Validity was tested by t-test and Cohen's D effect size used to compare the gait speed and app speed. Reliability was tested by paired sample t-tests and Cohen's D effect size to compare inter-trial reliability of each method.

Results: The timing gate and the hand measurements were not different on average ($t = 0.380$, $p = 0.709$). The app's speed was compared to gate speed using a paired t-test, which concluded that their speeds were not similar ($t = 3.405$, $p = 0.004$). The paired t-test for speed measured by the app and by hand showed that they were not similar as well ($t = 3.015$, $p = 0.009$).

Conclusion: The Sixth Vital Sign app demonstrated to be significantly reliable, however not valid, when compared to the timing gates and the hand measurements.

Introduction

In the United States of America (USA), there have been ongoing trends across many measures indicating that the population is becoming less healthy.¹⁻⁴ Therefore, allied healthcare professionals need to test clients prior to initiating primary and secondary prevention interventions and to track progress. Healthcare workers can use walking speed tests to predict current functional independence and future health deterioration,⁵ screen for chronic lifestyle diseases such as hypertension, and help with clinical decision making such as whether they will be homebound, the likelihood of hospitalization and the location of release after hospital visits.⁶⁻⁹

Typical laboratory or clinical gait testing methods, using sophisticated kinematic equipment such as optoelectronic motion capture systems, force plates, and instrumented walkways,¹⁰ are highly valid and reliable, but their expense and size make them prohibitive for use outside of a research laboratory. These types of equipment are not regularly accessible and call for trained personnel. An additional limitation is that they can only measure up to a few steps, which may not represent longer duration gait ability in individuals. Accelerometers are more accessible because they are cheaper and they are more practical because accelerometers can be used in more difficult settings. Today, most smartphones come installed with a 3-dimensional accelerometer, gyroscope, and a compass with equal sensitivity as research-grade biomechanical equipment.^{10,11} Using a smartphone as a testing device for movement velocity has become an appealing option for researchers and clinicians, and can be used by a patient to track their own health.¹⁰⁻¹⁵

In 2016, researchers from Duke University launched the 6th Vital Sign app for measuring walking speed and assessing public health.¹⁶ However, with their initial launch, they did not include preliminary validity or reliability data. The purpose of the current study was to test the reliability and validity of the 6th Vital Sign app.

Methods

This project was approved by the CSUMB Committee for the Protection of Human Subjects. All participants provided written informed consent before commencing the assessments. CSUMB students were asked to attend one session at the Exercise Physiology laboratory. Participants were instructed to refrain from eating, smoking, or ingesting caffeine or alcohol within 3 hours of their testing session, or from exercise prior to their testing session, and to wear athletic shoes and clothing. After 5 minutes of seated rest, participants were assessed for resting blood pressure and heart rate. They were then assessed for height using a stadiometer, and weight and body fat percentage using a Tanita BF-350 Total Body Composition Analyzer (Tanita, Tokyo, Japan). Participants were then equipped with a Polar Heart Rate (HR) Monitor and watch (Polar Electro Inc., Kempe, Finland) to monitor HR during the testing session.

Next, participants were brought to the track to complete the walk test. The track was permanently marked at the point where the timing gates needed to be set, the point where participants started, and at meter intervals from 150m to 300m to ensure accuracy and repeatability of measurements. Brower Timing Gates (Brower Timing Systems, Draper, USA) were placed at the starting line and at 100m. In this study, the app was downloaded to an iPhone 7 plus, measuring 158.2mm x 77.9mm x 7.3 mm and weighing 188 g. The same phone was used at all testing sessions. During the test, the smartphone was placed in a custom fitted pouch attached to a belt with a d-ring buckle that the participants fit snugly over their left iliac crest.¹⁰

When ready, participants started with their toes on a line marked 30 cm from the first timing gate.¹⁷ Participants were instructed to walk at their normal pace in the indicated lane for 2 minutes when they heard the starting signal from the app and stop when they heard the stopping signal from the app. Participants were instructed to stand still at the exact place they stopped until measurements were recorded. The smartphone was then placed in the pouch and the trial began. After 2 minutes, participants stopped in place and researchers hand measured

the total distance walked by placing a cone at the participant's toes. Distance was recorded by measuring the distance from the last meter mark on the track to the cone with a tape measure. Participants returned to the start and sat down until their heart rate returned to resting level or 5 minutes had elapsed, then the trial was repeated.

Speed was measured by the 6th Vital Sign app, Brower timing gates (this was used as the criterion standard), and by dividing the hand-measured distance by 2 minutes.

To assess concurrent validity, T-tests were used to compare (i) the means of gate speed and app speed and (ii) the means of gate speed and hand-measured speed. Cohen's D effect sizes were calculated to compare (i) gate speed and app speed, (ii) gate speed and hand speed, (iii) hand speed and app speed. This effect size quantifies how much the hand measurement and the app measurement deviate from the timing gate measurement.

In order to check for the reliability of hand measured speed, app speed, and gate speed, paired samples t-tests were used to compare inter-trial reliability of each method. Cohen's D effect sizes were calculated based on on trial 1 and trial 2 for each of the three walking pace measurements. This effect size quantifies the consistency of an individual's walking speed from trial to trial via each method of measurement.

Results

Fifteen participants (4 males, 11 females; 21.5 ± 2.75 years; $25.57\% \pm 12.22\%$ body fat percentage; 164.46 ± 8.90 cm tall) completed the assessments. There were two trials per subject. The average speed was taken from the app, hand measurements and gate speed. The average speed of the two trials are: gate speed 1.444 ± 0.166 m/s, hand speed 1.438 ± 0.200 m/s, and average app speed was 1.268 ± 0.189 m/s (Figure 1).

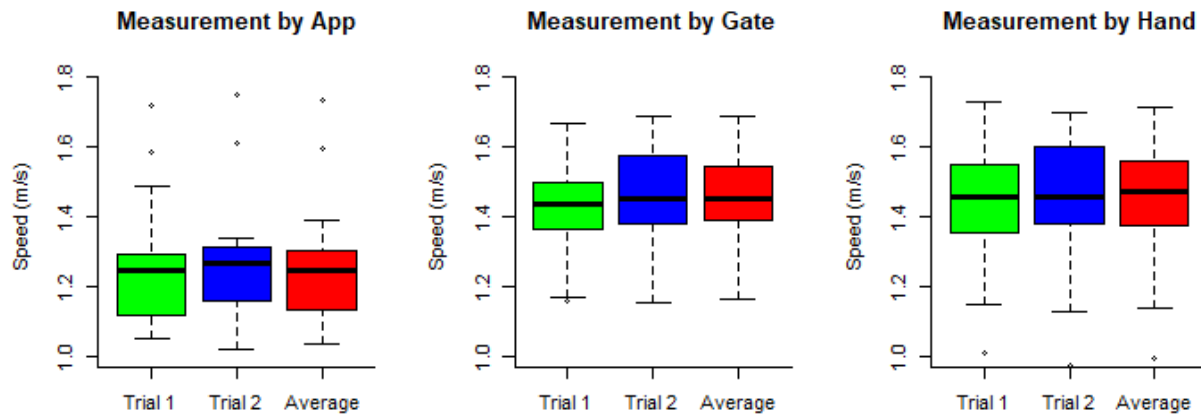


Figure 1: Boxplots for each of the three measurements and their trials along with their average of the two trials.

The t-test concluded that the timing gate and hand measurements are not different based on the T-statistic and P-value (Table 1). However, speed was significantly different when measured by the app and the timing gates based on the T-statistic and P-value (Table 1). Comparing the speed measured by app and by hand, the paired t-test showed statistical significance in difference (Table 1). The estimated Cohen’s D effect size indicated a small difference between gate and hand speed (Table 1). Effect size for comparing gate pace and app is considerably large (Table 1).

Table 1: Cohen’s D Effect size for the different compared measurements with the T-statistic value with their according P-value and 95% confidence interval.

Concurrent Validity	Cohen’s D Effect Size	T -statistic	P-value	95% CI
APP-GATE	0.879	3.405	0.004	(0.065, 0.287)
APP-HAND	0.778	3.015	0.009	(0.049, 0.292)
HAND-GATE	0.098	0.380	0.709	(-0.024, 0.035)

T-tests indicated no inter-trial difference for the gate, app, or hand measurements of speed (Table 2). From the reliability tests the effect size for inter-trial difference for the app and hand measurements were small, while the gate measurement effect size was medium.

Intertrials (trial 1 -trial 2)	Cohen's D Effect Size	T -statistic	P-value	95% CI
APP	0.124	-0.139	0.89	(-0.506, 0.441)
HAND	0.119	-0.111	0.912	(-19.237, 17.253)
GATE	0.493	0.402	0.691	(-5.288, 7.863)

Table 2: Cohen's D Effect size for trial 1 and trial 2 with the T-statistic with their according P-value and 95% confidence interval.

Discussion

We found that the 6th Vital Sign app was reliable but not accurate when compared to both the timing gates and hand measurements. This would imply that the app would not be fit to use in a clinical or recreational setting for authenticity. Other studies have found that the usage of smart phones to measure gait speed is both reliable and valid.¹⁰⁻¹⁵ Therefore, in comparison to other smart phone apps, the 6th Vital Sign app does not meet the standards for use in accurately measuring gait speed.

Conclusion

The 6th Vital Sign app demonstrated moderate reliability but poor validity, and therefore it should not be used in clinical or recreational settings as a measure for gait speed. Other smart phone apps could potentially be used as a measure of health if they are reliable and valid.

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3. Comparison of an iPad Application and 3D Body Scanner to the Bod Pod for Measurement of Body Fat Percentage

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Background: According to the American College of Sports Medicine (ACSM), body composition is an important health-related component of fitness, and as such a measurement of body fat percentage (%BF) is typically part of a fitness assessment (ACSM 2018). Novel and innovative imaging methods provide a rapid estimate %BF by using only photographs without the technician ever touching the client. Three-dimensional body scanners (3D SCAN) produce hundreds of anthropometric measurements in the matter of a few seconds, while two-dimensional iPad applications (2D APP) offer a portable, low-cost method for estimating %BF. Because of these appealing features, imaging methods are rapidly gaining popularity in health and fitness settings. However, research supporting the reliability and validity of these imaging methods for estimating %BF is still very limited.

Purpose: The purpose of this study was to evaluate the test-retest reliability of the %BF estimation from a 2D APP and a 3D SCAN, and compare both imaging methods to the %BF estimation from air displacement plethysmography (Bod Pod).

Design/Methods:

Participants - Adults from the general population volunteered to participate. Although defined stratified sampling techniques were not used, an effort was made to include a wide range of ages and body types.

Procedures - The study was reviewed and approved by the university's institutional review board, and participants provided voluntary written consent prior to participation. All three body composition methods (Bod Pod, 3D SCAN, 2DAPP) were completed in a single session for each participant.

Male participants wore compression shorts, and females wore compression shorts and a sports bra for all measurements. Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Seca 216, Seca Corp., Ontario, CA). Weight was measured to the nearest 0.1 kg with a digital scale (Seca 869, Seca Corp., Ontario, CA).

Bod Pod (Cosmed USA, Inc., Concord, CA) air displacement plethysmography with measured thoracic gas volume was used to measure body volume. Body density was calculated from the body mass and body volume data provided by the Bod Pod. Subsequently, %BF was estimated from body density with the Siri (1961) formula, and this %BF estimation served as the criterion

measure in this study. Manufacturer's guidelines were followed for Bod Pod testing procedures as described previously (McCroory et al. 1995).

An iPad (Apple, Inc., Cupertino, CA) with the LeanScreen app (PostureCo, Inc., Trinity, FL) was used to take photos of the participants according to the manufacturer's guidelines for the app. The technician stood 2.3 m away from the participant when taking photos, and the participant's entire body, head to toe, was captured in each photo. The photos of the front and right side of the body were used for analysis. The analysis involves using the touch screen of the iPad to mark specific anatomical landmarks on the photos. The landmarks using the frontal photo include the left and right borders of the neck, abdomen, waist, and hips. The landmarks using the lateral photo include the anterior and posterior borders of the same locations: neck, abdomen, waist, and hips. The photo can be zoomed-in, and the landmarks can be adjusted. The LeanScreen app automatically connects the border pairings with horizontal lines. The app then displays the estimated %BF from a proprietary equation. To evaluate test-retest reliability, the entire 2D APP procedure was repeated on each participant. For consistency and to eliminate issues of inter-rater reliability, the same technician performed all of the 2D APP tests. Participants were measured on the Fit3D body scanner (Redwood City, CA) according to the manufacturer's instructions. This involves standing on a turntable and grasping handles at the sides such that the arms will be fully extended and slightly abducted. Once in the correct posture, the participant initiates the test by pressing a button on the handles. The turntable slowly rotates while the scanner moves up and down, rapidly collecting images. The entire scan lasts about 40 s. A 3D digital image is created, and according to the manufacturer, more than 400 measurements including circumferences, heights, lengths, widths, volumes, and surface areas, are extracted from this digital image. An estimate of %BF from a proprietary formula is generated. The entire Fit3D scanning procedure was done in duplicate to evaluate test-retest reliability.

