THE EFFECT OF 5% HYPOHYDRATION ON MUSCLE CRAMP THRESHOLD FREQUENCY

A Thesis
Submitted to The Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Kyle William Braulick

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Advanced Athletic Training

June 2012

Fargo, North Dakota

North Dakota State University

Graduate School

The Effect of 5% Hypohydration on Muscle Cramp Threshold Frequency

By

Kyle William Braulick

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Kevin C. Miller, PhD, LAT, ATC
Chair

Jay M. Albrecht, PhD, LAT, ATC, HFS

Jared M. Tucker, PhD

James E. Deal, PhD

Approved:

06-21-2012
Date

Margaret Fitzgerald
Department Chair

ABSTRACT

Many clinicians believe exercise associated muscle cramps (EAMC) occur because of dehydration and electrolyte losses. Experimental research supporting this theory is lacking. Mild hypohydration (3% body mass loss) does not alter cramp threshold frequency (TF), a measure of cramp susceptibility, when fatigue and exercise intensity are controlled. No experimental research has examined TF following significant (3-5% body mass loss) or serious hypohydration (>5% body mass loss). TF and blood variables of ten subjects were measured pre-post exercise. Subjects exercised in an environmental heat chamber alternating between upper arm ergometry and non-dominant leg cycling every 15 minutes until they lost 5% of their body mass or were too exhausted to continue. Significant (n=5) or serious hypohydration (n=5) did not alter cramp TF, cramp intensity, or cramp EMG amplitude. Significant and serious hypohydration with moderate electrolyte losses does not alter cramp susceptibility when fatigue and exercise intensity are controlled.

ACKNOWLEDGMENTS

Thank you to my advisor and mentor, Dr. Kevin Miller, for always challenging me. Thank you for opening my eyes to research and the scientific process. The numerous skills I've learned from you over the last 2 years will benefit me in my future endeavors.

Thank you to the rest of my graduate committee, Drs. Albrecht, Tucker, and Deal. Your background, knowledge, and support made this possible.

Finally, thank you to NDSU's College of Human Development and Education, NDSU's Department of Health, Nutrition, and Exercise Sciences, and NDSU's Athletic Training Education Program for partially funding this research.

DEDICATION

I dedicate this Thesis to my loving family and fiancée Jessica for allowing me to chase my dreams, wherever they may take me. None of this would have been possible without your confidence and support. I look forward to my next journey knowing you'll support me throughout.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

INTRODUCTION

Exercise-associated muscle cramps (EAMC) are painful involuntary contractions of skeletal muscle occurring during or shortly following exercise.[1] EAMC can impair athletic performance,[2] and EAMC symptoms can last up to 8 hours post-activity.[3] EAMC typically occur in multi-joint muscles (e.g, hamstrings), and are a common injury.[4-5] Despite their prevalence, EAMC cause remains unknown.

The dehydration and electrolyte theory is the most popular theory to explain the onset of EAMC.[6] The theory states exercise-induced sweating causes fluid to shift from the interstitium to the intravascular space.[7-8] The interstitium then contracts increasing pressure on select nerves and altering excitability.[7] EAMC then ensue.[8] Support for this theory comes from research comparing fluid and electrolytes losses in crampers and non-crampers.[9-11]

Others have observed altered muscle spindle[12] and golgi tendon organ[13]activity in fatigued muscle. Thus, some scientists proposed EAMC occur when the nervous system fatigues and afferent activity causes a reflexive excitation at the alpha motor neuron pool.[1] Support for this theory comes from investigations linking EAMC risk with faster race times (and possibly greater fatigue) than dehydration.[14] Moreover, crampers have lower golgi tendon organ inhibition than non-crampers[15] and afferents activated by hypertonic saline reduced cramp threshold frequency (TF, minimum electrical stimulation needed to induce cramp).[16]

Not controlling exercise intensity or fatigue is a limitation of studies examining EAMC.[9-11,17-18] Some scientists have attempted to isolate the effects of dehydration and electrolyte losses from fatigue by electrically inducing cramps and measuring cramp TF.[19] These scientists[19] observed mild hypohydration (3% body mass reduction) did not alter TF. Cramp TF is thought to be a quantitative measure of cramp risk where lower TF indicates increased risk and vice

versa.[20] Since some athletes with a history of EAMC have high fluid and electrolyte losses,[9-11] determining cramp risk when individuals are significantly or seriously hypohydrated is needed. The National Athletic Trainers Association[21] defines significant and serious hypohydration as 3-5% and >5% body mass losses, respectively. No research has examined cramp TF following significant or serious hypohydration. Based on previous observations,[22] we hypothesized TF would be similar when euhydrated or significantly or seriously hypohydrated when peripheral fatigue and exercise intensity are controlled.

MATERIALS AND METHODS

Subjects

Thirteen males between 18 and 30 years old volunteered; three were excluded because cramps could not be induced in the flexor hallucis brevis (FHB) on the familiarization day. Ten males completed the study (age=24±4y, ht=184.2±4.8cm, pre-exercise mass=84.8±11.4kg).

Volunteers were excluded if: 1) they experienced injury to their legs in the 6 months pre-data collection; 2) they self-reported any cardiovascular, neurological, or blood borne diseases; 3) they did not self-report a history of leg cramps within the 12 months pre-data collection; 4) cramp could not be induced in the FHB; 5) they were not physically active (≥30 minutes of activity on all or most days per week);[23] 7) they were taking any medications; 8) they had a history of cardiac events, heat exhaustion, or heat stroke. All procedures were approved by our university's ethics board and subjects provided informed consent pre-testing.

Procedures

Subjects reported for one familiarization session and one testing day. For the familiarization session, subjects were asked to arrive hydrated and avoid caffeine, alcohol and exercise for 24 hours. They reported to a laboratory and had their leg dominance determined.

Subjects lay supine and had their dominant leg prepped for EMG analysis.[19] Subjects' medial ankle, lower leg, tibial tuberosity, and midbelly of the gastrocnemius were shaved (if necessary), debrided with fine sandpaper, and cleaned with alcohol. Two Ag-AgCl EMG electrodes (EL502, Biopac Systems Inc, Aero Camino, CA) were placed over the midbelly of the FHB with a 2 cm center-to-center distance. A single ground EMG electrode was placed over the ipsilateral tibial tuberosity. A single Ag-AgCl electrode (6750, Prometheus Group, Dover, NH)

was placed over the middle of the gastrocnemius and connected to a biofeedback unit (Pathway TR-10C; Prometheus Group, Dover, NH).

The ankle was placed in a foam block with a foot pad angled at 120º to keep the foot in slight plantar flexion. The big toe was placed into a toe harness. Subjects performed 20, 2-s duration FHB maximum voluntary isometric contractions (MVIC) with 1 minute of rest separating each MVIC. Following these MVICs, subjects rested 5 minutes and performed three, 2-s MVICs. The mean EMG amplitude of these contractions was averaged and used to determine cramp intensity.

To ensure subjects were using the correct muscle during MVICs, gastrocnemius activity was monitored; activity exceeding 8 mV indicated a failed attempt. If subjects performed MVIC incorrectly, a 1-min rest period was given and MVIC was re-attempted. Researchers have reported high MVIC intratester reliability using this method (ICC 3,3>0.81).[24] Following MVICs, subjects big toe and ankle were removed from the toe harness and foam block, respectively, and subjects were prepped for cramp induction. An 8-mm Ag-AgCl shielded electrode (EL258S; Biopac) was placed over the medial ankle distal to the medial malleolus where the tibial pulse was felt. An 8-cm, square dispersive electrode was placed over the lateral malleolus. To determine proper placement of the stimulating electrode, the tibial nerve was stimulated with 1-ms electrical stimuli at 80V (Grass S88 stimulator with SIU5 stimulus isolation unit, Astro-Med Inc., West Warwick, RI). The proper electrode location was the site causing the greatest hallux flexion.

Subjects were instructed to relax during cramp induction. Subjects received 1 second of rest followed by 2 consecutive bursts of 80V electrical stimulation. Initial burst frequency was 4 Hz. If cramp was not induced, subjects rested 1 minute and stimulation frequency was increased

by 2 Hz until FHB cramped. These procedures have been used previously and have high intratester (ICC 3,1=0.84), and intertester reliability (ICC 3,1=0.96).[25]

A muscle cramp was defined as an involuntary contraction of the FHB immediately following the end of the electrical stimuli and must have met three criteria. First, post-stimulus EMG amplitude must have been ≥50% of MVIC EMG amplitude, and must have maintained this intensity for >5 seconds. Second, subjects verified the cramp. Finally, sustained flexion of the first ray must have been observed post-electrical stimulation. The frequency used to induce a cramp meeting these criteria was deemed cramp TF. If a cramp did not spontaneously resolve after 5 seconds, the FHB was passively stretched. The electrode sites were marked for future testing sessions. Subjects reported for their testing session ≥36 hours following the familiarization day.

Twenty-four hours before their testing day, subjects avoided exercise. Twelve hours before the testing day, subjects were instructed to fast for 6 hours, drink water consistently, and avoid consuming any beverage other than water. On the testing day, subjects emptied their bladders completely and urine specific gravity was measured using a refractometer (Sur-NE, Atago VSA Inc., Bellevue WA). If hypohydrated (urine specific gravity>1.010),[21] subjects testing day was rescheduled. If euhydrated, subjects lay supine for 30 minutes for fluid compartment equilibration.[26] The dominant leg was prepped for cramp induction using the procedures described above. A sterile catheter was inserted into the subject's arm; a 5-mL blood sample was collected (euhydrated sample).

Subjects performed 10 practice MVICs (1 min rest between each MVIC), rested for 5 minutes, and performed three consecutive 2-s duration MVICs. After 15 minutes rest, subject's euhydrated TF was determined. Post-cramp induction, the electrodes were removed. Subjects

donned a heart rate monitor (Polar Electric Inc., Lake Success, NY), inserted a rectal thermistor (Yellow Spring Instruments 4600, Advanced Industrial Systems Inc., Prospect, KY) 10 cm past the anal sphincter, and were weighed nude. Both posterior mid-forearms were shaved, cleaned with deionized water and dried. Sterile sweat patches (occlusive dressing and sterile gauze) were placed at these sites.

Subjects donned sweat pants, socks, shoes, and a t-shirt and entered an environmental chamber (39.1±1.5°C; 18.4±3% humidity). They alternated exercising with an upper body cycle ergometer or non-dominant leg exercise every 15 minutes at 70% of their age-predicted maximal heart rate. Sweat patches were removed after 30 minutes of exercise. Following sweat patch collection, subjects donned a hooded sweatshirt for the remainder of the exercise protocol. After 60 minutes, subjects exercised for 5 minutes at a self-selected lower intensity to cool down. They exited the environmental chamber, removed their clothes, towel dried, emptied their bladders completely, and were weighed nude. Subjects dressed and rested for 10 minutes in a climate controlled room. They resumed exercising for another 60 minutes using the above protocol. These procedures continued until subjects lost 5% of their body mass or were too exhausted to continue. Upon 5% hypohydration or volitional exhaustion, subjects exited the environmental chamber, removed the heart rate monitor and rectal thermistor, and lay supine for 30 minutes.

During the rest period, the EMG and stimulation electrodes were placed over the previously marked locations. After the rest period, a 5-mL blood sample was collected (hypohydrated sample). Subjects performed 10 practice MVICs (1 min rest between each MVIC), rested for 5 minutes, and performed three consecutive 2-s duration MVICs. Cramp TF

was reassessed 15 minutes following MVIC. Following cramp induction, the electrodes were removed and subjects were excused. No fluids were given during testing.

Cramp and MVIC EMG Procedures

FHB muscle action potentials were sampled at 2000 Hz, amplified (gain=2000), and filtered (band pass, low frequency=10 Hz, high frequency=500 Hz) using the BioNomadix analog-to-digital system operated by Acqknowledge 4.0 software (Biopac). Amplifier impedance was 2 mΩ with a common mode rejection ratio of 11 dB and a signal to noise ratio of 0.75 dB.

Blood Analysis Procedures

Plasma osmolality (OSM_p), plasma sodium concentration ($[Na^+]_p$), plasma potassium concentration ($[K^+]_p$), hematocrit, and hemoglobin concentration ([Hb]) were measured to describe the extracellular compartment pre- and post-exercise.

Blood was collected into 6-mL lithium heparin vacutainers. Hematocrit and [Hb] were determined in triplicate immediately after sampling. To determine hematocrit, blood was drawn into heparinized microcapillary tubes and centrifuged at 3000 rpm (IEC Micro-MB; International Equipment Co., Needham Heights, MA) for 5 minutes. Hematocrit was read using a microcapillary reader (model IEC 2201; Damon/IEC, Needham Heights, MA). To measure [Hb], the cyanomethemoglobin technique was used. Percent change in plasma volume was estimated using the Dill and Costill equation.[27]

Any remaining blood was centrifuged at 3000 rpm at 3°C for 15 minutes. Plasma was removed and stored (-80°C) for later analysis of OSM_p (freezing-point depression osmometry;

model 3D3 Osmometer, Advanced instruments, Inc. Norwood, MA), $[Na^+]_{p,}$ and $[K^+]_p$ (analyzed in duplicate, NOVA 16, NOVA Biomedical, Waltham, MA).

Sweat Analysis Procedures

Sweat $[K^+]$, sweat $[Na^+]$, and sweat volume were measured to estimate fluid and electrolyte losses. Sweat patches were centrifuged for 10 minutes at 5000 rpm at 3°C and analyzed in duplicate for sweat $[Na^+]$ and $[K^+]$. Sweat $[Na^+]$ and $[K^+]$ were corrected using Baker et al's[28] equations which have high reliability (r=0.96 for Na^+ and r=0.9 for K^+) with the whole body wash down technique.

Sweat volume was estimated by subtracting the final post-exercise body mass from subject's pre-exercise mass and correcting for urine volume produced.[21] It was assumed 1 kg of body mass lost equaled 1 L of fluid lost.

Statistical Analysis

Repeated measures ANOVAs were used to determine differences between euhydrated and hypohydrated TF, cramp intensity, cramp EMG amplitude, and MVIC as well as calculate reliability of the MVIC (ICC 2,3) and TF (ICC 2,1) data. Shapiro-Wilk tests confirmed normality. Significance was accepted when $P<0.05$ (NCSS 2007, Kaysville, UT).

RESULTS

Subjects self-reported compliance with pre-test instructions. Subjects began exercise well-hydrated and became significantly or seriously hypohydrated (exercise duration=3.9±0.8 h; Table 1). Five subjects experienced volitional exhaustion before achieving 5% body mass reduction; thus, they were only significantly hypohydrated.

The familiarization day's MVIC EMG amplitude, cramp TF, and cramp EMG amplitude were compared to the testing day's euhydrated condition to calculate reliability. MVIC EMG amplitude ($ICC_{2,3}$=0.85), cramp TF ($ICC_{2,1}$=0.91), and cramp EMG amplitude ($ICC_{2,1}$=0.79) were reliable. However, cramp intensity was inconsistent ($ICC_{2,1}$=0.10). Blood and sweat data are reported descriptively in Tables 2 and 3, respectively.

Significant or serious hypohydration did not alter TF ($F_{1,9}$=3.0, P=0.12, power=0.34), cramp intensity ($F_{1,9}$=1.9, P=0.2, power=0.24), or cramp EMG amplitude ($F_{1,9}$=0.07, P=0.79, power=0.1; Table 1). However, MVIC EMG amplitude was higher when euhydrated ($F_{1,9}$=9.04, P=0.02).

Table 1. MVIC and Cramp Variables While Euhydrated or Hypohydrated.

Subject	Pre-Exercise U_{sg}	H (% of body mass lost)	Cramp TF (Hz)		MVIC EMG Amplitude (μV)		Cramp EMG Amplitude (μV)		Cramp Intensity (% of MVIC Activity)	
			E	H	E	H	E	H	E	H
1	1.005	5.1	8	4	0.15	0.12	0.23	0.34	153.3	275.8
2	1.004	5.0	14	8	0.22	0.17	0.16	0.26	74.4	152.9
3	1.005	4.1	16	10	0.22	0.16	0.13	0.12	58.4	74.4
4	1.003	5.0	12	14	0.17	0.15	0.16	0.08	97.2	50.7
5	1.008	4.0	18	20	0.33	0.24	0.23	0.19	70.3	80.0
6	1.004	4.1	24	18	0.29	0.20	0.15	0.14	50.0	71.8
7	1.003	4.5	10	12	0.22	0.11	0.31	0.20	138.6	176.8
8	1.004	5.3	10	8	0.14	0.15	0.22	0.25	158.9	163.3
9	1.004	5.0	16	12	0.19	0.19	0.15	0.10	78.8	50.0
10	1.008	4.6	22	24	0.17	0.17	0.10	0.11	62.2	63.7
Mean	1.005	4.7	15	13	0.21*	0.17	0.18	0.18	94.2	115.9
SD	0.002	0.5	5	6	0.06	0.04	0.06	0.09	41.0	73.9

E=Euhydrated; H=Hypohydrated; MVIC=Maximum Voluntary Isometric Contraction; U_{sg}=Urine Specific Gravity; Ex=Exercise
*Euhydrated > Hypohydrated (P<0.05).

Table 2. Descriptive Data of Blood Variables While Euhydrated or Hypohydrated.

	Euhydrated	Hypohydrated
$[Na^+]_p$ (mmol*L^{-1})	141.9 ± 3.1	149.5 ± 1.8
$[K^+]_p$ (mmol*L^{-1})	4.9 ± 0.4	5.0 ± 0.4
OSM_p (mOsm*kg^{-1} H_2O)	287 ± 7	301 ± 5
Δ PV (% from baseline)	0 ± 0	-11.8 ± 4.9
Hct (%)	45 ± 4	47 ± 4
[Hb] (g*dL^{-1})	15.9 ± 1.0	17.4 ± 1

Values are Means ± SD (n=10). $[Na^+]_p$=plasma sodium concentration; $[K^+]_p$=plasma potassium concentration; OSM_p=plasma osmolality; ΔPV= change in plasma volume; Hct=hematocrit; [Hb]=hemoglobin concentration.

Table 3. Descriptive Data of Sweat Variables and Total Fluid Lost.