Statistical Analyses - All data were analyzed using SPSS version 25 (IBM Inc., Armonk, NY). Statistical significance was accepted at $P < 0.05$. Means and standard deviations were calculated for all variables, and normality of sample distribution was assessed with the Shapiro-Wilk test.

Test-retest reliability of the 2D APP and 3D SCAN were assessed with intraclass correlation ($ICC_{3,2}$) with a two-way mixed average measures model and absolute agreement. Coefficient of variation (CV) was also calculated. Additionally, the standard error of measurement [$SEM = SD\sqrt{(1-ICC)}$] was calculated in order to obtain the minimal difference ($MD = SEM \times 1.96 \times \sqrt{2}$).

The *MD* is a valuable test-retest variable because it sets the baseline for “real” change that exceeds the error of measurement when evaluating measurements over time (Weir 2005). The validity of the LeanScreen app and Fit3D body scanner to estimate %BF were evaluated against the %BF obtained from the Bod Pod. The following evaluation criteria described by Heyward and Wagner (2004) were used: a) a substantial relationship between the test method and criterion method as evidenced by a Pearson correlation coefficient (r) > 0.80, b) no significant mean difference between the three methods (e.g., nonsignificant F from repeated measures ANOVA with sex as a covariate), c) the regression slope and intercept should not be significantly different from 1.0 and 0.0, respectively, d) the standard error of estimate (SEE) and total error (TE) should be small (< 3.5% BF), and e) Bland and Altman (1986) plots of residual scores should result in small, nonsignificant correlation coefficients and small 95% limits of agreement.

Results: Seventy-nine adults (37 females, 42 males), varying widely in age (18-65 y) and body type (BMI of 18.2 to 41.8 kg/m²), completed the study.

The *ICCs* for test-retest reliability of the 2D APP and 3D SCAN were both 0.993 (95% CI of 0.989 to 0.996 for the app and 0.989 to 0.995 for the scanner). The 2D APP had an SEM = 0.98% BF and an MD = 2.70% BF. The 3D SCAN had an SEM = 0.81% BF and an MD = 2.25% BF. The CV was 4.7% for the 2D APP and 2.8% for the 3D SCAN. Given the high test-retest reliability of both methods, the two trials were averaged; consequently, the average 2D APP %BF and average 3D SCAN %BF were compared to the %BF from the Bod Pod.

The %BF estimations from the 2D APP and the 3D SCAN correlated with each other ($r = 0.923$), and both were highly correlated with the %BF estimation from the Bod Pod; $r = 0.857$ and $r = 0.899$, respectively. However, the three methods produced %BF estimations that were significantly different from each other ($F = 8.996$, $p = 0.001$, $\eta^2 = 0.105$) such that the %BF estimation from the LeanScreen app ($19.9 \pm 8.2\%$ BF) was significantly ($p = 0.001$) less than the estimation from the Bod Pod ($21.9 \pm 9.4\%$ BF), but the %BF estimation from the Fit3D body scanner ($24.0 \pm 6.8\%$ BF) was significantly ($p < 0.001$) greater than the Bod Pod value.

Furthermore, the method x sex interaction was also significant ($F = 3.666$, $p = 0.037$, $\eta^2 = 0.045$). The difference between methods was more pronounced for men than women, with neither the app ($p = 0.607$) nor the scanner ($p = 0.091$) being significantly different from the Bod Pod for the women. Linear regression for the 2D APP with Bod Pod as the dependent variable resulted in a slope of 0.981 and y-intercept of 2.4 with an $R^2 = 0.735$, $SEE = 4.86\%$ BF, and $TE = 5.22\%$ BF. Regression for the 3D SCAN resulted in a slope of 1.238 with a y-intercept of -7.854, an $R^2 = 0.809$, $SEE = 4.13\%$ BF, and $TE = 4.88\%$ BF.

The Bland and Altman (1986) plots of individual error scores revealed that there was a significant bias for both the 2D APP ($r = -0.253$, $p = 0.024$) and the 3D Scan ($r = -0.597$, $p < 0.001$) to overestimate the %BF of lean participants and underestimate it for fatter participants. Furthermore, the 95% limits of agreement were large for both the 2D APP (-11.7 to 7.6% BF) and the 3D SCAN (-6.7 to 11.0% BF).

Discussion: Previous investigators have compared the Fit3D body scanner (Ng et al. 2016) and the LeanScreen app (MacDonald et al. 2017) to dual-energy X-ray absorptiometry (DXA), but this is the first known study to compare both of these imaging devices to the Bod Pod. Despite excellent test-retest reliability and acceptable correlations with the Bod Pod, the 2D APP underestimated %BF by 2% while the 3D SCAN overestimated it by about the same amount. Additionally, the prediction errors and limits of agreement were large. Our findings are similar to those who used DXA as the reference method (MacDonald et al. 2017; Ng et al. 2016). The good reliability suggests that these imaging methods might be useful for tracking change in %BF over time; however, such longitudinal studies have yet to be conducted.

Conclusions: Although highly reliable, neither the 2D APP nor 3D SCAN provided valid estimates of %BF compared to the Bod Pod. More research is needed to determine if the algorithms associated with the imaging devices can be modified to improve the accuracy of the %BF estimates.

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4. Differing Impact of Weight Cycling on Ambulatory Blood Pressure versus Conventional Blood Pressure Assessment: A Possible Explanation to Controversy

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Introduction

In consequence to the recent blood pressure (BP) guideline changes, the prevalence of HTN in America is now estimated at roughly 46% (17). Since obesity and HTN often co-exist, and with approximately 36% of adults in the United States considered obese (9), weight loss is recommended as treatment for HTN (17). Thus, weight loss attempts are extremely prevalent, and the data consistently demonstrates that women report more weight loss efforts than men (18). Dishearteningly, failure rates of weight loss are tremendously high (7). Thus, dieters' relapse into the predictive pattern of weight loss followed by weight regain sequences, termed weight cycling (WC). WC has been highlighted as a contributor of increased BP (4-6, 20). A 2010 review (1) analyzed the available data on the impact of WC on BP in overweight/obese adults. Of the five included articles, three studies showed no impact of WC on BP while two showed WC increased BP. The authors determined there is not enough evidence of acceptable quality to draw any definitive conclusions and that additional research is needed looking at the impact of WC on BP. Surprisingly, none of the involved studies included the superior technique of ambulatory BP (ABP) monitoring.

Methods

Subjects. Sixty-five healthy overweight/obese nonsmoking weight stable Caucasian women with a body mass index (BMI) > 25 kg/m² and aged 25-60 years were recruited for this study.

Study design. Subjects came to the laboratory between 0600 and 0900 hours for one visit. Anthropometric measures of body weight, height, waist and hip circumferences and body fat were then measured.

Demographics. Body composition and body weight were measured by whole body air displacement plethysmography (Bod Pod, Cosmed, Concord CA, USA). Following the Bod Pod, a VO_{2peak} test was completed. Waist circumference was measured at the level of the umbilicus while hip

was measured at the largest protrusion. Following these assessments laboratory BP was then measured.

Laboratory BP assessment: Participants were directed to sit quietly for 20-min and then two BP readings were taken to ensure hemodynamic stability. Two additional BP readings were then taken and averaged together to give a resting laboratory BP value. Appropriate cuff sizes were used for all subjects.

VO_{2Peak} assessment: VO_{2peak} was assessed to acquire an objective measurement of fitness. Overlooking fitness has been highlighted as a weakness in past literature looking at WC and BP (1). . The chosen test was a ramp cycle ergometer protocol. Subjects began cycling at 25 watts for 5 min for a warm-up. Every minute the wattage increased by 20 watts until the subject reached failure.

Measures of WC: Following the exercise test subjects were given a questionnaire related to personal weight history. The Weight and Lifestyle Inventory (WALI) questionnaire was used in this study (15) and has been found to reliably report the number of diets (test-retest reliability $r = 0.77$) and amount of weight lost (test-retest reliability $r = .87$ both P values < 0.001) (14). Subjects recorded all diets/exercise programs they had undertaken that resulted in a weight loss of ≥ 10 lb. (4.5 kg), and this value was considered a single WC. Weight loss associated with menstrual cycle, illness, or pregnancy was excluded. Subjects were classified as weight cyclers (WC) if they reported a weight loss of ≥ 4.5 kg followed by weight regain at least 3 times (2, 6). Those who reported less than this were classified as non-weight cyclers (NWC).

Ambulatory Blood Pressure Monitoring. Following completion of the questionnaire, subjects were equipped with an ABP cuff. The *Oscar 2* ABP System (SunTech Medical, Morrisville, NC) was used in this study. The *Oscar 2* has been validated in accordance to the standards of British Hypertension Society (3). The *Oscar 2* was programmed to take readings every 15 min throughout the day (0900 h – 2200 h). Subjects wore the ABP device from roughly 0900 h to 2200 h. Appropriate cuff sizes were used for all subjects.

Statistical analysis

Statistical analyses were performed using SPSS software version 24 (SPSS 24.0 IBM Corporation, Armonk, New York, USA). Data are expressed as means \pm standard deviation (SD) unless otherwise specified. Data were analyzed for normality and values with skewed or kurtotic distributions were transformed to achieve normality. Descriptive statistics were used for the demographics of the participants. All *P* values were calculated assuming two-tailed hypothesis and *P* < 0.05 was considered statistically significant. Statistical analysis included ABP data collected from 0900 h – 2200 h of the same day. Mean day time ABP between groups were compared via independent t-test. Confounding variables of age, body fat, and VO₂peak were then included as covariates via General Linear Models. Due to the small sample size and collinearity of BMI and bodyfat in the current study (*r*=0.80, *p* <0.001) body fat was used in place of BMI as a confounding variable. Bivariate Pearson correlations were used to assess if linear relationships existed between the number of WC's, as a continuous variable, and systolic and diastolic laboratory BP and mean ABP.

Results

Laboratory Data

Sixty-five overweight/obese female subjects completed the study (N=31 for WC and N=34 for NWC). WC women were older (39.7 ± 8.9 vs. 33.1 ± 11.3 yr, *P*=0.012), carried more body fat overall (47.1 ± 6.2 vs. $41.4 \pm 7.8\%$, *P*=0.002) and in the abdominal region (waist-to-hip ratio; $.83 \pm .12$ vs. $.71 \pm .23$, *P*=0.015) and were not as fit as NWC women (21.2 ± 5.4 vs. 26.7 ± 7.6 ml.kg.min, *P*=0.002). There was no statistical difference in resting (laboratory) SBP (*P*=0.499) and DBP (*P*=0.529) between groups. The WC woman did however have a statistically higher mean systolic ABP (130.1 ± 13.6 vs. 122.0 ± 8.2 , *P*=0.006) and diastolic ABP (76.2 ± 8.9 vs 70.0 ± 9.0 mmHg, *P*=0.011). After adjusting for age there were still differences between groups for systolic ABP (*P* = 0.040) and borderline significant difference for diastolic ABP (*P* = 0.056). There was a trend for statistical significance on systolic ABP when adding body fat as a covariate (*P* = 0.062) while significance was completely lost for diastolic ABP (*P* = 0.123). There were still significant systolic and diastolic ABP differences between groups

when adjusting for waist-to-hip-ratio ($P = 0.014$, $P = 0.017$, respectively). The addition of VO_{2peak} into the model negated all significance for systolic ABP ($P = 0.182$) and diastolic ABP ($P = 0.243$).

When all subjects were entered into a correlation analysis and the number of WC's was used as a continuous variable, no significant correlation between laboratory SBP and the number of WC's is witnessed ($P = 0.830$). However, a significant correlation between the number of WC's and mean systolic ABP exists ($r = .326$, $P = 0.010$). Number of WC did not correlate with laboratory DBP ($P = 0.997$) but there was a trend for the number of WC to correlate with mean diastolic ABP ($r = 0.238$, $P = 0.065$). After adjusting for age, body fat and waist-to-hip ratio, a trend still existed between the number of WC and mean systolic ABP ($P = 0.080$). After adjusting for VO_{2peak} , all significance was lost ($P = 0.206$).

Discussion

The major finding of this investigation was that WC, analyzed as both a dichotomized variable and when using the number of WC's as a continuous variable, did not impact laboratory BP but did have an impact on daytime ABP, including BP Load. Even after adjusting for confounders such as age, waist-to-hip ratio, and body fat, it appears that WC still plays some role in elevated systolic ABP.

Pickering et al. observed that: "Any clinical measurement of BP may be regarded as a surrogate measure for the 'true' BP of the patient, which may be defined as the mean level over prolonged periods" (11). Thus, laboratory or clinic BP assessments are a poor prognostic tool to determine CV risk and may not best reflect the patients true BP values. For example, approximately 16% of those who have non-elevated BP in the clinic setting, averaged over 3 visits, have elevated average awake ABP and therefore have masked HTN (12). On the other hand, ABP measuring can identify and better classify those with white coat syndrome (10). Thus, it could be that past literature assessing the impact of WC on BP has been inconclusive due to the method of BP measurement.

One theory as to why WC exerts deleterious impacts on health is because periods of weight gain and loss increases sympathetic activity (8). A heightened sympathetic activity would more than likely impact ABP because of the inclusion of assessment during activities of daily living which include stress

induced situations, opposed to a single clinic BP assessment at rest. Consequently, the true impact of WC on BP may be missed when only assessing BP in the laboratory or clinic.

It is well established that abdominal adiposity is linked with increased risk of cardiometabolic abnormalities (13). One theory suggests that the harmful impacts of WC could be a consequence of a redistribution of body fat to more of an android shape (16, 19). The current study supports the role of WC on abdominal obesity in that the WC group had a significantly higher waist-to-hip ratio compared to the NWC group.

Our study has many strengths, one being the objective measurement of fitness. Fitness and PA should be considered in the WC research but rarely, if ever, is (1). Our study showed that NWC women had higher fitness levels than WC women and when adjusting for fitness, all statistical significance was lost. Empirical evidence shows obese subjects with increased cardiorespiratory fitness have lower all-cause mortality and lower risk of CV and metabolic diseases when compared to leaner unfit individuals. Another strength was the use of ABP devices. In our study it was found that measures of WC were not associated with laboratory BP but was associated with ABP. Prior research in this area have focused on a one-time BP assessment assuming it represents the impact of BP on CV health when in fact they may have missed deleterious effects of WC on these measures. This may partially be responsible for the divergent findings in the literature when it relates to WC and BP.

The current study is not without weaknesses. The assessment of WC was self-reported and collected retrospectively. We do believe that the methods to obtain weight history however are reliable as shown in prior reliability assessment (14). Also, there is no universal definition among clinicians and researchers on how to define a single WC and who constitutes a weight cyclist. Also, multiple regression analysis was not able to be ran due to the small sample size thus limiting our ability to attribute a variance amount explained by the independent variables.

In conclusion, the current study suggests that WC may increase measures of ABP. The current paper also provides evidence that to truly gage the impact of WC on BP, researchers should be using ABP monitoring, not a laboratory measurement. It also appears that increasing fitness may be

somewhat protective against the damaging effects of WC on BP control. From a societal perspective, it may be easier to get people to increase fitness than lose body mass.

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5. Downhill Running: An Effective Countermeasure to Limitations of Exercise in Acute Hypoxia?

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Preconditioning involves exposure to external stressors that produce physiological and functional adaptations (5). One example of preconditioning is heat acclimation (HA). HA reduces physiologic strain in a hot environment via higher sweat rate, earlier onset of sweating and lower resting and exercising core temperature (7). Cellular adaptations exhibited by HA include an increase in heat shock protein 72 (Hsp72) (12). Hsp72 are intracellular chaperone proteins that protect the cell against a variety of stressors. An increase in the cell's basal stores of Hsp72 characterizes an enhancement in a cell's ability to endure stress without the need for new protein synthesis and is a recognized indication of an organism's adaptation to stress (6).

Previous studies have suggested that specific physiologic adaptations to a single environmental stressor, such as heat, can induce cross adaptations to other stressors (i.e. hypoxia) (3, 4, 11). For example, 3 days of HA were sufficient to increase monocyte Hsp72 levels, reduce exercising heart rate, and increase O₂ saturation during exercise in acute hypoxia (3000m) (3).

Further, 10 days of HA increased Hsp72 and significantly improved cycling performance (~2:02 minutes over 16.1 km) in hypoxia (14% fraction of inspired oxygen (FiO₂)) (4). Similarly, downhill running (DHR) increased Hsp72 and reduced exercise heart rate, core temperature, and increased sweat response during subsequent exercise in the heat (9). Taken together, these data suggest that exercise stress that results in increased Hsp72 may confer protection against exercise in a different environment. While DHR has been shown to increase Hsp72 and provide protection against exercise in the heat, the impact of DHR on exercise in hypoxia is unknown. Since best practices suggest two weeks are needed to acclimate to exercise in

hypoxia (8), DHR could be an effective countermeasure to reduce the time necessary to acclimate. The purpose of this study was to analyze the effect of DHR on exercise performance in normobaric hypoxia. We hypothesized that DHR would increase basal Hsp72 and improve exercise performance in hypoxia.

Methods

Participants

8 healthy and active males (Table 1) participated in this study. All participants were trained runners, familiar with pacing and race pace efforts. All procedures were approved by the institutional review board.

Table 1: Participants' Characteristics. Data are mean \pm SD.

Age (years)	Height (cm)	Weight (kg)	Body Fat (%)	VO _{2max} (ml kg ⁻¹ min ⁻¹)	V _T (ml kg ⁻¹ min ⁻¹)
23.8 \pm 5.8	180.4 \pm 8.1	80.7 \pm 10.9	13.6 \pm 5.2	54.1 \pm 5.1	39.2 \pm 5.5

Preliminary Testing

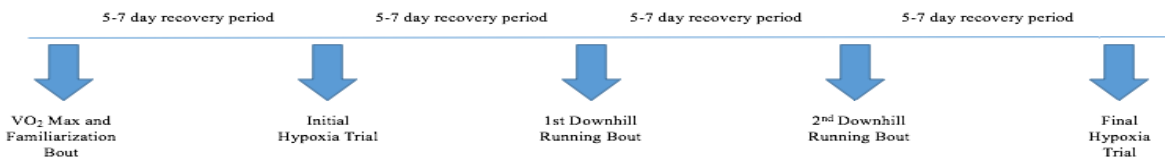
Demographic data included age, height, weight, and body fat % estimated via three site skinfold measurement (1). Aerobic capacity was quantified through indirect calorimetry (Parvo Medic, Sandy UT). Subjects ran on a treadmill at increasing speeds and gradients until volitional fatigue. Additionally, ventilatory threshold (V_T) was assessed using the three following techniques: ventilatory equivalents method, ExCO₂ method, and the V-Slope method (2). V_T was measured to establish the speed in which each subject would be running downhill. After resting for ~15 minutes, subjects performed a familiarization (FAM) time trial (TT). This was done to accustom participants to the environment and washout any learning effect of pacing or treadmill use. The FAM TT consisted of running 5km in a 20° C, 35% relative humidity environment, and 20.9% FiO₂.

Experimental Design

Two normobaric hypoxic (16% FiO₂) 5 km TT were performed: one before any DHR and one 5-7 days after the last bout. Subjects performed two 45-minute DHR bouts (12.5% downhill grade) in the speed that elicited V_T while running downhill. Each DHR trial was separated by 5-7

days (Fig 1). Muscle soreness (DOMS) was assessed 24 and 48 hours after each downhill bout according to a validated Likert scale (10).

Figure 1: Timeline of experimental study.



Hypoxic Time Trials

Subjects abstained from exercise 24 hours prior to the TT. Hydration (via specific gravity of urine) was assessed before the TT while blood lactate (Nova Biomedical) was measured before and immediately after each TT. During the TT, heart rate, RPE and O₂ saturation (SaO₂) (Nellcor) were recorded every 1 km. The hypoxic environment was created through nitrogen dilution (creating normobaric hypoxia) and monitored through two independent O₂ sensors (BioSpherix P360). The O₂ sensors were set to maintain FiO₂ levels of 16% (simulating ~7,500 ft elevation). Subjects were allowed to warm up under normal conditions before entering the chamber. They were instructed to treat each TT as a race. Subjects were blinded to the speed and time on the treadmill, but knew the distance they had covered.

Flow Cytometry Analysis

Venous blood (2 ml) was obtained before (basal) and immediately post DHR. Red blood cells were lysed before adding antibodies to determine the population of monocytes and quantifying Hsp72. Samples were analyzed utilizing a BD Accuri C6 Flow Cytometer.

Statistics

Data are presented as mean \pm standard deviation. Results were obtained via paired sample t-tests (TT data) and ANOVA (Hsp72 data). Statistical significance was accepted at $p < 0.05$. Calculations were performed on Microsoft Excel.

Results

Monocyte Hsp72 Responses

Blood concentration of monocyte Hsp72 (Figure 2) showed no significant change across time ($p=0.53$). Specifically, basal concentration from downhill bout I to downhill bout II were not different (3.5 ± 2.3 to 2.9 ± 1.5).

Figure 2: Monocyte Hsp72

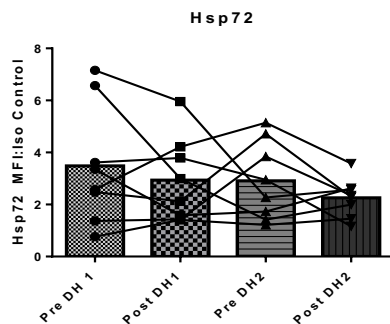
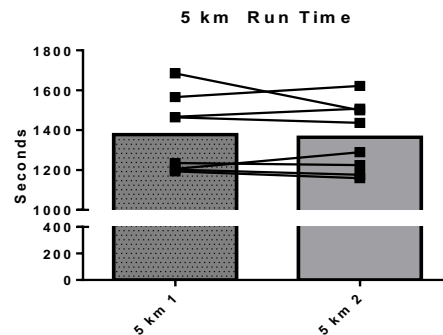


Figure 3: TT Performance



Perceptual and Metabolic Responses

TT performance (Figure 3) was similar between conditions. Hydration, RPE, HR, and blood lactate were similar in both TTs in hypoxia, however, SaO_2 significantly increased from TT1 to TT2 (Table 2). Perceived soreness response was significantly lowered 24 (5.1 ± 0.8 to 3.5 ± 1.4 , $p = 0.00$) and 48 (4.6 ± 1.0 to 2.6 ± 1.5 , $p = 0.00$) hours following the second DHR trial when compared to the first trial.

Table 2: Mean \pm SD physiological and perceptual measures during exercise in hypoxia. *denotes a significant difference from Hypoxia I ($p < 0.05$).

	Pre-Blood Lactate (mmol/L)	Post-Blood Lactate (mmol/L)	Urine Specific Gravity (SG)	Average HR (b/min)	Average RPE	Average SaO_2 (%)
<i>Hypoxia I</i>	2.1 ± 0.5	11.6 ± 1.8	1.018 ± 0.007	178.7 ± 8.5	14.9 ± 1.1	84.5 ± 4.0

<i>Hypoxia II</i>	2.4 ± 0.7	12.0 ± 3.1	1.013 ± 0.009	179.2 ± 8.7	14.6 ± 1.3	87.2 ± 2.3 *
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Discussion

The key finding of this study was that two-45 minute bouts of DHR did not improve running performance in acute hypoxia. This was potentially the result of no change in monocyte Hsp72 after two bouts of DHR. However, DHR did result in decreased perceived levels of soreness 24 and 48 hours post exercise and improved SaO₂ during hypoxia TT's.

Previous data has suggested that exercise capacity in hypoxia can be improved through heat acclimation (4, 11). Further, DHR in hot conditions demonstrated an increase in the heat acclimation phenotype (9). These works suggested the increase in monocyte Hsp72, that accompanied both HA and DHR, was a primary variable associated with these improvements. The current data show no change in monocyte Hsp72 after DHR and no improvement in performance at hypoxia. This could be the result of significant variation in Hsp72 production and 5k performance between subjects. Specifically, 5 of the 8 participants improved performance while 4 of 8 increased Hsp72 (R²=0.15; p=0.38; data not shown).

Despite basal Hsp72 levels being unaffected, DOMS data suggest adaptations were made as perceived muscle soreness was lowered after downhill two compared to downhill one. In addition, SaO₂ during the TT was improved after DHR. Increased SaO₂ during exercise in hypoxia was also observed after 3 and 10 days of HA (3, 11). This could be a meaningful finding and warrants further investigation.

In conclusion, two bouts of DHR did not result in increased running performance in hypoxia, nor an increase in basal Hsp72 expression. However, SaO₂ during exercise was improved. Therefore, we reject our hypothesis since these data suggest that two bouts of DHR is not an effective countermeasure to exercise in acute hypoxia.

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6. Effect of Fed State on Affective Response to Exercise Following Public Health Guidelines

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Introduction

Obesity has become a worldwide epidemic, inflicting tremendous cost at the individual and public levels (Locke et al. 2015). For example, obesity rates within the United States from 2000-2015 significantly increased in both youth and adults (Hales et al. 2017). Physical activity is an effective strategy to prevent or decrease severity of obesity (Hoor et al. 2017), yet only about 20% of adults participate in regular physical activity (PA) (CDC 2014).

One factor linked to regular PA participation is enjoyment; increasing individuals' enjoyment of PA may be a way to increase adherence to exercise (Ekkekakis and Lind 2006; Williams et al. 2008). Exercise enjoyment was higher during self-paced exercise versus prescribed exercise (Ekkekakis and Lind 2005; Rose and Parfitt 2010) likely due to greater autonomy. In addition, when exercisers are given autonomy, they choose intensities high enough to elicit physiological adaptations (Rose and Parfitt 2007). However, the PA guidelines for adults do not offer the ability to self-pace; instead, exercise is prescribed based on fractions of maximal heart rate, peak power, or oxygen consumption (VO_2). One additional aspect related to exercise enjoyment is dietary intake before exercise. In endurance-trained athletes, Backhouse et al. (2005), reported that pre-exercise carbohydrate (CHO) consumption increased feelings of pleasure during prolonged exercise versus the fasted state which suggests that fed state may be important to optimize exercise enjoyment. However, to our knowledge, no study has determined whether fed state alters perceptual responses to exercise when intensity is self-selected instead of imposed.