$[Na^+]_{sw}$ (mmol*L^{-1})	53.2 ± 16.7
$[K^+]_{sw}$ (mmol*L^{-1})	4.6 ± 0.6
Sweat Volume (L)	3.2 ± 0.5
Sweat Rate (L^*h^{-1})	0.87 ± 0.3
Total Fluid Lost (L)	3.9 ± 0.5

Values are Means ± SD (n=9); one subject's sweat electrolyte concentrations could not be measured due to technical difficulties. $[Na^+]_{sw}$=sweat sodium concentration; $[K^+]_{sw}$=sweat potassium concentration. Sweat $[Na^+]$ and $[K^+]$ values were corrected using Baker et al's equations.[28]

DISCUSSION

Significant or serious hypohydration did not alter cramp TF. Cramp TF is often used as an indicator of cramp susceptibility in scientific studies where changes reflect increased or decreased cramp risk.[16,19,29] Miller et al[19] observed an insignificant 2 Hz reduction in TF when subjects were euhydrated (24±2 Hz) or hypohydrated (20±2 Hz) and subjects lost 3% of their body mass and ~3 g Na^+. Our study expands Miller et al's[19] results by having subjects' hypohydrated to 4.7±0.5%, exercising for ~2 hours longer in similar conditions, and losing 4 g Na^+. The strength of the current and previous study[19] is the induction of cramps in a rested muscle before and after dehydration. Other studies examining cramping investigate differences in hematological or other variables pre- and post-exercise in athletes.[9-10,14,17-18,30] These experimental designs do not allow scientists to make valid conclusions regarding cramp susceptibility because fatigue and dehydration occur concomitantly.

Our data do not support the theory that dehydration and electrolyte losses cause cramping. A lack of fluid or electrolyte losses cannot explain our observations. Our plasma data confirm significant hemoconcentration of the extracellular space following exercise. Moreover, our subjects lost similar or more fluid and electrolytes than other studies examining crampers and non-crampers. Bergeron[10] observed tennis players with a history of EAMC lost 2.7 g of Na^+ and 2.6% of their body mass via exercise-induced sweating during match play. Similarly, Stofan et al[11] observed total sweat Na^+ losses of 5.1 g and fluid losses of ~3% in athletes with a history of cramp following 5 hours of American football. Our subjects lost 48% more Na^+ and 81% more fluid than other authors[10] observing fluid and electrolyte losses in cramp-prone athletes. Differences in Na^+ and fluid losses are likely due to other authors[10-11] allowing subjects to

consume fluids during data collection where our subjects were fluid restricted for the duration of testing.

Though the majority of clinicians believe dehydration and electrolyte losses cause EAMC,[6] many observations outside of the current study argue against dehydration and electrolyte losses causing EAMC. First, $[Na^+]_p$, $[K^+]_p$, serum magnesium concentration and serum calcium concentration are often similar in crampers and non-crampers pre and post-competition.[17,30] In comparison to others' cramping athletes post-exercise blood,[17] our subjects $[Na^+]_p$ and $[K^+]_p$ were 6% and 2% higher, respectively. Second, even when $[Na^+]_p$ was lower in runners suffering from EAMC than non-crampers,[18] $[Na^+]_p$ were within normal clinical ranges. Third, crampers often have similar body mass reductions following exercise as non-crampers.[17-18,30] Furthermore, sweat $[Na^+]$ of crampers is often within normal clinical range.[11] Fourth, stretching rapidly relieves cramp,[31] yet adds no fluids or electrolytes to the body. Finally, subjects experienced cramping even when subjects replaced their sweat losses with a carbohydrate-electrolyte solution.[32] Furthermore, cramps occurred ~15 minutes into the protocol equating to ~500 mL of fluid lost (1% body mass lost).[32]

Our data are more aligned with the theory cramps are the result of neuromuscular changes. Scientists[1] propose as muscle fatigue develops a combination of increased excitation from Ia and decreased inhibition from Ib afferents cause altered α-motor neuron activity leading to the onset of cramps. Both quasi-experimental[2,33] and experimental research support this theory.[12-13,15,22,34-35] For example, a triathlete's chronic hamstring cramping was relieved following 8 neuromuscular reeducation sessions when fluid and Na^+ intake proved to be unsuccessful.[2] Pre-therapy, hamstring activation during terminal swing and first half of the stance phase of running was ~36% of MVIC (normal = 19%) suggesting cramps were due

overactivation of the hamstrings.[2] By increasing gluteal activation, hamstring activity decreased and the athlete reported 3 triathlons without cramp incidence. Furthermore, Nelson and Hutton[12] observed increases in Ia firing frequency when stretches were applied to fatigued muscle. In a follow-up study,[13] Ib activity was lower when fatigued. Scientists[15] have also demonstrated cramps can be inhibited by tendon afferent stimulation and TENS application at a site away from cramping.[33] Moreover, cramp TF was significantly greater when the tibial nerve was blocked (18±3 Hz vs. 13±3 Hz)[35] suggesting afferents play an important role in cramp genesis. Since TF remained unchanged following significant and severe hypohydration, it is possible afferent activity was unaffected in the rested leg and similar electrical stimuli were required to induce cramp.

Two limitations must be addressed. First, cramps were induced in the FHB with percutaneous electrical stimulation rather than exercise. However, authors have observed high correlation with FHB cramp TF when comparing subjects with a previous history of EAMC and subjects with no prior history.[20] Second, not all subjects achieved 5% hypohydration. However, even the subjects who were significantly hypohydrated lost amounts of fluid and electrolytes similar to those reported in the literature for crampers.[9,11]

In summary, significant and/or serious hypohydration does not increase cramp risk, as indicated by TF. Therefore, cramps occurring to significantly or seriously hypohydrated individuals may be more related to muscle fatigue. Strategies to increase neuromuscular endurance or correct muscle imbalances may be more successful at minimizing the onset of EAMC than rehydration or electrolyte replenishment strategies.

ACKNOWLEDGMENTS

We thank NDSU's College of Human Development and Education, NDSU's Department of Health, Nutrition, and Exercise Sciences, and NDSU's Athletic Training Education Program for partially funding this research.

REFERENCES

1. Schwellnus M, Derman E, Noakes T. Aetiology of skeletal muscle 'cramps' during exercise: A novel hypothesis. *J Sports Sci* 1997;15:277-85

2. Wagner T, Behnia N, Ancheta W, et al. Strengthening and neuromuscular reeducation of the gluteus maximus in a triathlete with exercise associated cramping of the hamstrings. *J Orthop Sports Phys Ther* 2010;40:112-19

3. Dickhuth H, Rocker K, Niess A, et al. Exercise-induced, persistent and generalized muscle cramps. *J Sports Med Phys Fitness* 2002;42:92-94

4. Cooper E, Ferrara M, Broglio S. Exertional heat illness and environmental conditions during a single football season in the Southeast. *J Athl Train* 2006;41:332-36

5. Kantarowski P, Hiller W, Garrett W. Cramping studies in 2600 endurance athletes. *Med Sci Sports Exerc* 1990;22:S104

6. Stone M, Edwards J, Stemmans C, et al. Certified athletic trainers' perceptions of exercise associated muscle cramps. *J Sport Rehabil* 2003;12:333-42

7. Jansen P, Joosten E, Vinerhoets H. Muscle cramp: main theories as to aetiology. *Eur Arch Psychiatr Neurol Sci* 1990;239:337-42

8. Bergeron M. Muscle cramps during exercise--Is it fatigue or electrolyte deficit? *Curr Sports Med Rep* 2008;7:S50-S55

9. Bergeron M. Heat cramps during tennis: A case report. *Int J Sport Nutr* 1996;**6**:62-68

10. Bergeron M. Heat cramps: fluid and electrolyte challenges during tennis in the heat. *J Sci Med Sport* 2003;6:19-27

11. Stofan J, Zachwieja J, Horswill C, et al. Sweat and sodium losses in NCAA football players: A precursor to heat cramps? *Int J Sport Nutr Exerc Metab* 2005;15:641-52

12. Nelson D, Hutton R. Dynamic and static stretch responses in muscle spindle receptors in fatigued muscle. *Med Sci Sports Exerc* 1985;**17**:445-50

13. Hutton R, Nelson D. Stretch sensitivity of golgi tendon organs in fatigued gastrocnemius muscle. *Med Sci Sports Exerc* 1986;**18**:69-74

14. Schwellnus M, Drew N, Collins M. Increased running speed and previous cramps rather than dehydration or serum sodium changes predict exercise associated muscle cramping: a prospective cohort study in 210 Ironman triathletes. *Br J Sports Med* 2011;**45**:650-56

15. Khan S, Burne J. Reflex inhibition of normal cramp following electrical stimulation of the muscle tendon. *J Neurophysiol* 2007;**98**:1102-07

16. Serrao M, Arendt-Nielsen L, Ge H, et al. Experimental muscle pain decreases the frequency threshold of electrically elicited muscle cramps. *Exp Brain Res* 2007;**182**:301-08

17. Schwellnus M, Nicol J, Laubscher R, et al. Serum electrolyte concentrations and hydration status are not associated with exercise associated muscle cramping (EAMC) in distance runners. *Br J Sports Med* 2004;**38**:488-92

18. Sulzer N, Schwellnus M, Noakes T. Serum electrolytes in Ironman triathletes with exercise associated muscle cramping. *Med Sci Sports Exerc* 2005;**37**:1081-85

19. Miller K, Knight K, Mack G, et al. Three percent hypohydration does not affect the threshold frequency of electrically-induced muscle cramps. *Med Sci Sports Exerc* 2010;**42**:2056-63

20. Miller K, Knight K. Electrical stimulation cramp threshold frequency correlates well with the occurrence of skeletal muscle cramps. *Muscle Nerve* 2009;**39**:364-68

21. Casa D, Armstrong L, Hillman S, et al. National Athletic Trainers Association position statement: Fluid replacement for athletes. *J Athl Train* 2000;**35**:212-24

22. Miller K, Mack G, Knight K, et al. Reflex inhibition of electrically-induced muscle cramps in hypohydrated humans. *Med Sci Sports Exerc* 2010;42:953-61

23. Whaley M. ACSM's guidelines for exercise testing and prescription. *7th ed.* Philadelphia: Lippincott Williams & Wilkins, 2006:4.