The purpose of this study was to examine if fed state affects self-selected intensity, exercise enjoyment, and affect during exercise.

Methods

Recreationally active women ($n = 12$) and men ($n = 13$) (age and BMI = 22.0 ± 2.0 yr. and 24.3 ± 3.3 kg/m²) participated in this within-subject crossover study. They completed a health questionnaire and provided informed consent before participating in the study. Trial order was randomized across participants using a web-based application (<http://www.randomizer.org>). Initially, a familiarization visit was completed which required subjects to perform 15 min of treadmill exercise at an intensity equal to rating of perceived exertion (RPE) = 13 (“moderately hard”) (Borg 1982).

For the 2 subsequent trials, subjects arrived at the lab after an overnight fast and either consumed 12 ounces of water (FAST) or ingested a small (180 kcal) carbohydrate rich (39 g CHO) meal (Quaker Chewy granola bar, PepsiCo, Barrington, IL, USA; 12 oz Gatorade, PepsiCo, Barrington, IL, USA) (FED). Thirty minutes later, participants exercised on a treadmill for 30 minutes at a self-selected intensity equal to RPE of 13 (Borg 1982). They were free to adjust the speed/grade as necessary to maintain this intensity. During exercise, gas exchange data were acquired by a metabolic cart (ParvoMedics True One, Sandy, UT, USA), and heart rate (HR) was measured via telemetry (Polar T31, Woodbury, NY, USA). Pre-exercise and every 10% of work completed, RPE (Borg 1982), affect (FS) (Hardy and Rejeski 1989), and arousal (Felt Arousal Scale, Svebak and Murgatroyd 1985) were recorded using validated scales. Two and five-minutes post-exercise, affect was also recorded. Fifteen minutes post-exercise, enjoyment was recorded using the Physical Activity Enjoyment Scale (PACES) (Kendzierski and DeCarlo 1991).

Data were analyzed using SPSS version 24 (IBM Corporation, Armonk, NY, USA) and are expressed as mean \pm standard deviation. A two-way (time and fed state) analysis of variance with repeated measures was used to compare differences in outcome measures between FED and FAST. If a significant F ratio was obtained, Bonferroni’s post hoc test was used to identify significant differences between conditions. For mean exercise data and PACES data, paired t-tests were used to assess differences between conditions. Statistical significance was set at $p < 0.05$.

Results

Exercise responses between FED and FAST were similar (Table 1). In addition, treadmill speed and grade during exercise were not significantly different ($p > 0.30$ for both).

	FED	FAST	P-value
VO ₂ (L·min ⁻¹)	2.22 \pm 0.65	2.19 \pm 0.70	0.54
RER	0.97 \pm 0.04	0.96 \pm 0.05	0.29
Energy expenditure (kcal)	328 \pm 104	332 \pm 98	0.54
Heart rate (b·min ⁻¹)	172 \pm 12	170 \pm 15	0.35

VO₂ = oxygen uptake; RER = respiratory exchange ratio

Psychological responses across conditions are shown in Figure 1. Affect showed a main effect of time ($p < 0.001$) as it decreased during exercise yet rebounded in recovery. There was no main effect of condition ($p = 0.12$) or condition*time interaction ($p = 0.19$). Arousal displayed a main effect of condition ($p = 0.007$), but no effect of time ($p = 0.34$) or condition*time interaction ($p = 0.21$). Mean arousal was lower during FED than FAST. Exercise enjoyment measured through PACES was not significantly different between conditions (81.2 \pm 10.3 vs. 85.1 \pm 12.9, $p = 0.22$). Blood glucose was significantly different ($P < 0.001$) across FED and FAST as there was a condition*time interaction.

There were no gender differences ($p > 0.05$) in any outcome between conditions.

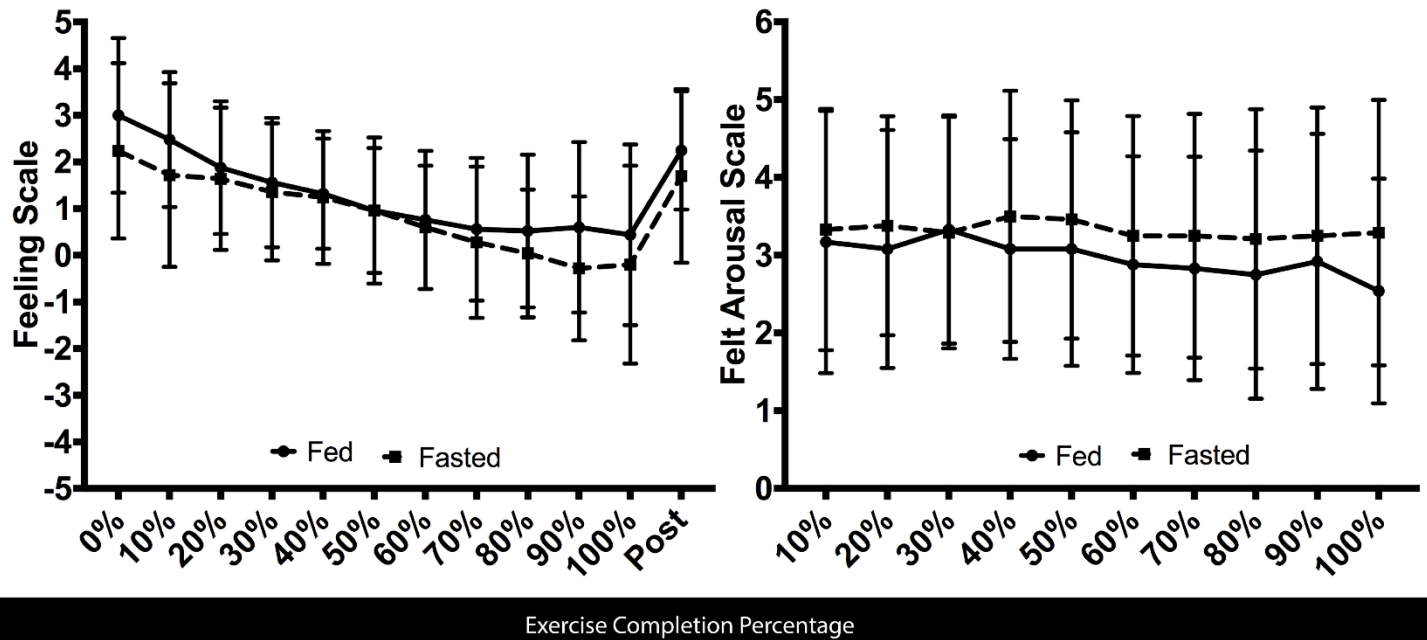


Figure 1: Change in affect and arousal during 30 min of moderate exercise in the fed and fasted state (mean \pm SD).

Discussion

This study examined if fed state affects self-selected intensity, exercise enjoyment, and affect during exercise. Data show no effect of fed state on affect or enjoyment during 30 min of “somewhat hard” exercise, although there was a significant difference in arousal. Therefore, eating before moderate exercise does not augment feelings of pleasure or enjoyment to sustained aerobic exercise, yet arousal is altered.

Like other studies measuring affect during exercise, our data show a decline in affect with a rebound post exercise (Rose and Parfitt 2007; Parfitt et al. 2000). However, our data show no significant difference in affect between conditions. Ekkekakis et al. (2011) examined the relationship between affect and RPE, and indicated that at a “clamped” RPE, changes in affect may be unexpected. Rose and Parfitt (2007) showed that suprathreshold intensities cause participants to become more concerned with the discomfort of exercise, leading to lower affect. An alternative explanation for lower affect during exercise is shown by previous studies (Coggan and Coyle 1989; Backhouse et al. 2005), which examined changes in perceptual responses with alterations in blood glucose. Backhouse et al. (2005) showed a higher exercise affect in cyclists who ingested CHO pre-

exercise versus fasting. Coggan and Coyle (1989) showed that CHO intake during prolonged exercise prevented an increase in RPE versus fasting and resulted in improved performance in cyclists. Future studies are needed to determine if post-exercise enjoyment is altered by fed state or with ingestion of CHO or other nutrients; for example, Schubert et al. (2014) reported increased enjoyment during exercise with pre-exercise caffeine intake.

Data show that exercise intensity was not different between conditions, which was expected with a fixed RPE. Rose and Parfitt (2010) showed when given the opportunity to self-select the intensity, subjects choose an intensity above the ventilatory threshold, which long-term would elicit significant physiological adaptations.

A limitation of our study is that despite “clamping” RPE, the intensity was high as illustrated by HR ~ 88% predicted HRmax and end-exercise blood lactate concentration equal to 4.0 (FED) and 5.2 (FAST) mmol/L. Given the familiarization visit, it is unclear why the physiological strain was so high. Another limitation of the current study was that the meal’s caloric intake was identical across participants and not based on body mass. However, dosing by body mass removes some practicality since people consume energy based on packaged items.

Overall our data show no significant effect of fed state on affect or enjoyment, yet arousal was modified. However, it is plausible that a small meal before exercise may improve mood during exercise due to increased blood glucose availability. We recommend that subjects freely choose whether to eat a small meal before exercise. Dishman and Buckworth (1996) explained that enjoyment may be a better motivator to increase exercise adherence versus knowledge of exercise benefits. This may be particularly useful to combat the obesity pandemic. Yet, further studies are necessary to determine changes in exercise adherence and psychological responses in response to manipulation of fed state.

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Friday, October 26, 2018

Graduate Student Award Competition – Oral Presentation

1. Effect of Two Regimes of Sled Sprinting on 40-Meter Sprint Performance in Collegiate Soccer Players

*Patrick Mara and Todd Astorino, Ph.D.
California State University San Marcos, California*

2. Effect of a Golf Intervention on Center of Pressure Control

*Jared Moore¹, Andrea DuBois, M.S.¹, Nicole Marcione, M.S.¹, Hyun Lee¹, Steven Castle, M.D.², George Salem, Ph.D.²
¹University of Southern California, California and ²VA Greater Los Angeles Healthcare System - GRECC, California*

3. The Genetic Profile of Individuals with Traumatic Spinal Cord Injury Following an 8-Week Group Exercise

*Ruiz L¹, Dy C¹, J Ramirez¹, L Le¹, D Salas¹, Won D², Pebdani R³, de Leon R¹, S Keslacy¹.
¹School of Kinesiology and Nutritional Sciences, ²College of Engineering, Computer Science and Technology, ³Charter College of Education, California State University, Los Angeles, California*

4. Cardiovascular Adaptations in a Male-to-Female Transgender Athlete Before and During Estrogen Therapy: A Case Study

*Shannon L. Wilson¹, Theresa M. Jorgensen¹, Joanna Harper², Andrew C. D'Lugos¹, Glenn A. Gaesser FACSM¹, Jared Dickinson FACSM¹, and Siddhartha S. Angadi FACSM¹
¹Arizona State University, Arizona and ²Providence Portland Medical Center Portland, Oregon*

5. Physiological response to exercise intensity in spinal cord injured compared to able-bodied individuals

*Ramirez, Joel; Keslacy, Stefan; De Leon, Ray; Defiesta, Dominic; Ruiz, Lloyd; & Dy, Christine
California State University, Los Angeles, California*

1. Effect of Two Regimes of Sled Sprinting on 40-Meter Sprint Performance in Collegiate Soccer Players

*Patrick Mara and Todd Astorino, Ph.D.
California State University San Marcos, California*

Introduction

Speed is one of the most important factors dictating athletic performance especially in field based team sports including soccer (Upton 2011). Although soccer players jog and walk for upwards of 90% of the game (Bloomfield et al. 2007, Bradley et al. 2010), the outcome is often determined by who can win a series of short competitive sprints that range from 5 to 40 meters (Upton 2011). Sports performance coaches continue to design training programs to enhance this ability. In addition to lower body resistance training and plyometrics, one frequently-employed method is some form of resisted sprinting. However, data concerning efficacy of this method of training is equivocal and there is much debate over the proper resistance to prescribe.

Early research concerning kinematics of resisted sprinting (Alcaraz et al. 2009) shows that any load that reduces speed by more than 10% alters the mechanics of sprinting leading to a reduction in performance. Consequently, strength coaches have been afraid to prescribe a load greater than 10% BW. However, Kawamori et al. (2014) challenged this notion by comparing changes in 5 and 10 meter sprint performance between athletes using light (10% loss of velocity) and heavy (30% loss of velocity) sleds, with results demonstrating more improvement in the heavy group. Cross et al. (2016) identified that the optimal load for generating maximal power output in resisted sprinting is equal to 70 – 90% BW for field-based athletes, a value that is markedly higher than currently practiced in the strength and conditioning literature.

The aim of this study was to compare the effects of two 5-week sled sprinting programs varying in load on sprint performance and jumping ability in male collegiate soccer players. It was hypothesized that heavy sled sprinting will reveal greater improvements in speed versus light sled sprinting, especially during the early and late acceleration phase.

Methods

This intervention was a 5-week, longitudinal quasi-experimental study held during athletes' off-season strength and conditioning program. Subjects were 20 male soccer players at a Division II school in California ranging from 18 – 22 years old who had ≥ 1 year of resistance training and collegiate soccer experience.

On the Monday of the 1st week, their sprint speed and leg power were tested using the 40-meter dash and broad jump. Athletes sprinted 40 meters as fast as they could, with time recorded at 10, 20, and 40 meters. Times were measured with the Brower Speed Trap 2 (Draper, Utah, USA), an electrically timed laser system accurate to 0.01 seconds per manufacturer specifications. Athletes were then ranked from slowest to fastest, matched, and randomly placed into the “light” (n=10) or “heavy” (n=10) resistance group. The same tests were performed on the Monday following their final sled training session.

Two days per week (Wednesday and Friday) after a standardized warm up, athletes performed all out sled sprinting at their respective loads. The light group sprinted with a sled (SKLZ, Carlsbad, CA) weighing between 10 – 20% of bodyweight; whereas, the heavy group used a sled (Rogue Fitness, Columbus, OH) weighing between 70 – 80% of bodyweight. The exact progression of sled sprints is shown in Table 1. Sled sprinting was

completed concurrently with full body strength-training held on Monday, Wednesday, and Friday and soccer-specific running and conditioning drills on Tuesday and Thursday.

Data are expressed as mean \pm SD and were analyzed using SPSS Version 24.0 (Chicago, IL). Two way repeated measures ANOVA was used to examine changes in outcomes from pre to post-training, with group as a between subjects variable. Cohen's d was used as a measure of effect size, and statistical significance was set at $p < 0.05$.

Table 1: Sled training regimes with both light and heavy loads

^amen performed eight 12.5 m sprints at load = 10 % body weight

Week	Light Wednesday	Light Friday	Heavy Wednesday	Heavy Friday
1	8 x 12.5m @ 10% ^a	8 x 12.5m @ 10%	8 x 12.5m @ 70%	8 x 12.5m @ 70%
2	10 x 12.5m @ 10%	10 x 12.5m @ 10%	10 x 12.5m @ 70%	10 x 12.5m @ 70%
3	8 x 12.5m @ 20%	8 x 12.5m @ 20%	8 x 12.5m @ 80%	8 x 12.5m @ 80%
4	10 x 12.5m @ 20%	10 x 12.5m @ 20%	10 x 12.5m @ 80%	10 x 12.5m @ 80%
5	10 x 12.5m @ 20%	10 x 12.5m @ 20%	10 x 12.5m @ 80%	10 x 12.5m @ 80%

Results

Results showed a significant effect of time for the 20-meter ($p = .005$) and 40-meter distances ($p = .008$), as well as for the broad jump ($p = .002$). 10 meter sprint times remained unchanged, and there was no group \times time interaction for any variable. Very large effects were seen for 20 and 40 meter performance in response to heavy sled training, with a huge effect seen on broad jump (Table 2). Medium effects were seen in 10 meter sprint times and broad jump in response to light sled training, with large effects seen at the 20 and 40 meter distances.

Table 2: Changes in performance measures from light and heavy sled sprinting

Variable	Pre training	Post training	Effect Size	Meaning
Light Load				
10 Meter (s)	1.66 ± 0.05	1.64 ± 0.08	0.47	medium effect
20 Meter (s)	2.85 ± 0.07	2.81 ± 0.10*	0.94	large effect
40 Meter (s)	5.02 ± 0.15	4.97 ± 0.20*	0.83	large effect
Broad Jump (cm)	242.8 ± 12.2	246.9 ± 16.5*	0.65	medium effect
Heavy Load				
10 Meter (s)	1.62 ± 0.07	1.61 ± 0.08	0.24	small effect
20 Meter (s)	2.82 ± 0.10	2.77 ± 0.11*	1.18	very large effect
40 Meter (s)	5.02 ± 0.20	4.95 ± 0.20*	1.17	very large effect
Broad Jump (cm)	232.7 ± 15.5	243.8 ± 10.4*	1.82	huge effect

*= p < 0.05 compared to pre-training within group

Discussion

Our data show that a 5-week sled sprinting intervention significantly improves sprinting performance and broad jump in collegiate soccer players. There were no differences between regimes which suggests that light and heavy sled sprinting is efficacious to enhance these outcomes.

The optimal load to use during sled sprinting is unknown, and it is suggested (Cross et al. 2016; Cross et al. 2018), individual variation mediates adaptation to sled sprinting rather than specific aspects of training. Our findings that resisted sprinting improves late

acceleration and the maximal velocity phase rather than early acceleration agrees with data from Upton et al. (2011) in Division 1 female soccer players, but contradicts research from Harrison and Bourke (2009) showing improved early acceleration. Data from Cross et al (2018) showed minimal improvements in response to an 8 week heavy and light sled sprinting intervention, but it was performed in a heterogeneous group of field athletes differing in gender. To our knowledge, this is the first study to compare heavy versus light resisted sprinting in a homogenous population performing identical strength and conditioning regimes. Another interesting finding was the marked improvement in broad jump in response to heavy sled training. Morin et al. (2017) observed that heavy sled sprinting improved mechanical efficiency to produce greater horizontal ground reaction forces than light sled sprinting, which could explain these results.

There were some limitations of our study, including the small sample. Ideally, a control group participating in resistance training and soccer-specific training but not sled sprinting would be included in addition to the heavy and light sled group. This would allow us to differentiate effects of the sled sprinting intervention from a traditional strength and conditioning program. Also, with only 10 athletes in each experimental group, we may have been underpowered to detect any significant differences in responses between groups, including changes in 40 m performance.

Future research could include a true control group, or perhaps compare the effects of an optimally loaded resisted sprinting intervention in other homogenous groups of athletes where speed is a top priority, such as in Sprinters or American football wide receivers.

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2. Effect of a Golf Intervention on Center of Pressure Control

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INTRODUCTION: Aging is associated with numerous physiological changes including decreased balance, which is associated with increasing fall risk.¹ This may be exacerbated in veterans who have poorer health compared to non-veterans.²⁻⁵ This impaired balance is evident as a reduced ability to decrease force variability during the static phase of a tandem stance balance task.⁶ A semi-tandem balance task can be used to investigate the contribution of ankle and hip muscles to quiet standing, and both medio-lateral (ML) and anterior-posterior (AP) balance capabilities can be quantified.⁷

Golf is a multimodal, dynamic activity that encompasses a wide range of movement patterns. This is seen in the multiple aspects of a complete golf round: traversing uneven terrain, bending to pick up and place golf ball, twisting during swing, quiet standing during putting, and cognitive processing required to adjust to environmental factors such as wind, terrain, noise, and slope of the green. We hypothesized that a golf intervention could be used to improve balance during quiet standing because other similar multimodal activities have been shown to improve balance in older adults.⁸ Moreover, golfers have better static and dynamic balance compared to non-golfers,⁹ and similar balance of Tai Chi participants.¹⁰ The purpose of this study was to investigate the changes in ML and AP center of pressure (COP) dynamics⁷ in older military veterans following a comprehensive 12-week golf intervention.

Methods: Nine older (70.8 ± 5.3 y), military veterans whom did not currently golf, performed three trials of a semi-tandem (heel of lead limb placed 10cm anterior and 10cm lateral to toe of trail limb) balance task both before and after the golf intervention. The golf intervention consisted of complimentary exercises and dynamic warmup that prepared the participants for the demands of the activity in addition to golf-specific training. Sessions were 90 minutes, two times per week. All sessions were led by a Professional Golf Association (PGA) instructor and included an introduction to golf rules and etiquette, swing training, and progressive golf play. During balance testing, the participants were instructed to keep their arms across their chest and look directly ahead. They were instructed to stand as still as possible and only use their arms for balance if necessary. The participants were asked to stand in this position for a maximum of thirty seconds, and the trial would end if the participant took a step or either arm moved. Two AMTI (Newton, MA) force platforms measured COP (1500Hz). COP and ground reaction forces were filtered using a 4th order low-pass Butterworth filter with a 5Hz cutoff. The last 20 seconds of each semi-tandem stance were used for analysis. Results are presented as means \pm standard deviation. Paired t tests were used to detect changes in COP range (maximum COP position – minimum COP position) and COP mean velocity (total excursion/time) in the AP and ML directions.¹¹ An alpha of 0.05 was considered significant.

Results: All participants were able to stand for the maximum of 30 seconds on all trials. COP range and mean velocity are presented in Table 1 as changes from pre to post testing. Paired t tests revealed no significant changes in medio-lateral ($p=0.407$) or anterior-posterior ($p=0.130$) center of pressure range. There were also no significant

changes in medio-lateral ($p=0.927$) or anterior-posterior ($p=0.331$) center of pressure mean velocity.

	Δ Range (mm)	Δ Mean Velocity (mm/s)
ML	1.56 ± 5.36 ($p=0.407$)	-0.13 ± 4.07 ($p=0.927$)
AP	5.39 ± 9.59 ($p=0.130$)	1.63 ± 4.73 ($p=0.331$)

Table 1: Group Average center of pressure range and mean velocity changes from pre to post during tandem stance task. Mean \pm SD

Shown below (Figures 1 & 2) are the individual changes for each participant from baseline testing to after the golf intervention. As can be seen, some participants vary but a majority of the participants show no change in balance performance.

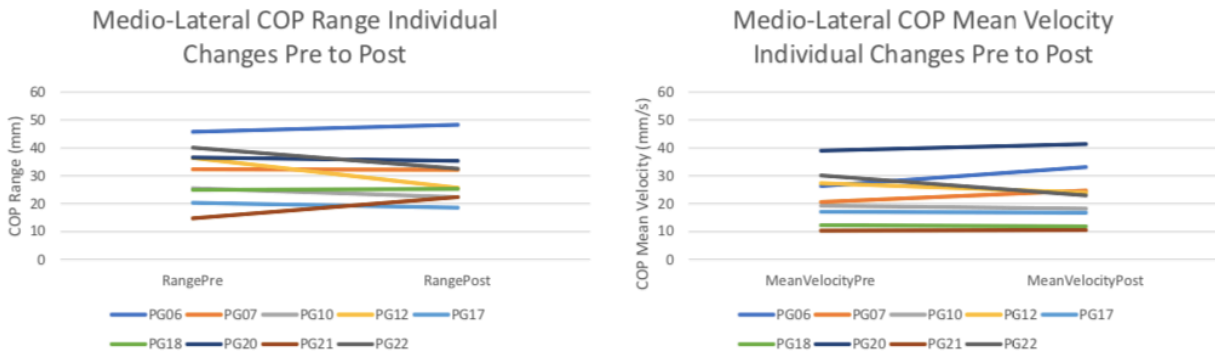


Figure 1: Changes in COP range and mean velocity in the medio-lateral direction for each participant during baseline testing (Pre) and following the golf intervention (Post).

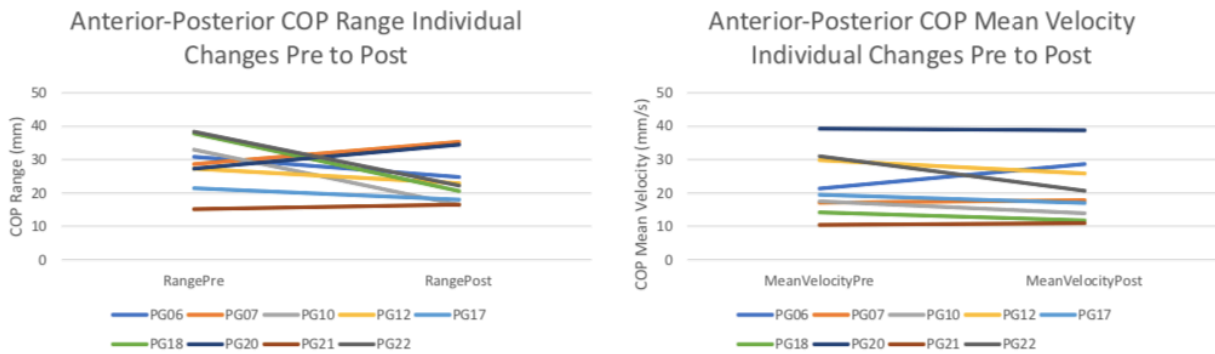


Figure 2: Changes in COP range and mean velocity in the anterior-posterior direction for each participant during baseline testing (Pre) and following the golf intervention (Post).

Discussion: Golf is a dynamic task that may have more pronounced effects on a dynamic balance task. Golf requires walking over uneven terrain as well as bending and twisting while standing. Very little time is spent in quiet standing during a game of golf (putting). Exercise improvements are very task specific,¹² so it could be argued that since little time is spent controlling balance in quiet standing during golf, there were no differences seen in COP range or mean velocity in a semi-tandem balance task. Improvements in balance may be more apparent in a dynamic balance test.