24. Miller K, Knight K. Initial electrical stimulation frequency's effect on cramp threshold frequency and force. *J Athl Train* 2012:In press

25. Stone M, Edwards J, Babington J, et al. Reliability of an electrical method to induce muscle cramp. *Muscle Nerve* 2003;27:122-23

26. Hagan R, Diaz F, Horvath S. Plasma volume changes with movement to supine and standing positions. *J Appl Physiol* 1978;45:414-18

27. Dill D, Costill D. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974;37:247-48

28. Baker L, Stofan J, Hamilton A, et al. Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. *J Appl Physiol* 2009;107:887-95

29. Stone M, Edwards J, Huxel K, et al. Threshold frequency of an electrically induced cramp increases following a repeated, localized fatiguing exercise. *J Sports Sci* 2010;epublication:1-7

30. Maughan R. Exercise induced muscle cramp: A prospective biochemical study in marathon runners. *J Sports Sci* 1986;4:31-34

31. Bertolasi L, De Grandis D, Bongiovanni L, et al. The influence of muscular lengthening on cramps. *Ann Neurol* 1993;33:176-80

32. Jung A, Bishop P, Al-Nawwas A, et al. Influence of hydration and electrolyte supplementation on incidence and time to onset of exercise-associated muscle cramps. *J Athl Train* 2005;40:71-75

33. Mills K, Newham D, Edwards R. Severe muscle cramps relieved by transcutaneous nerve stimulation: A case report. *J Neurol Neurosurg Psychiatry* 1982;45:539-42

34. Ge H, Zhang Y, Boudreau S, et al. Induction of muscle cramps by nociceptive stimulation of latent myofascial trigger points. *Exp Brain Res* 2008;187:623-29

35. Minetto M, Holobar A, Botter A, et al. Mechanisms of cramp contractions: peripheral or central generation. *J Physiol* 2011;23:5759-73

APPENDIX A. PROSPECTUS

Introduction

Exercise associated muscle cramps (EAMC) are painful involuntary contractions of skeletal muscle occurring during or shortly following exercise.[1] Exercise associated muscle cramps can impair athletic performance,[9] and in some instances EAMC symptoms can last up to 8 hours after activity.[2-4,] Exercise associated muscle cramps typically occur in unilateral multi-joint muscles (e.g. gastrocnemius, hamstrings).[1,5-6] Exercise associated muscle cramps are a common injury affecting active individuals. In American football athletes, 73% (102 of 139) experienced EAMC during physical activity.[7] In triathletes,[8] 67% (1631 of 2438) complained of EAMC during a triathlon. Despite the prevalence of EAMC their cause remains unknown.

The dehydration and electrolyte theory is the most popular theory among clinicians to explain the onset of EAMC.[10] Proponents of this theory state exercise-induced sweating and sodium (Na^+) losses causes fluid to shift from the interstitial space to the intravascular space.[11-12] This fluid shift is said to make the interstitial fluid compartment contract thereby increasing the pressure and excitability of select nerves.[12] This increase in excitability is said to cause EAMC. Support for this hypothesis comes from observational research in which fluid and electrolytes losses in crampers and non-crampers. Sodium has been the focal point of many research studies examining electrolyte depletion because of the large losses of sodium in sweat compared to any of the other electrolytes.[16] Stofan et al[13] measured sweat sodium loss, fluid intake, and sweat sodium concentration in 10 American football players (5 players suffering from muscle cramps and 5 that did not). The athletes with EAMC lost more total sodium in their sweat than athletes that did not suffer from muscle cramps over the course of the day (total Na lost: -10.4 ± 4.1(g) vs. -4.9 ± 2.3(g)).[13] Sweat sodium concentration was also greater in the athletes experiencing

EAMC (54.6 ± 16.2 mmol/L vs. 25.3 ± 10.0 mmol/L).[13] There were no differences in fluid intake between crampers and non crampers (2.6 ± 0.8 L vs. 2.8 ± 0.7L).[13] Bergeron[14] noted a 20-30% sodium deficit when observing a tennis player with chronic EAMC. In a follow up observation of multiple tennis players suffering from chronic EAMC, EAMC symptoms were relieved with sodium and fluid intake.[15]

Despite the common belief of dehydration and electrolyte depletion imbalances causing EAMC, many researchers question the validity of these studies. Seven observations cast doubt on the validity of the dehydration and electrolyte depletion theory. First, serum sodium concentration, serum potassium concentration, serum magnesium concentration and serum calcium concentration are often similar in crampers and non crampers before and after races.[6,17-19] Second, even when scientists observe decreases in plasma sodium concentration levels in runners suffering from EAMC as compared with non crampers,[20] the plasma sodium concentrations are within normal clinical ranges. Third, sweat sodium concentration of athletes experiencing EAMC are never recorded when EAMC occurred.[20] Fourth, the researchers that did observe differences in sweat sodium concentrations between crampers and non crampers[13-15] had small sample sizes creating the possibility that a type 1 error occurred. Fifth, it is impossible to infer cause and effect from quasi experimental or cross sectional research studies. Sixth, stretching is the predominant treatment for EAMC but does not add fluids or electrolytes to the body yet quickly relieves EAMC.[21] Seventh, studies observing electrolyte and dehydration in crampers and non crampers often fail to control for exercise intensity and fatigue. [20]

Many causes of EAMC have been hypothesized, however they remain difficult to discern because of the several control variables that need to be involved(i.e. exercise intensity, fatigue, dehydration and electrolyte losses). Recent research has attempted to isolate the effect of

dehydration and electrolyte depletion from fatigue by inducing cramps with electricity. These authors examined the effects of 3% hypohydration on muscle cramp threshold frequency[23] (i.e. the lowest electrical stimulation frequency needed to induce a muscle cramp), because threshold frequency is thought to be a quantitative way of assessing an individual's cramp risk (a lower threshold frequency indicates an increased risk of muscle cramps).[24] To control for fatigue subjects performed non dominant single leg cycling until 3% hypohydrated. Muscle cramps were then electrically induced in the flexor hallucis brevis in the dominant leg. Authors observed no differences in threshold frequency between euhydration and 3% hypohydration trials (23.7 ± 1.5 Hz vs. 21.3 ± 1.4 Hz).[23] One limitation of this study was that subjects were only 3% hypohydrated (mild hypohydration). Voluntary ingestion of fluids available during exercise can result in wide ranges of fluid intake, however many athletes only replace 50% of fluid loss[25] and begin the next exercise session in an already hypohydrated state.[26] It may be possible that they dehydrate themselves to 5 percent. No research has examined the effects of more than 3% hypohydration on muscle cramp threshold frequency.

Research Questions

1. Does 5% hypohydration decrease muscle cramp threshold frequency?

Research Hypothesis

1. We hypothesize cramp threshold frequency will be unaffected by 5% hypohydration

Assumptions

1. One kg of body mass lost is equivalent to 1 L of fluid lost.
2. Electrically induced cramps are similar to exercise-associated muscle cramps.

3. Physically active individuals suffering from exercise associated muscle cramps dehydrate themselves to 5%.
4. Athletes experience EAMC at 5% hypohydration.

Limitations

1. Athletes are not normally fasted for 12+ hours prior to exercise.
2. Muscle cramps were induced electrically.
3. Muscle cramps induced in the flexor hallucis brevis have different threshold frequency than other muscles.

Delimitations

1. Only healthy men ages 18-30 with a history of muscle cramps in the 6 months prior to this study will be included.
2. Only those that could have muscle cramps electrically induced will be included.
3. Subjects who are free of injury to their legs (injuries that affect their activities of daily living for longer than 7 days) in the 6 months prior to data collection will be included in this study.
4. Only subjects free of any cardiovascular, neurological, or blood borne diseases will be included in this study.
5. Only subjects that are physically active (> 30 minutes on 3 or more days of the week) will be included in this study.

Operational Definitions

1. Chloride- electrolyte ion with a negative charge with symbol Cl.
2. Cyanomethemoglobin reagent- a reagent used to determine the hemoglobin concentration by measuring the absorption of light using a spectrophotometer.
3. Electrolyte- an ion with a positive or negative charge.
4. Euhydration- state of being hydrated
5. Eversion- turning of the ankle outward.
6. Extracellular- fluid that is not contained in the cells with a high concentration of sodium.
7. Hematocrit-the volume of blood that contains red blood cells (expressed as a %).
8. Hemoglobin-protein molecule in red blood cells that carries oxygen.
9. Hypohydration- state of being less than hydrated.
10. Intracellular- body fluid contained in the cells with a high concentration of potassium.
11. Inversion- turning of the ankle inward.
12. Maximum voluntary isometric contraction- muscle contracting against an immovable object.
13. Osmolality- The concentration of particles dissolved in a fluid expressed as osmoles of solute per liter of solution.
14. Potassium- the most common intracellular cation with the symbol K.
15. Sodium-the main positive ion present in extracellular fluids with the symbol Na.
16. Threshold Frequency- the lowest electrical stimulated frequency needed to induce a muscle cramp in the flexor hallucis brevis.
17. Urine Specific Gravity- measurement of the concentration of contents in the urine to determine hydration status.

Abbreviations

1. MVIC- maximum voluntary isometric contractions
2. EAMC- exercise associated muscle cramps
3. EMG- electromyography

Review of Literature

The purpose of this literature review is to discuss muscle cramps with an emphasis on exercise associated muscle cramps. The literature review will be organized by the following topics:

Databases and key words searched

Definition of a muscle cramps

Classification of muscle cramps

Definition of muscle cramps

Epidemiology

Causes

- Dehydration
- Electrolyte Imbalance
- Muscle Fatigue
- Nitric Oxide
- Metabolic Disorders
- Creatine Supplementation
- Hypomagnesaemia
- Environmental Theory
- Psychological Factors
- Biomechanics

Prevention

- Rehydration
- Electrolyte replenishment
- Calcium Supplementation
- Decrease in Muscle Fatigue
  - Lower Exercise Intensity
  - Change in Biomechanics
  - Increase in Level of Conditioning
- Increases in Range of Motion

Plyometrics

Proper Equipment Fitting

Relaxation Techniques

Quinine

Magnesium Supplementation

Decrease use of Caffeine

Taking a Good History

Treatment

Pickle Juice

Mustard

Bananas

Transcutaneous electrical nerve stimulation (TENS)

Stretching

Honey/Apple Cider Vinegar

Rehydration

Summary

Databases and Key Words Searched

The following databases were used in the search of journal articles: Ebsco Host, JSTOR, Sport Discus,Proquest, and Science Direct. The following words were used in search of this topic:

Calcium

Carbohydrates

Causes

Dehydration

Diet

Electrolyte deficiency

Environmental theory

Etiology

Exercise associated cramps

Exercise induced cramps

Fatigue

Honey

Hypomagnesaemia

Metabolic disorders

Muscle cramps

Muscle tetany

Mustard

Pickle juice

Plasma volume

Plyometrics

Potassium

Prevention

Definition of Muscle Cramps

Muscle cramps can be defined as painful spasmodic involuntary contraction of the skeletal muscle, that occurs with exercise or other pathophysiological conditions.[1] There are many types of muscle cramps ranging from night cramps, which most often occur in the elderly population, and exercise associated muscle cramps that occur in select number of athletes or active individuals. Exercise associated muscle cramps typically occur in unilateral multi-joint muscles.[1,5-6] There are also electrically induced cramps which are most often used in research studies because of the consistency of the equipment. Muscle cramps are a very debilitating condition and can cause a decrease in performance in any athlete or active individual. Psychological factors may also occur in the onset of muscle cramps. This has led to many research studies to find the exact cause, prevention, and treatment of muscle cramps.

Epidemiology of Muscle Cramps

In a recent survey 73% (102/139) of heat related injuries experienced by football athletes were diagnosed as exercise associated muscle cramps.[7] In an observational study done by Kantarowski et al[8], 67% (1631/2438) of triathletes complained of exercise associated muscle cramps in a variety of conditions and settings. In past surveys, the athletes with the highest incidence of muscle cramps were triathletes (67%), followed by cyclists (60%), Rugby at (52%) and lastly marathoners (39%).[15,25]

Causes

Dehydration was the first theory attempting to explain the cause of exercise associated muscle cramps. Dehydration was hypothesized as the cause of cramps from an observational

study of men working on steamboats and mines where the environmental conditions were hot and humid.[26] It was also noted that muscle cramps not only happened in the heat but were also accompanied by large amounts of fluid losses from sweating.[26] However, when water was given to treat the onset of cramps, no relief was seen which led researchers to hypothesize that the electrolytes that were being lost in the sweat due to dehydration was causing the onset of cramps.[14,27]

Our bodies use electrolytes to maintain normal function of the cells and to aid in the process of creating threshold frequency which create action potentials used for contraction and relaxation of the muscles.[28] The electrolytes related to exercise associated muscle cramps are sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and chloride (Cl).[12]Researchers agree that the loss of electrolytes is important, but mainly the loss of sodium that causes the onset of muscle cramps.[1,13,29-30] The theory behind electrolyte depletion is that certain concentrations of sodium are lost in the sweat causing an imbalance.[11] The concentration of the sodium in sweat varies from person to person. As sweating increases so does sodium loss.[11] The loss of sodium is even greater if loss of sodium through sweat exceeds dietary intake of sodium.[29,31-32] Sweat loss causes a decrease in plasma volume creating a shift of fluids from interstitial space to the intravascular space.[11-12] This shift of fluids is said to make the interstitial fluid compartment contract causing the neurons to become excited resulting in a muscle cramp.[12,27] In a study of football players done by Stofan et al[13], he concluded from his results that the football players that cramped had a sweat sodium concentration that was two times greater than the concentration of the non-cramping football players. Bergeron[12,31-32] also observed a high sweat sodium concentration in a tennis player during a competition in hot environmental conditions as well as during climate controlled indoor setting. It was concluded that the tennis player was suffering

from cramping due to high losses in sodium and a deficit in dietary intake.[12,31-32] Increasing dietary sodium appeared to alleviate cramps, seemingly supporting Bergeron's theory.[32]

A later theory suggests that the higher sweat sodium concentrations were due to the fact that the athletes with the higher concentrations of sodium were unacclimated or not well conditioned as the athletes with lower sodium concentrations in their sweat.[11-12,32] Bergeron and Stofan also observed that the athletes with a history of cramping had higher sweat sodium concentrations.[12-13] There is a lack of research on why athletes with a history of cramping, or that are unacclimated, have higher sweat sodium concentrations.

There have been few recorded cases where other researchers also observed sweat sodium concentrations to be different between conditioned or unconditioned athletes. Research conducted by Schwellnus[16] observing plasma sodium volumes in marathoners showed lower plasma sodium concentrations in cramping athletes post-race than when compared to non-cramping athletes. Other researchers found similar results when looking at plasma sodium volume in marathoners.

In separate studies done by Schwellnus, Maughan, Sulzar, and Nicol, when looking at marathoners, there was not a significant difference pre-race and post-race in serum concentrations of sodium, potassium, calcium, or magnesium between crampers and non crampers.[6,15-17] In a recent review of literature by Schwellnus[18], he states that there is not a published study that has shown serum electrolyte concentrations to be abnormal in athletes at the time of exercise associated muscle cramp. Any decrease seen in serum sodium concentration levels in athletes were with in normal ranges.[18] A problem with these studies is that none of the researchers took samples from within the muscle. Instead they took a blood sample from the arm and are making the assumption that the blood that is circulating the body is the same in the arm

as it is in the cramping muscle. The effective treatment of muscle cramps also casts doubt on electrolyte imbalance theory. Muscle cramps are relieved by stretching but stretching the muscle does not cause any fluid or electrolyte shift inside the body.[19] Talbot, who was one of the first to study muscle cramps, observed that there was a decrease in sodium in the urine because the body was trying to maintain its sodium balance.[25] This theory would support other research studies which saw no significant decrease in plasma sodium volumes in crampers and non crampers because the body is trying to maintain its sodium levels.[16-18]

Arguments made by Bergeron [12,14-15,31-32] state that sodium deficit is usually not detectable by measuring plasma electrolytes and especially not detectable after exercise bouts when sodium concentration is either normal or elevated. Post exercise sodium concentrations are influenced by free water that shifts between compartments through diffusion and not because of electrolyte losses.[11] Therefore serum sodium concentration should not be used to indicate a presence or absence of plasma sodium levels.[11] Bergeron[14,32] makes the argument that sodium status should be determined solely on sweat sodium concentrations and dietary intake of sodium. Bergeron[14,32] states that other researchers in support of the electrolyte depletion theory did not monitor sodium intake. Bergeron further argues that measurements taken in Sulzar study were not measuring electrolyte losses but instead the fluid shift between compartments and therefore their assumptions are wrong because fluid shift has to do with dehydration rather than with electrolyte depletion.[14]

Junget al.[35] conducted a study of13 healthy man with a history of exercise associated muscle cramps went through a cramping protocol and measurements were taken throughout. The subjects were randomly assigned to hypohydration group or carbohydrate/electrolyte group and taken through an exercise protocol.[35] Jung observed no difference in hydration or plasma

concentration of electrolytes between individuals.[35] It appears that hydration and electrolyte supplementation has no effect on preventing the incidence of muscle cramps as the subjects still cramped even when given an electrolyte and carbohydrate solution.[35] In the study the carbohydrate solution was ingested at a rate that matched the sweat loss of subjects. Jung's inference of the results was the exercise was so intense that electrolyte and hydration of subjects may not have had time to take effect.[35] This could be due to the decrease in gastric emptying or increase in exercise intensity because the blood flow is directed away from the gastrointestinal system out to the working muscles.[35] The problem with this study is that sweat electrolyte concentration was not measured. Jung only replaced fluid lost but not sodium lost. Jung also hypothesized that is electrolyte depletion and dehydration are the causes for muscle cramps than it takes more than 1.5-2.0% hypohydration.[35] Miller et al[21] hypothesized that it takes more than 3% hypohydration from a study he performed where he could not get an electrically induced cramp at 3% hypohydration, assuming that electrically induced muscle cramps are the same as exercise associated muscle cramps. Further research is needed to determine if levels of hypohydration greater than 3% can cause an onset of muscle cramps.