Another argument is that there could be a ceiling effect occurring due to the semi-tandem task not being challenging enough for the participants. This is supported by the fact that all participants were able to complete the 30 second trials. The participants in this study performed very well on the task with only minor fluctuations in COP range and mean velocity for both ML and AP directions. The golf intervention may not have enough of an impact on quiet standing balance in older adults that are already relatively well-balanced. Larger differences may be seen with a more challenging static balance task (i.e. single leg stance) or using a dynamic balance task.

Future studies should investigate changes in dynamic balance following a golf intervention and/or more challenging static balance tasks such as a single-limb or full tandem balance task. These more demanding tasks may be useful to better tease out static balance effects of golf play.

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3. The genetic profile of individuals with traumatic spinal cord injury following an 8-week group exercise program

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Introduction: A traumatic spinal cord injury (SCI) is a devastating event with lifelong consequences. Approximately 17,700 people experience each year in the United States with about 288,000 people currently living with SCI. We have completed the first systemic level genome-wide study of spinal cord injury in human. Infection, pneumonia, cardiopulmonary event, and metabolic disorders are plaguing the quality of life of those with spinal cord injury. Recent evidence supports a link between the immune system and reduced life expectancy for those with SCI². Given that exercise may delay the onset of comorbidities such as cardiovascular disease⁶, improve insulin sensitivity⁷, reduces cytotoxic NK cells following 30 minutes of aerobic activity⁴, and overall has been shown to improve the health of those with SCI^{3,4,6,7} our goal was i) to compare SCI to control and ii) to identify the pathways underlying the effect of exercise in SCI.

Participant characteristics						
Participant ID	Age	Sex	Weight (kg)	Ethnicity	Level of SCI	Completeness
1	41	M	70.5	A.A.	C3	Incomplete
2	28	M	51.5	H	C4	Incomplete
3	22	M	48.3	AA	C5	Incomplete
4	48	M	69.9	N.A.	C5	Incomplete
5	52	F	83.9	A	C7	Incomplete
6	58	M	116.0	H	L5	Incomplete
7	44	M	84.6	H	T12	Incomplete
8	42	M	62.7	H	T2	Complete
9	32	M	66.0	H	T4	Complete
10	26	F	74.7	H	T6	Complete
11	34	M	79.9	A	T7	Complete

Methods: Subjects: This study was approved and performed in accordance with the California State University, Los Angeles IRB. Inclusion criteria: Age 18-55 y/o, traumatic SCI, time of injury >6months, weight< 250lbs, no medical conditions including arthritis, autoimmune disorders, diabetes, hypertension, Cardiopulmonary diseases, liver or kidney disease, musculoskeletal injuries, unhealed

fractures, pressure sores, or untreated infections. Exclusion criteria included the use of baclofen, neurotonin, and medications to regulate blood pressure. Able-bodied (AB) participants were >18 years old, without a history of SCI or any other medical conditions. Participants with SCI were requested to participate in a battery of assessments including anthropometric measures, dual energy X-ray absorptiometry (DXA, GE Lunar Prodigy), resting metabolic rate (Cosmed Quark CPET), and VO₂max assessment via arm ergometry (Lode), and blood draws prior to and after engaging in an 8-week on-campus group exercise protocol (N=11) designed

for wheelchair users. Participant characteristics can be found in Table 1. Whole-Blood was collected into PaxGene tubes according to manufacturer's protocol (Qiagen) by a trained phlebotomist, then stored at -80C°. RNA was extracted, amplified and sequenced through next-generation sequencing (Illumina HiSeq 3000).

Exercise Protocol: The 8-week exercise protocol was 2x/week for 1h. The duration of aerobic exercise began with 15 minutes, increased by 5 minutes weekly until a total of 30-minutes was reached. Aerobic exercise was segmented into intervals ranging from 55-80% HR_{max}, (Wahoo Fitness), and 30-minutes of resistance exercise (TheraBand) performed in 3 continuous rounds. Each round of resistance consisted of 6 exercises (Military press, Horizontal row, Chest fly, Latissimus pulldown, Dip press, & Bicep curl) with 8-10 repetitions at a tempo of 3 seconds for each concentric and eccentric phase. Each participant was teamed with three research fellows to monitor exercise intensity, anchor resistance bands, provide stability and motivate the subjects.

Results:

Impact of Spinal Cord Injury: The overall gene expression difference between SCI and control is shown in a

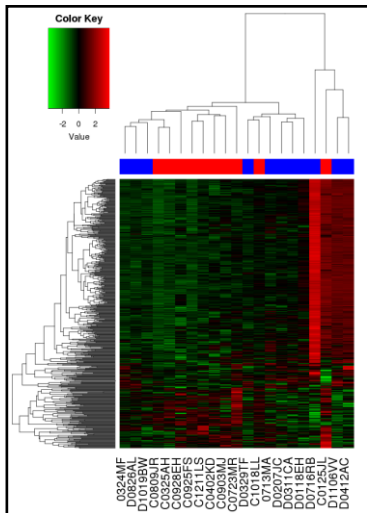


Figure 1: Heat map generated from DNA sequencing comparing SCI and control subjects

heat map (Fig. 1). We can observe that most of the genes were downregulated with SCI and many are grouped in clusters showing the biological signature of SCI. These genetic changes have then been combined using IPA software and the effect of SCI yields notable change to >150 canonical pathway genes known from the Kyoto gene bank. The greatest change was associated with interferon signaling ($p = (-\log(2.11))$), and Pyrimidine Ribonucleotides pathways ($p = (-\log(1.74))$) (Fig. 2). Analysis of genes associated with function and diseases yield

500 genes with significant changes. Cell signaling ($p < 0.0001$), and developmental disorder, hereditary disorder, ophthalmic disease, organismal injury and

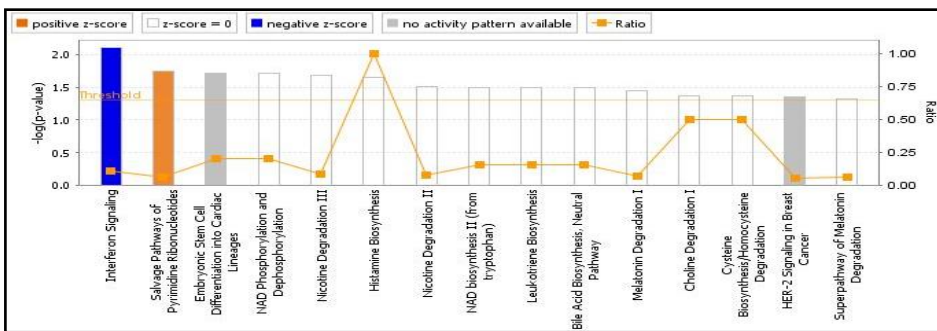


Figure 2 Canonical pathway between SCI and controls

abnormalities ($P < 0.0001$) displayed

the greatest change when comparing

AB with SCI. Diseases and functions

of cell signaling, inflammatory disease

neurological disease, cell movement,

antimicrobial response, and inflammatory response produce

the most involved networks ≥ 25 molecules. The upstream regulators of MAPK1 pathway, an important

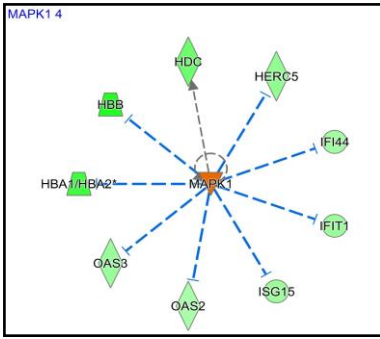


Figure 3: MAPK1 pathway downregulated in SCI when compared to control

abnormalities ($p < 0.05$). From network assembly, 5 distinct molecular networks emerged with >12 molecules. The largest network (23 molecules) relates to cancer, organismal injury, abnormalities and cellular development.

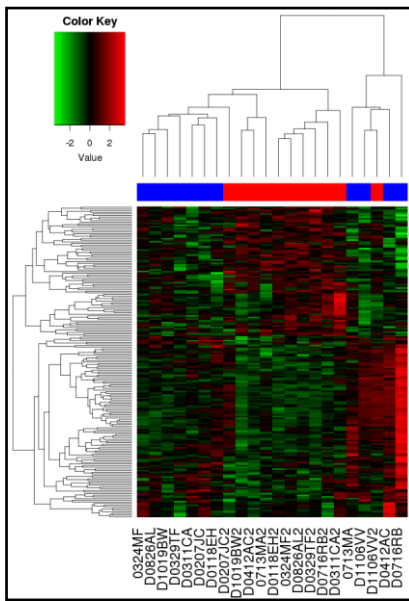


Figure 5: Heat map comparing SCI before and after exercise

pathway involved in the immune system appear to be downregulated following injury (Fig. 3). **Effect of training:** The overall impact of exercise on SCI subjects is shown with a heat map on Figure 4. More than 200 canonical pathways are significantly impacted by the 8-week training program, of which Synaptic long term depression $p = (-\log(2.76))$ and endothelin-1 signaling $p = (-\log(2.54))$ express the greatest change (Fig.5). Functional and disease analysis yields the most change to cancer, gastrointestinal disease, organismal injury and

Discussion: This study is the first genome-wide analysis comparing SCI and able-bodied. It is also the first study to assess gene expression following a group exercise-based intervention in those with spinal cord injury. There is a significant change in a large number of genes, but very surprisingly, the pathways involved in SCI are closely related to the immune system. Noteworthy, the most significant is the interferon signaling, a critical pathway involved in viral defense (Bloom, 2018). Furthermore, we have identified potential new targets that may contribute to understanding the involvement of the immune system response to SCI (table 2). Following exercise, genes associated to synaptic long-term depression, endothelin-1 and gastrointestinal diseases express the most change.

Although mortality rates from other factors such as cancer, CVD, stroke, or suicide are declining, they are being offset by endocrine, metabolic and nutritional diseases, nervous system disorders, and mental disorders. Exercising in a group setting clearly provide a social support group and reduce the effects of depression or depressive behaviors in participants. Following exercise, downregulation of the endothelin-1 (ET-1) signaling pathway, an important regulator of vasoconstriction may be indicative

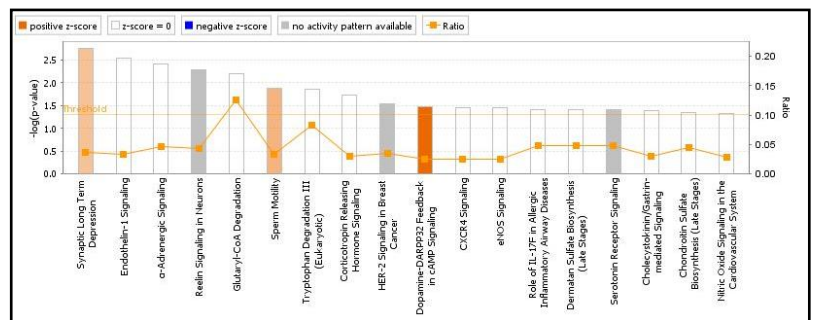


Figure 4 Canonical pathway for SCI after exercise

SCI vs control	SCI sed vs Ex
IFNL1	PGF
IFNA2	HDL
PRL	IGF-1
IFNL4	IL-8
IFNA1/IFNA13	PDCD1
IFNB1	PTN
IL1RN	BMP7
IFNA10	KITLG
IFNA21	CCL2
IFNA5	TNFSF10
IFNA7	CCL21
IFNA14	IL23A
IFNA4	IL36A
IFNA6	TNFSF15
IL7	
IL22	
IL12B	
IFNA17	
IFNA8	
IFNA16	
IFNG	

Table 2 Potential therapeutic targets

of an improvement to the vascular system of those with SCI. A Previous study examining plasma endothelin in SCI has shown that blockade of ET receptors produces a significant change in blood flow of the observed limb, while having no effect on able-bodied controls⁸. Here we found that genes associated with ET pathways seem to be involved in the effect of exercise in SCI.

Conclusion: This study is important because it demonstrated that a genome-wide analysis at the systemic level showed gene expression difference between SCI participants and control. It unmistakably demonstrates the need for a paradigm shift in the field of SCI. Indeed, most researchers focus on the spinal cord while mortality is mostly linked with systemic and chronic conditions. There is gap that need to be filled in better understanding the role of molecular pathways in the quality of life of people suffering from SCI. Our data showed that the effect of long-term SCI results

in changes in the first, the interferon signaling pathway, and second in the pyrimidine ribonucleotides. Cell signaling, developmental disorders, hereditary disorder, ophthalmic diseases, organismal injury and abnormalities have the greatest significant difference between SCI and control. An 8-week group-based exercise training also yields genome-wide alterations in which the greatest change observed is synaptic long-term depression and endothelin-1 signaling. Finally, exercise appears to have a profound role on cancer and gastrointestinal disease genes for those with long-term traumatic SCI. We have identified new targets for potential prophylactic and therapeutic pharmaceutical strategies. Future analysis needs to delve into the upstream regulators of these genetically altered pathways, molecular networks of interest, and the physiological response to exercise.

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4. Cardiovascular Adaptations in a Male-to-Female Transgender Athlete Before and During Estrogen Therapy: A Case Study

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Background: Estrogen is a potent steroid hormone that is known to have a wide range of effects on the cardiovascular system. However, the relationship between the cardiovascular risk and estrogen is not conclusive. For instance, women tend to have a lower prevalence of coronary artery disease, which has led to the thought that estrogen may be a “cardioprotective” hormone [1]. However, hormone replacement therapy in postmenopausal women is associated with an increased CVD risk [2]. Although low cardiovascular (CV) event rates are linked to estrogen, it is important to keep in mind that estrogen is associated with a higher incidence of morbidity and mortality [3].

The transgender population in the United State is increasing, and current estimates indicate that 1 out of every 250 adults in the United States identify as transgender or are gender dysphoric[4]. Thus, the need to fully understand the effect of estrogen on the cardiovascular system in male to female transgender individuals needs to be addressed. A recent review demonstrates that there may be an increased risk for myocardial infarction in transgender subjects when compared to age matched cisgender subjects [5]. Despite this research, changes in biventricular myocardial performance and mechanics (an early marker of CV dysfunction) following estrogen administration in males during transition are currently unknown as well as estrogen’s impact on determinants of aerobic fitness.

Purpose: To comprehensively phenotype before and during estrogen treatment for gender reassignment in an aerobically trained male-to-female transgender athlete.

Methods: The case study subject is a 28-year old, biologically male endurance runner currently undergoing gender reassignment. Outcome variables were assessed on two separate occasions prior to beginning hormone therapy, which were averaged to represent pre-estrogen therapy data. Sequential visits were averaged on two-month intervals. Data presented are from pre to 12 months of estrogen therapy.

All testing was carried out following an overnight fast and at the same time of day to minimize the effects of circadian variation on cardiovascular markers. All tests performed have been validated and shown to have good test-retest reliability within the testing lab. The following measures were performed during every testing visit:

Central and Peripheral Blood Pressure and Pulsewave Velocity: Central aortic and peripheral blood pressure waveforms were assessed using the non-invasive SphygmoCor system (AtCor Medical). Pulse wave analysis and augmentation index was derived by using brachial and central pulse pressure oscillations. Pulse wave velocity, was calculated by acquiring the carotid pulse with applanation tonometry and using the volumetric displacement waveform acquired from the femoral artery. This method has been shown to have excellent agreement with invasive aortic blood pressure with a mean difference of <1 mmHg [6]. Three measurements were taken at each visit and the closest of the two are averaged.

Resting Echocardiogram- 2D and Doppler measures of the Apical four and two chamber views were obtained by a registered diagnostic sonographer using the Terason uSmart 3300 system and following techniques outlined by the American Society of Echocardiography [7, 8]. All measures were averaged over 3-cardiac cycles. Images were analyzed using Echoinsight™ Software (Epsilon Imaging) in a blinded fashion [8-10]. Left Ventricular (LV) and Right Ventricular (RV) systolic and diastolic function were assessed at rest.

Anthropometrics- Height and weight were determined using a Detecto Balance Beam scale (Webb City, MO, USA) and a Stadiometer (Seca, Chino, CA, USA). After 15 minutes of lying flat a whole-body Dual-energy X-ray absorptiometry (DXA) (GE Lunar iDXA) scan was performed by a certified radiology technician. DXA has been demonstrated to have a high degree of precision measuring fat and lean mass with a coefficient of variation of 1.0% and 0.5% respectively [11].

VO_{2 peak} Testing and Physioflow™- After a 5 min warm up, the subject completed 3, 5-minute running economy tests at 7, 8, and 9 mph, respectively. Running economy tests were followed by a ramp test at 9 mph (1% grade increase per min) to exhaustion. Expired gases were continuously analyzed for ventilating, %O₂ and %CO₂ via the Oxycon Mobil™ metabolic cart [12, 13] and the Physioflow

Enduro™ [14] was used to non-invasively estimate cardiac output, stroke volume, systemic vascular resistance, and heart rate every 15 seconds during the running economy and ramp tests.

Results: All results are presented in 2 visit averages.

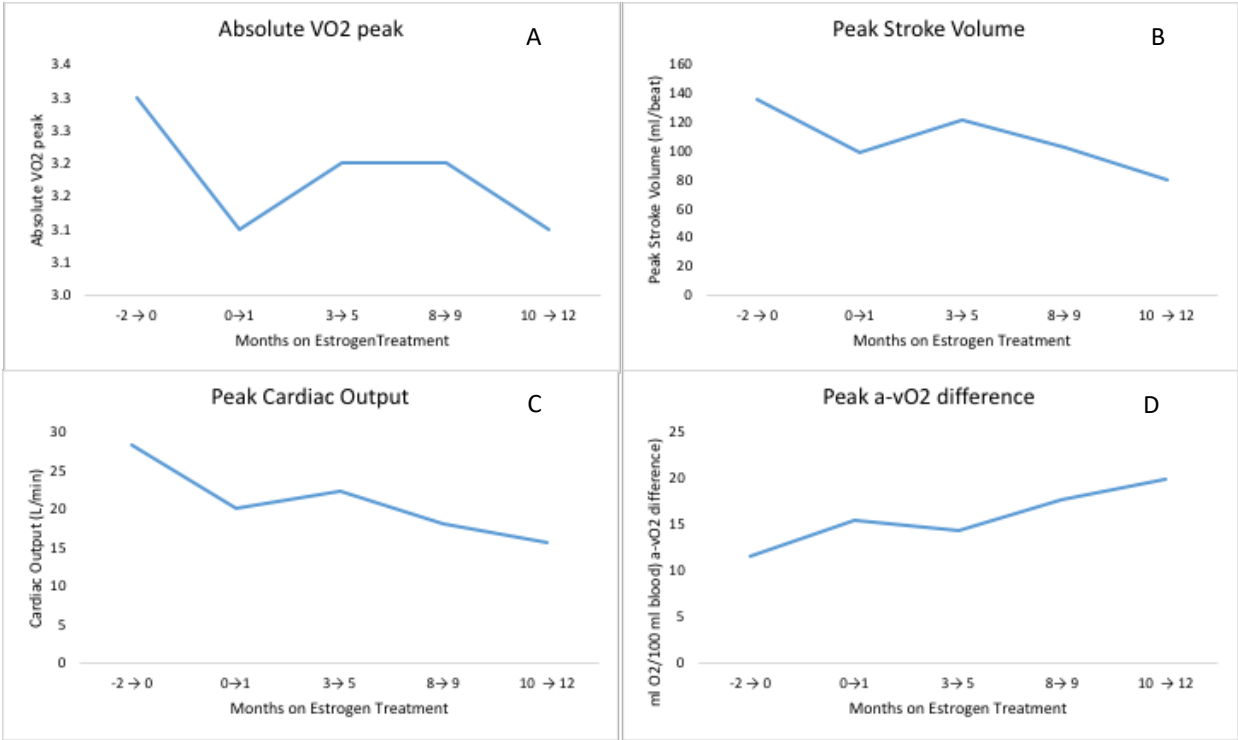


Figure 1: Aspects of the Fick Equation taken during peak exercise testing prior to and throughout estrogen treatment A) Absolute VO_{2 peak} – 6% reduction from baseline B) Peak Cardiac Output-42% reduction from baseline C) Peak Stroke Volume- 42% reduction from baseline D) Peak a-vO₂ Difference- 42% increase

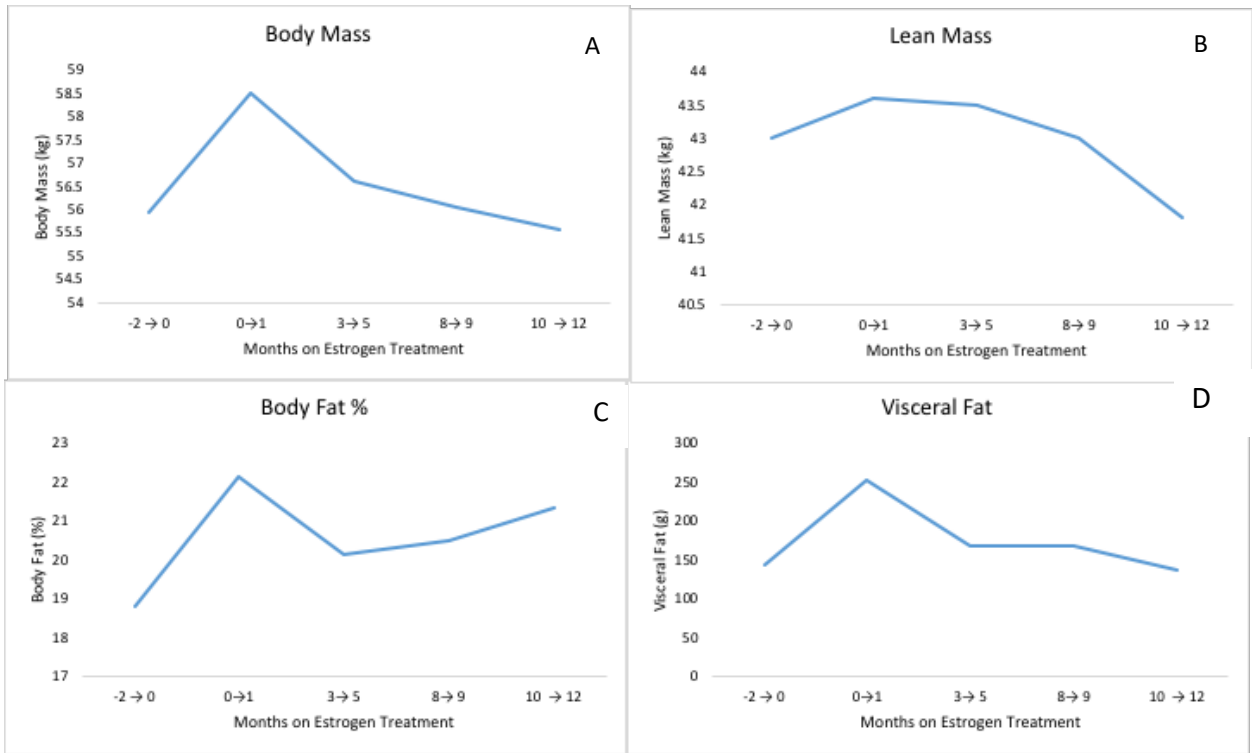


Figure 2: Changes in body composition A) changes in body mass- negligible change B) changes in lean mass- 3% reduction from baseline C) Fat %- 12% increase from baseline D) Visceral Fat – 12% reduction from baseline

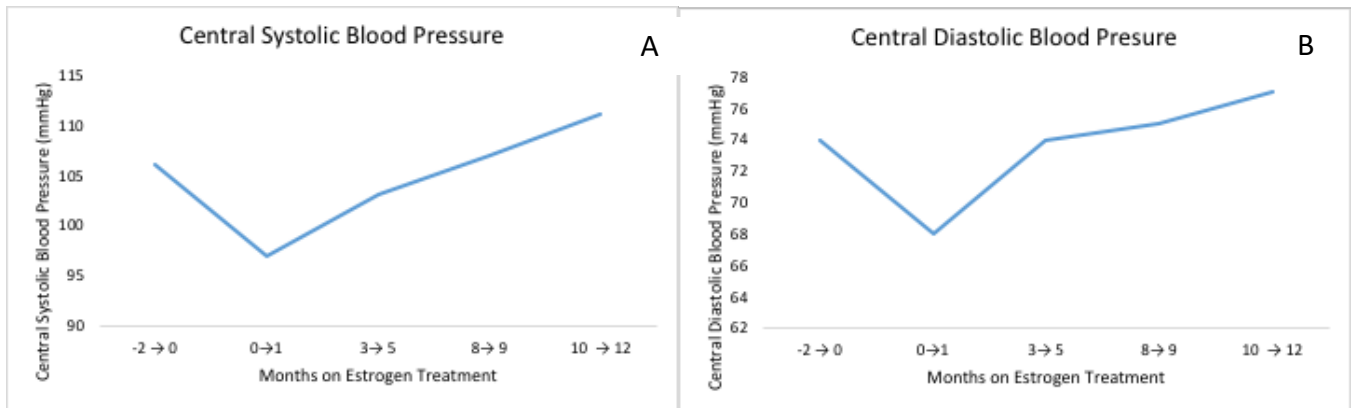


Figure 3: Changes in Central Blood Pressure A) changes in central systolic blood pressure B) changes in central diastolic blood pressure

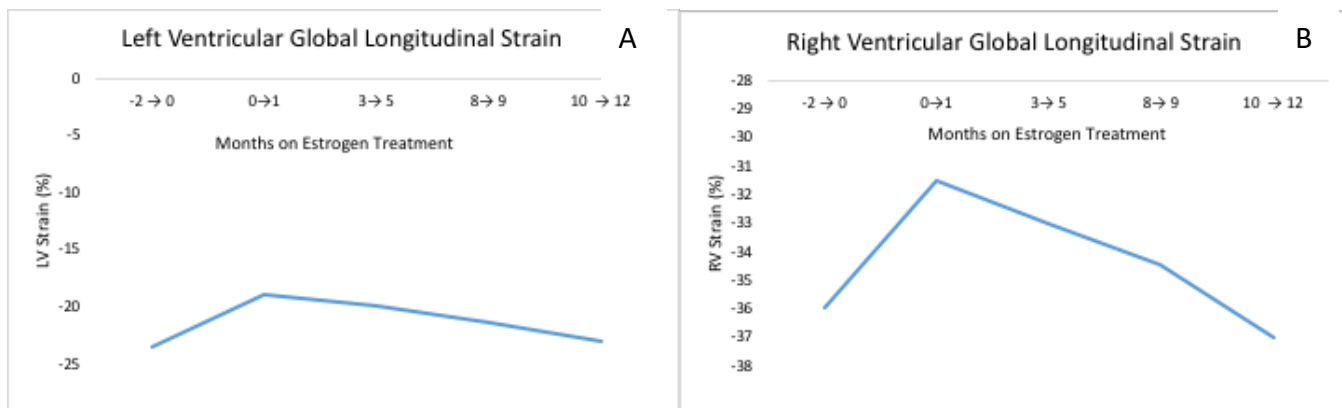


Figure 4: Global longitudinal strain (GLS)- measure of systolic function. A) Left Ventricular GLS B) Right ventricular GLS

Discussion: While still ongoing for one more year and will continue with the addition of an androgen blockade, this case study indicates that initiation of estrogen therapy resulted in significant reductions in peak cardiac output and stroke volume with compensatory increases in peak a-vO₂ difference. While significant changes in aspects of the Fick equation were seen, there was only modest declines in absolute VO_{2 peak} and lean mass were also seen while relative VO_{2 peak} was preserved, despite no changes in aerobic training. Similar results have been observed in postmenopausal women receiving hormone replacement therapy [15]. Further, transient worsening of myocardial strain was observed following the initiation of estrogen therapy. Given myocardial strain serves as a marker of systolic function, the strain data demonstrate that there is a need to consider cardiovascular function and estrogen's impact on the heart and the heart's function prior to transitioning.