With further investigation and growing disbelief that electrolyte depletion and dehydration were the causes of the onset of muscle cramps, researchers have hypothesized a new theory of muscle fatigue. Support for this theory comes from observations of the development of exercise associated muscle cramps while contracting muscle that are already in a shortened position.[1] Muscle fatigue is said to influence this by altering neuromuscular control responsible for muscle activation and inhibition.[18] This acts directly on the golgi tendon organs and muscle spindles.[18] The golgi tendon organ detects muscle tension and is designed to inhibit muscle contractions.[36] When the golgi tendon organ becomes disrupted or inhibited by muscle fatigue,

its ability to detect tension in the muscle decreases.[36] When an increase in muscle spindle activity or hyper-excitability of the muscle happens, the fatigued golgi tendon organ fails to cause inhibition of the muscle to prevent muscle cramps .[1,37-38] Muscle cramps are said to happen frequently in jobs or activities where repetitive motions place stresses on the muscle causing muscle cramps to occur.[39]

Predisposing risk factors are thought to be associated with muscle fatigue and related to muscle cramps. These factors include: older age, poor stretching habits, insufficient conditioning, history of cramping, excessive exercise intensity, long duration of exercise, related metabolic disturbances, family history, high body mass index, longer running history, and hot environment.[5,25,37,40] All of these factors are just observations made by researchers. Most of the risk factors have never been tested in a research study. The long list of potential risk factors being an aiding factor in the cause of muscle cramps presents the idea that there are many possible variables and an exact cause is unknown. In many observational studies, the occurrence of exercise associated muscle cramps increases with intensity and duration of activity and overloading the muscle.[1,5,10,40-41] Insufficient conditioning will also create fatigue in the muscle, which can prompt muscle cramping.[1,5,10,37,40-41] In a study by Sulzer et al[15] , there was a significant increase in cramping runners electromyography activity compared to non crampers at certain timed intervals. An increase in electromyography activity is a result of an increase in muscle spindle activity causing the muscle to become hyperexcitable.[15] This causes the muscle to be more susceptible to cramps.[15] In another experimental study, executed in an animal model, Hutton[36] observed a delay Golgi Tendon Organ firing rate. It was concluded that fatigue decreased the inhibition from the Golgi Tendon Organ.[36] Results of testing the muscle fatigue

theory are promising but further research is needed to verify that muscle fatigue does in fact cause the onset of muscle cramps.

There are researchers that question if muscle fatigue is the cause of exercise associated muscle cramps. In reviewing the research, other researchers have found flaws in the experimental design or they have done studies themselves that show maybe muscle fatigue is not the cause of exercise associated muscle cramps. There are several limitations that must be noted for the muscle fatigue theory. First, report of altered activity between Golgi Tendon Organ and muscle spindle activity relies on difficult methodology, none of which have produced consistent results. It is unclear how fatigued a muscle has to be for an exercise associated muscle cramp to occur. Quantifying muscle fatigue is difficult for researchers to do and every researcher may quantify it differently which may explain why there are inconsistent results. The study that does have good evidence to support fatigue theory was done on a cat and therefore can only assumed to be similar in humans. To increase support of muscle fatigue as the cause of muscle cramps, there needs to be more studies with reproducible findings to increase reliability and validity of this theory.

After the big three theories of dehydration, electrolyte depletion and muscle fatigue as the cause of exercise associated muscle cramps comes other theories which have not had very much research or are not as supported by the research community. Nitric oxide has been noted by few researchers to be the cause of exercise associated muscle cramps. The role of nitric oxide is to cause vasodilation and it also is a signal transmitter between cells.[42] It is hypothesized that change in the amount of nitric oxide after activity will cause the onset of muscle cramps.[42] There is no research to show how nitric oxide causes muscle cramps but only observed to be a change in post exercise measurements. It was also hypothesized in the study done by Maddali[42] that the

concentrations of nitric oxide were independent of the changes of electrolytes and serum chemicals. Maddali[42] studied football players during preseason camp. He collected baseline blood samples from the players and only took other blood sample collections from the athlete's that cramped. The problem with measuring nitric oxide is that it has a half-life of 3 to 5 seconds.[42] Therefore nitrite was measured in all the blood samples because it is assumed to be similar to nitric oxide and lasts longer.[42] According to Maddali,[42] there was a significant increase in nitrite (300%) compared with the baseline measurements that were taken and was not secondary to dehydration. There were also increases in blood urea nitrogen, creatine, calcium, lactate, glucose and other chemicals but they were within normal limits.[42] There was also a decrease in sodium, potassium, magnesium and chloride.[42] Results from this study should be taken lightly as there were many variables that were not controlled for. Blood samples were taken from the arm and not from the muscle that was cramping. It is an assumption that blood flow in the arm has similar contents as that of the cramping muscle. Post exercise nitrite was not measured in non crampers post exercise. Therefore Maddali[42] could not infer cause and effect because it is also possible that nitric oxide was just as high in the non-cramping athletes. Maddali[42] also did not control for other factors such as carbohydrate, electrolyte or water intake, or fatigue. As a result Maddali[42] could not conclude that nitric oxide caused the onset of cramps because it could have been from dehydration, fatigue, and depletion of sodium or other electrolytes in the body. It is also unknown where the source of the nitric oxide is.

Metabolic disorders have been noted as a possible cause of muscle cramps. Metabolic disorders impair the body's metabolism, which is used for converting food into energy for the muscles during exercise. According to Maquirriain[43], metabolic disorders affecting the glycolytic pathway are more prone to cramps with exercise because of the inability for the

muscle to receive energy. As of 2009, according to Schwellnus,[18] no studies have explored the depletion of muscle energy stores and development of exercise associated muscle cramps. The only related test that was done was by Jung. In Jung's study, there was a delay of the onset of muscle cramping when subjects were given a carbohydrate and electrolyte solution.[35] The limitation of the study is that Jung cannot infer cause and effect because the subjects were given carbohydrate and electrolyte fluid. Therefore it is not known whether the delay of exercise associated muscle cramps came from the electrolyte solution, carbohydrate, or both. This is the same study that was discussed in the fatigue theory where Jung took 13 healthy men with a history of exercise associated muscle cramps and went through a cramping protocol. Future research to determine that metabolic disorders and/or the lack of glucose for energy to the muscle is the cause of exercise associated muscle cramps. Researchers have suggested that a biopsy from the muscle is needed because whatever is happening inside the muscle may be different than another measurement.[35] Therefore in future research studies it is important for the researchers to take a biopsy of the muscle to get an accurate result of what is happening inside the muscle.

With the increase in popularity of using creatine as a supplement researchers questioned if taking supplements such as creatine could be the cause or a risk factor in the onset of muscle cramps. Creatine has been suggested as a possible factor in the onset of muscle cramps in observational studies.[44-45] The hypothesis behind creatine being the cause is that it promotes fluid retention which may alter electrolyte balances and cause the onset of cramps.[46] Dalbo studied the use of creatine in football players. In his study 560 athletes were taking the creatine supplement and 19% (108/560) reported muscle cramps.[44] However it was reported that some athletes exceeded the recommended dosage.[44] The players were also exercising in hot humid

conditions for long durations.[44] Therefore there is speculation whether the creatine caused the cramp, the exceeded dosage, the environment, muscle fatigue, or electrolyte depletion. In a four month study done by Greenwood[45], he studied football players at a NCAA division 1A university. All athletes were medically cleared and 38 of them volunteered to take creatine while the others took a placebo electrolyte drink. The results of the study showed 10 of the 38 subjects on creatine cramped whereas 18 of the 34 subjects taking placebo group experienced muscle cramps.[45] Creatine users also had lower core temperatures and were more hydrated than non creatine users.[45] The limitation with his study is the history of cramping in subjects was unknown and could have possibly skewed the results. Greenwood concluded that creatine was not the cause of muscle cramps and future research should be done to verify his findings.[45] He also stated that there must be other underlying conditions to the causes of muscle cramps.

The electrolyte depletion theory mainly focused on sodium loss. Researchers have also hypothesized that loss of other electrolytes could be the cause of muscle cramps. Researchers believe low magnesium concentration causes increased irritability of the nervous system.[47] Bibley[47] observed in 3 case reports where subjects suffering from recurring cramps had a magnesium deficit. In all three cases the cramps were relieved when the magnesium supplementation was given. Further support of this theory came from a double blind study done by Fehlinger[48] where 64 patients suffering from chronic muscle cramps were randomly assigned to treatment group (366mg magnesium per day) and placebo group for 4 weeks. It was reported that 75% (24/32) subjects in the treatment group saw improvement.[47]

A theory that correlates with dehydration is the environmental theory. Though this theory is not strongly supported by researchers, it is one of the early hypothesized factors as a cause for exercise associated muscle cramps. It was believed that exercising in the heat will

cause dehydration and electrolyte depletion and therefore cause the onset of muscle cramps.[25] The support for this theory comes from observations that athletes suffer from muscle cramps on hot humid days because they are not acclimated to the heat.[12,14] However it must be noted that dehydration and electrolyte depletion can happen at cooler ambient temperatures.[29,37] It was observed later by Schwellnus[1] that passive heating does not cause muscle cramps which further casts doubt on this theory. Although cramps also happen during cooler temperatures it is possible for the environment to be a secondary factor in the generation of muscle cramps.

The final thoughts of the cause of muscle cramps are all anecdotal and have little to no research to support. The first of which is psychological factors. It was thought that since cramps occur frequently during competition that the stress could cause a physiologic response and may be a predisposing factor.[49] During times of stress and anxiety the brain releases chemicals that could cause the cells to become hyperexcitable.[49] If these chemicals released in a chain reaction can cause neurons to become hyperexcitable then it could be a factor to consider as a predisposing factor.

Other causes that place stresses on the muscle, which causes prolonged contraction of the muscles and leads to fatigue are poor biomechanics, lack of stretching, and static deformities of the body.[5] These causes would be factors to consider in the fatigue theory.

The cause of muscle cramps is still unknown. Majority of the research is on the 3 theories of dehydration, electrolyte depletion, and muscle fatigue but some of the studies have flaws and limitations that leave questions for future researchers to answer. All other theories rests largely on observations in case studies, case series, and assumptions made from lack of research or lack of knowledge. Some researchers have come to the conclusion that the most important factor in exercise associated muscle cramps is previous history. It is believed that

muscle cramps are all different and have a variety of causes. With a detailed history of the athlete it could be possible to make an informed guess at what the underlying cause might be.

Prevention

According to a recent survey 73 % (45/62) of Athletic Trainers use water and 87% (54/62) use a sports drink making it the most widely known and most popular prevention strategy.[10] The problem with using just water is that the body is inefficient at detecting the level of rehydration. It is possible for the body to expel water before being properly rehydrated. In a study done by Mitchell[50], the results pointed to the conclusion that rehydration of 100% only took place after the volume ingested was 1.5 times the amount lost. When water was given to treat the onset of cramps, no relief was seen which led researchers to hypothesize that the electrolytes that were being lost in the sweat due to dehydration was causing the onset of cramps.[11-12,14]

As stated earlier the theory behind electrolyte depletion is that the sodium concentration in sweat is elevated causing a decrease in plasma sodium which creates a shift of fluids from interstitial space to the intracellular space.[30] This shift of fluids is said to make the interstitial fluid compartment contract causing the causing the nerves to become excited resulting in a muscle cramp.[12] The electrolyte that is lost the most during exercise is sodium.[31] Sodium is a positive charged ion found on the outside of cells.[50] It is used in important body functions such as the brain, nervous system and muscles which act on electrical signals.[50] The movement of sodium can generate these electrical signals. That's why replacement of sodium is hypothesized to be a prevention of exercise associated muscle cramps. Bergeron et al[12,14] supports this hypothesis as seen in the tennis player whose cramps were relieved after sodium was replaced.

Ingesting sodium will be absorbed into the extracellular fluid making the plasma more concentrated. This causes a fluid shift from the intracellular space back to the interstitial space thereby restoring plasma volume.[31,50] It must be noted however that ingesting just sodium will cause a greater fluid shift from the interstitial fluid which could cause further dehydration.[50] Therefore you must have a fluid to help rehydrate while ingesting salt. That is why the National Athletic Trainers Association suggests that you adding 3 grams per liter of salt to sports drinks for added benefit.[51] This way you are replacing plasma volume with the fluid and replacing sodium lost to help with cell function. Drinking fluid with sodium in it helps the body retain more water by causing the nephron to be more leaky.[52]

It is important not to rely solely on sports drinks like Gatorade to replenish sodium. Gatorade has a low sodium content and to replace sodium lost in the body would equate to very large amounts of fluid to be ingested which is not very plausible.[53] A well balanced diet helps replenish sodium and other electrolytes because most of the replenishment happens during meals and is therefore more plausible than trying to replace all of the electrolytes with fluid alone.[5,30,39,43,54-55]

With the newest hypothesis that exercise associated muscle cramps are related to muscle fatigue comes new prevention strategies. As mentioned earlier muscle fatigue causes the Golgi Tendon Organ to become disrupted or inhibited thereby decreasing the ability to detect muscle tension which could lead to the onset of muscle cramps.[1,36-37] Using this theory some plausible prevention strategies have been made to reduce muscle cramps. They are lower overall exercise intensity, [18,20,28-29,54] changes in biomechanics,[54]and increase in level of conditioning.[5] Dumke [5] states that poorly conditioned muscles are the most vulnerable to cramps. That is why it has been assumed that majority of exercise associated muscle cramps are seen in the early training

phases because the muscle is not conditioned enough for the high intensity or long duration. The purpose behind lowering overall intensity is to delay the onset of muscle cramps. Higher intensities increases the rate of fatigue which then causes an earlier onset of exercise associated muscle cramps.[1,16,18-19] Correcting bad biomechanics is also thought to be important. Bad biomechanics can cause an increase of stress on the muscles which then can cause in increase in fatigue.[56]

Correcting things such as posture and achieve the most efficient biomechanics for specific activities will help alleviate any added stress and should in theory delay or possibly prevent exercise associated muscle cramps from happening. Increasing the level of intensity is the most important when talking about fatigue. An increase in conditioning will allow for a longer duration of exercise as well as an increase in intensity. An increase in conditioning allows for the delay of muscle fatigue which can help delay or prevent exercise associated muscle cramps. An increase in plasma volume is seen in an increase in conditioning.[57] With conditioning come cardiovascular benefits. These benefits include increase in diameter of blood vessels, increase cardiac output, increase in stroke volume, decrease in blood pressure and an increase in the blood plasma volume.[57] Increasing plasma volume is done by moderate duration and high intensity exercises.[57] As seen in a study done by McCutcheon,[58] a 13.8% increase in plasma volume was seen after 10 days. It must be noted however that the study was done on horses. It is only an assumption that it is applicable to humans.[58] Increasing plasma volume and exercising would therefore take longer to deplete your plasma volume which will delay the shift of fluids thereby delaying the onset of exercise associated muscle cramps. These ideas however are all theories which need further research to test their efficacy.

Improving range of motion by stretching the muscles at risk has been hypothesized as a plausible preventative measure in exercise associated muscle cramps. Stretching is the most effective treatment and therefore thought to be a plausible preventative measure.[59] Stretching the muscle activates the golgi tendon organ which thereby inhibits muscle spindle activity which reduces contractility and helps the muscle relax.[59] Muscles that are in a shortened position are said to be most vulnerable to muscle cramps.[1] The theory behind range of motion is that tight muscles are in a shortened position. Tight muscles can also cause a change in biomechanics.[56] As discussed in the fatigue section, bad biomechanics can cause muscle fatigue which leads to the onset of muscle cramps. Therefore improving the range of motion will lengthen the muscle, help maintain good biomechanics and possibly prevent muscle cramps. Further research is needed to determine if this is an effective prevention measure or if this combined with another theory would be more effective.

Plyometric exercises are exercises that have an eccentric load which is immediately followed by a concentric contraction.[56] Plyometric exercises are thought to effect the stretch reflex and the Golgi Tendon Organ.[56] The Golgi Tendon Organ is involved in the stretch reflex by detecting rate of tension.[56] The Golgi Tendon Organ works by protecting the body from injury and when stimulated causes the muscle to relax.[56] According to Chimera,[56] after plyometric training the Golgi Tendon Organ is desensitized. This is a problem for those who suffer from muscle cramps because the Golgi Tendon Organ is thought to be the relief for muscle cramps by sensing the tension and causing the muscle to relax. Woodrup[60] contrasts the thought of the Golgi Tendon Organ being desensitized and states that plyometric training causes de-inhibition of the Golgi Tendon Organ. If the Golgi Tendon Organ was more sensitive then it could keep muscle cramps at bay by detecting changes in tension earlier and causing the muscle

to relax before muscle cramps can occur. The problem with these two theories is that there is no research on plyometric training and the effects on the Golgi Tendon Organ. The cause of muscle cramps is also unknown which could mean that plyometrics could have nothing to do with preventing muscle cramps. Future research in this area will help determine the role of plyometrics or even the cause of muscle cramps.

Properly fitted equipment and clothing have also been discussed as being possible preventative measures for muscle cramps. Equipment that does not fit properly can place additional stresses on the muscles that can lead muscle to fatigue earlier.[28-29] As discussed earlier fatigue is one of the factors that is thought to be one of the causes of the onset of exercise associated muscle cramps. Therefore properly fitted equipment will reduce stresses placed on the muscles which will delay fatigue as well as exercise associated muscle cramps. It was also hypothesized that restriction of blood flow to the muscle causes a buildup of lactate which could cause the onset of muscle cramps.[13,39] Wearing tight clothes could cause a restriction of blood flow and lead to the onset of muscle cramps so it is theorized that wearing loose fitted clothing will help maintain blood flow to the muscle thereby preventing muscle cramps. Further research is needed to determine if this has any effect on muscle cramps.