Conclusion: With initiation of estrogen therapy, significant impacts on performance and function were seen. There is a need for a larger cohort study with comprehensive cardiovascular phenotyping and long-term follow-up to better understand the effects of estrogen on the cardiovascular system and the impact on long term health.

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5. Physiological response to exercise intensity in spinal cord injured compared to able-bodied individuals

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Introduction

Engagement in regular physical activity beyond the activities of daily living is essential to maintain and improve cardiometabolic health and fitness (Garber et al. 2011). Guidelines on volume of cardiorespiratory and resistance exercise specify not only the duration and frequency of activity, but also the level of intensity needed for healthful adaptations. Responses to modification in exercise intensity may be different, however, in individuals with altered neurophysiological function. Specifically, individuals with spinal cord injury (SCI) often have motor-, sensory-, and autonomic dysfunction that can affect not only their ability to participate in the exercise, but also their ability to draw physiological benefit from training (Jacobs, P. L. and Mark S. Nash 2004). For example, individuals with upper thoracic or cervical injury may not have the sympathetic drive needed to support the energetic demands of physical activity (Cowley 2018, Sisto et al. 2012, Lehmann et al. 1987). Individuals with lower thoracic or lumbar level injury may retain the somatic and autonomic ability to perform upper body movement with force, but may encounter limitations from due to hypokinesia (Hjeltnes N, Vokac Z). These complications not only limit the quality and quantity of exercise, but may predispose individuals to psychological and physiological stress (Ginis, K. A. M., et al. 2003, Gerhart KA, et al. 1993).

Participation in regular exercise is associated with numerous benefits, including, increase in muscle strength, healthy change in body composition, and improvement in physical and psychological well-being (Hicks et al. 2003, Jacobs et al. 2009, Davis et al. 1987, Nash 2002, Warburton et al 2006). Individuals with SCI report barriers to participating in exercise such as cost, accessibility, and transportation (Scelza, W. M., et al. 2005). While the benefits of maintaining exercise training after SCI is

clear, it is imperative to understand how the energetic demands experienced by the SCI population may differ from those of able-bodied individuals (Kehn, M. & Kroll, T. 2009, Mulroy, S.J., 2011).

Purpose: This study is one in a series evaluating exercise training in neurologically intact and spinal cord injured persons. The purpose of this study is to compare the physiological response to increasing intensity in two upper body resistance exercises, triceps dips and biceps curls, performed in the seated position by the two populations.

Hypothesis: Metabolic response to a change in exercise intensity may be deficient in SCI compared to able-bodied participants.

Methods: Ten active participants (eight males and two females, age: 32.3 ± 7.6 years, height: 170.8 ± 7.4 cm, mass: 80.8 ± 19.3 kg; participant demographics located in table 1) currently engaged in leisure physical activity volunteered for this study. Inclusionary criteria: based on age (18–65 years), and ability to perform the exercise. Exclusionary criteria: contraindications to exercise, and modifications to exercise that resulted in a different movement. The study protocol was approved by the California State University Los Angeles's Internal Review Board, with the written consent given by participants before the commencement of testing. Indirect calorimetry: Breath by breath measurements were collected and averaged every five seconds on a Cosmed Quark CPET system; the proprietary software and provided outcome measures. Biotelemetry was provided by Wahoo TICKR (Wahoo Fitness) proximal to the xiphoid process and synced to COSMED via ANT+. An Actigraph GT9X collected accelerometry outcomes placed on the self-reported non-dominant hand of each participant, METS and energy expenditure were provided via the Actilife 6.13.3 software using the Freedman 1998 algorithm at 100 Hz and one epoch to give accelerometry the fairest comparison. Electromyography was collected using a BTS FreeEMG system at seven sites at 2000 Hz and synced to a Vicon 8 camera system using nexus 2.6.1 software and a modified upper body plug in gate model. All Participants were required to complete two visits. The

first visit consisted of consenting and collecting anthropometrics and participant characteristics, familiarization with exercises to be performed, and a VO₂ peak assessment. The second visit consisted of participant preparation for the placement of markers and electrodes; 10-minute seated resting metabolic rate, static calibration, MVICs for each muscle collected on; three rounds of a circuit resistance training protocol adapted from previous work that pairs agonist and antagonist emphasized exercises with interspersed aerobic arm cycling, and 10-15 min EPOC collection. For the exercises performed, participants performed arm ergometry on a Lode Corival arm cycle and resistance exercises on a red and black Theraband®. Statistics: Preliminary results were provided by organizing data in excel 2016 for OSx and Prism 7.0. A prefatory statistical analysis was performed using a t-test for each group. Results: We observed that Levels of VO₂ during exercise were normalized to peak and resting levels to determine the intensity of the exercise as a percentage of the individual's VO₂reserve. Levels of VO₂ higher in SCI compared to AB during performance of both DP and BC exercises when presented as a function of VO₂reserve.

It was also observed that participants in both the SCI and AB groups experienced significant increases in VO₂ and HR with increasing level of resistance during the DP exercise (figure 1A and 1B, left-side of graphs, $p < 0.05$). Performance of BC exercise was associated with increase in VO₂ and HR (Figure 1A and 1B, right side of graphs), but the change was found to be statistically significant only for SCI heart rates. Levels METS and EE also increased as a function of intensity, but only when the value was calculated from indirect calorimetry (Figure 1C and 1E). Using accelerometry to calculate METS and EE (Figure 1D and 1F) resulted no significant change in either measure for either exercise.

Discussion: It has been observed in previous studies that levels of peak oxygen consumption (VO₂peak mL/kg/min) during a test of maximal exertion on an arm crank ergometer is less in individuals with SCI compared to AB. The reason for the lesser performance may be attributed to lesser level of physical fitness in the SCI. It may also be that AB persons have an advantage over SCI that they can access

stabilizing muscles in the trunk and lower body when performing upper body exercise, despite being asked to refrain from using the trunk and lower body muscles in any of the exercises. In turn, levels of VO₂reserve, i.e. difference between peak and resting metabolic rate, were smaller in SCI compared to AB. Therefore, regardless of whether participants with AB were able to outperform those with SCI in either exercise, the individuals with SCI were working at a higher level of intensity.

We found differences in the significance of VO₂ and HR between the two types of exercise (DP vs BC).

Observation of muscle activity data, not reported here, indicated that the performance of the DP exercise resulted in the activation of triceps, trapezius, and deltoid muscles, whereas performance of the BC exercise tended to activate the biceps muscle in isolation. Activation of relatively more muscles may explain the finding of significant change in the VO₂ and HR levels in the DP, but not the BC exercise in this study.

Finally, with respect to the inability of accelerometry to detect change in intensity. Participants were instructed to maintain a strict 3-0-3 tempo throughout the eccentric-to-concentric phases of both resistance exercises in order to diminish the use of momentum during the movement. This eliminated the utility of accelerometry to accurately reflect the intensity of exercise for either exercise.

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Participant	Sex	Age (yr)	Height (cm)	Mass (Kg)	Injury Level	Completeness
1	F	22	156.2	60.3		INTACT
2	M	28	177.8	119.8		INTACT
3	M	40	167.6	92.2		INTACT
4	M	30	178	99.4		INTACT
5	M	25	177	81.7		INTACT
6	M	42	177.8	58.6	T11	C
7	M	34	167.6	75.4	T4	C
8	F	26	162.6	72.5	T6	C
9	M	32	172.7	63.1	T4	C
10	M	44	170.2	84.9	T12	I

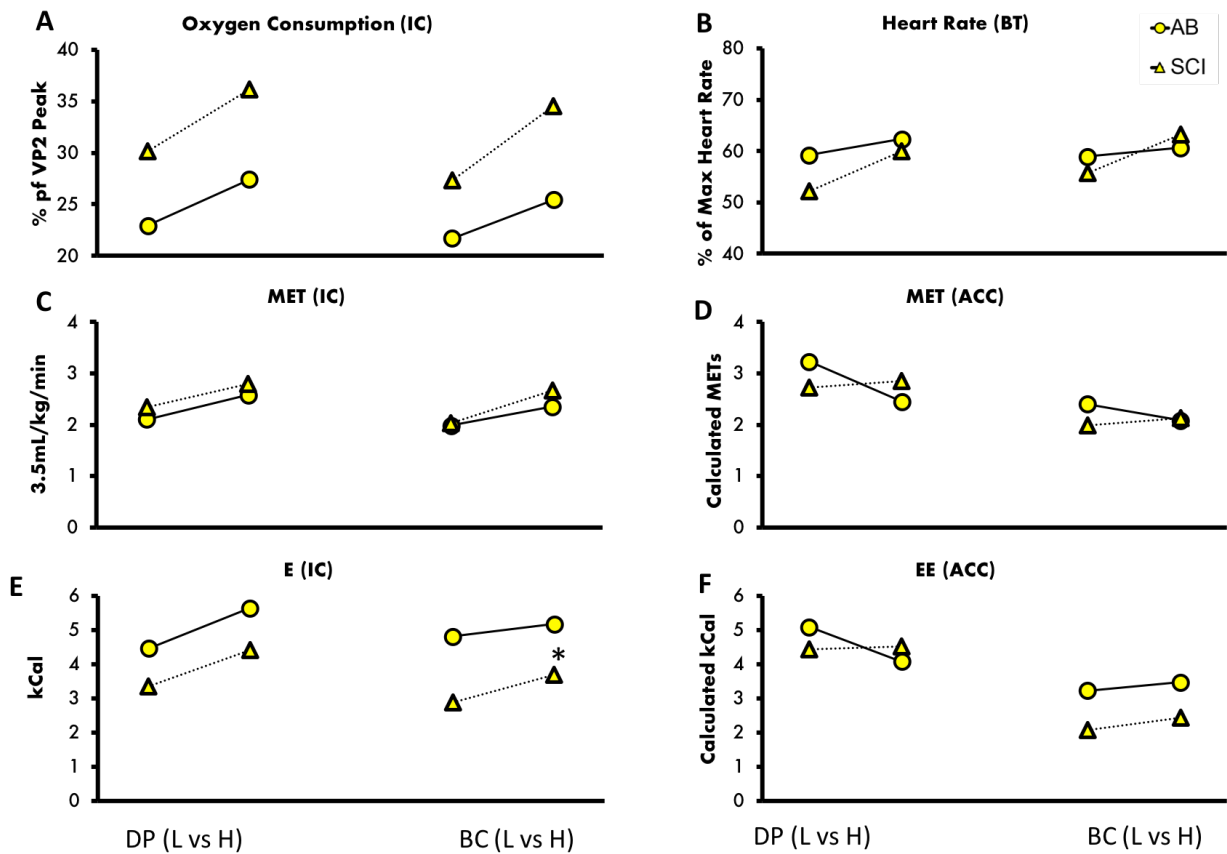


Figure 2: Comparison of able-bodied (solid line) and spinal cord injured (dashed line) tracked responses during triceps dip (Dips), and biceps curl (Curl) exercises using a light (L) or heavy (H) resistance band. Tracked data is: A) oxygen consumption using indirect calorimetry (IC), B) heart rate using a biotelemetric chest strap monitor, C) METS calculated by indirect calorimetry, D) METS calculated via an accelerometer attached to the wrist (ACC), E) energy expenditure calculated via indirect calorimetry, and F) energy expenditure calculated via the accelerometer (ACC).