As mentioned by Parisi,[49] stresses could be a contributing factor to the onset of muscle cramps and therefore relaxation has been mentioned as a possible remedy to prevent muscle cramps. Stress causes the release of adrenocorticotropic hormone from the pituitary gland.[61] As a result this hormone causes the release of cortisol from the adrenal gland.[61] Cortisol is a stress hormone and is causes hyperexcitability in the muscle cell as it is used in the fight or flight response.[61] Therefore it seems plausible that stress could cause muscle cramps with an increase in excitability in the muscle cell. The ability to control stress or ways to relieve stress then

would be the best preventative measure for muscle cramps if that is the case. Relaxation and peace of mind would decrease the amount of adrenocorticotropic hormone which would then decrease the amount of cortisol released from the adrenal gland.[61] Further research is needed to determine if this is an effective preventative measure.

A proven substance to prevent muscle cramps is Quinine. Quinine was a drug that was first used in the treatment of malaria in World War 2.[62] It was later discovered to be a remedy for muscle cramps. Quinine works by decreasing the excitability of the muscle cells.[62] In many research studies it has been shown to work but it must also be noted of the various side effects of the drug. It has been known to cause birth defects, deficiency in platelets, nausea, vomiting, headaches, deafness, vision irregularities and heart irregularities.[62] Quinine has been banned by the Food and Drug Administration as an over the counter drug due to its various side effects and toxicity.[62] Though it is no longer recommended, quinine can prevent the onset of muscle cramps but there are safer prevention measures that have been hypothesized.

Magnesium supplementation has been shown to treat muscle cramps as seen in the 3 case reports in review by Bibley.[47] It was also seen by Zeana[63] in study of 14 swimmers where magnesium supplementation relieved muscle cramps. Therefore it is hypothesized that maintaining magnesium levels during exercise could prevent muscle cramps. Magnesium is a cation that is used in reactions that require ATP and plays an important role in nerve conduction.[47] Low magnesium levels are thought to cause excitability of the muscle cell which would then cause the onset of muscle cramps.[64] Replacing the magnesium will bring the cell back to homeostasis and relieve muscle cramping by decreasing nerve excitability.[64] Kargus[65] states that chocolate, caffeine, and alcohol should be avoided because they could cause a decrease in magnesium absorption. Though it has been proven effective in other studies, further

research is needed the efficacy of magnesium supplementation on the prevention of muscle cramps.

Caffeine is thought to cause hyperexcitability of the muscle cell which could then cause the onset of muscle cramps.[65] Therefore avoiding caffeine should prevent muscle cramps by keeping the muscle cell from becoming hyperexcitable.

The most important thing to remember in the prevention of muscle cramps is taking a good history. Taking a good history is important in trying to determine whether cramps are exercise related or a type of disorder.[19] By taking a good history and identifying any risk factors it will be easier to determine an approach to take in preventing or treating the onset of muscle cramps. Here are a list of questions to ask:

Does cramping happen more with exertion or at rest?

Are there any symptoms that precede muscle cramps such as pain, decreased sensation, or muscular weakness?

When do they happen and how long do they last?

Does passive stretching relieve the muscle cramps?

Is there a strong family history of cramping?

Are you taking any drugs or supplements?

There hasn't been much or any research at all on the instance that muscle cramps are genetic. It would be interesting to know if people who are more susceptible to muscle cramps have a strong family history and if there is a certain gene that causes the onset of cramps more easily in some people than in others.

Treatment

If you know how to treat muscle cramps then it is possible have an idea how to prevent muscle cramps. There are many home remedies used in the treatment of muscle cramps, many of which have no scientific research to verify its efficacy. One remedy that has been studied is pickle juice. In a survey, Miller found that 25% (92/370) of Athletic Trainers use pickle juice to treat and prevent muscle cramps.[66] The original thought behind pickle juice is that the sodium content would replenish the sodium lost in sweat.[67] The replenishment of sodium would then have effect on plasma volume, electrolytes, and plasma osmolality.[67] In a study done by Williams[67], pickle juice was said to relieve cramps in 30-35 seconds. Miller did a similar study on the effects of pickle juice and concluded that there were negligible changes in plasma electrolytes, plasma volume, and plasma osmolality until thirty min post ingestion.[68] Plasma electrolytes and osmolality did not change enough with the volume of pickle juice ingested and therefore would need a larger volume to replace the sodium that was lost.[68] Miller also found that pickle juice relieved cramps 45% faster (85sec vs. 153sec) than when no fluid was consumed.[68] The problem is that 85 seconds is not enough time for pickle juice to be absorbed by the small intestine due to a decrease in gastric emptying. Since 85 seconds is not enough time for gastric emptying to take place but muscle cramps were still relieved, another hypothesis was made. The thought is that the acetic acid of pickle juice, which is found in vinegar, causes an oropharyngeal reflex that triggers the central nervous system response which causes the cramps to cease.[68] Further research is needed to test this hypothesis and to find if indeed an oropharyngeal reflex is occurring.

Another treatment that is related to pickle juice in the terms of how it is hypothesized to work is mustard. Mustard has an acetic acid content of about 3%.[69] There is also 200mg of

sodium per packet which is about 1 tablespoon.[69] Like pickle juice one hypothesis is that it causes an oropharyngeal reflex because of the acetic acid. Other theories have been developed in how mustard relieves muscle cramps. If the electrolyte theory is true and a loss of sodium is the cause of muscle cramps, then taking enough mustard should replenish the plasma sodium volume. Another theory is that muscle cramps are caused by a decrease in production of acetylcholine.[69] The theory is that acetylcholine production is influenced by fatigue because as you become fatigue the muscle energy stores are being depleted which leads to a decrease in production of muscle fatigue.[69] It is said that acetic acid aids in the acetylcholine production which would then cause a cessation of muscle cramps.[69] It was also stated that other ingredients in mustard may cause the relief of muscle cramps.[69] Turmeric, a native to the ginger family, gives mustard its yellow color and is a dietary supplement that could aid in the relief of muscle cramps.[69] Most of these observations and theories are anecdotal and have no research to date to support these theories. Future research is needed to determine if mustard is effective at treating and preventing muscle cramps.

Bananas have been used for their high potassium content in the treatment and prevention of muscle cramps. Going back to electrolyte theory, many believe that potassium plays a role in muscle cramps. Potassium plays critical role in generating nerve signals, maintaining electrolyte balance and pH balance.[65] If potassium is lost during exercise then replenishment with bananas or other source rich in potassium would restore any potassium losses causing a cessation of cramping.[28,41] Further research is needed to determine if potassium replacement is effective.

A remedy that is used to treat and not prevent is the transcutaneous nerve stimulation (TENS). TENS is used to break the pain spasm pain cycle by lessening mechanical pressure placed on nerve endings.[70] TENS also works through the gate control theory. The gate control

theory works by activating the A-beta fibers.[71] By activating the A-beta fibers they override the C fibers that produce pain and spasms.[71] Transcutaneous electrical nerve stimulation is only useful for treatment of muscle cramps.[70] It does not seem very ideal when stretching the affected muscle provides relief as well.

The best and most effective treatment for muscle cramps to date is stretching. Stretching activates the golgi tendon organ which decreases the muscle spindle activity.[59] Increases in muscle spindle activity are thought to be the cause of muscle cramps.[72] Therefore stretching is an effective way to treat cramps. Future research is needed to determine if stretching a muscle that is at risk as a preventative measure of muscle cramps.

There has been some theories on the medicinal value of honey. There have been reports of people using honey as treatment and prevention of muscle cramps. In most cases it is added to apple cider vinegar. Apple cider vinegar is high in potassium and has a high acetic acid value.[73] Honey could just be used to sweeten the taste of the apple cider vinegar or maybe there is a greater medicinal value to honey. Honey mixed with warm water is said to have a relaxing effect and calm the nerves.[73] This could be a good treatment for stress which is mentioned as a possible mechanism in the onset of muscle cramps. Future research is needed to determine if honey alone can treat and prevent muscle cramps.

The other most commonly used treatment of muscle cramps is rehydration with just water. Strong believers in the dehydration theory or people who are uneducated in muscle cramps, use water as a treatment method. Water replenishes plasma volume but does not replenish sodium loss. Though water is not effective as a treatment, as a preventative method it is thought to be effective. It is thought that cramps happen because athletes are not 100% hydrated when they start activity due to losses from previous practice or games.[49] When an

athlete is 100% hydrated, they have an increased plasma volume when compared to those who are not.[49] Therefore in theory those that are more hydrated should have delayed onset of muscle cramps when compared to their less hydrated counterpart.

Summary

There are many theories to the causes, treatment, and prevention of muscle cramps. With all the research that has been done, muscle cramps still remain a mystery. The cause remains unknown with the most popular theories being electrolyte-dehydration theory and muscle fatigue. Some believe that many factors combined to cause the onset of muscle cramps. It remains certain that future research is needed to find a definite cause to this common problem. The only definitive treatment of muscle cramps thus far has been stretching. Other treatments of muscle cramps are anecdotal and need further research to determine if they are effective. The new and upcoming theory that has promise is the use of pickle juice in the treatment of muscle cramps. More evidence to support this treatment is needed. Without having definite causes and lack of effective treatment options makes prevention of muscle cramps difficult. There are many theories about the prevention of muscle cramps with little evidence to support. It seems that the best thing a clinician can do is take a detailed history. With this detailed history it will be possible to identify possible solutions to the problems whether it is adding more sodium in the diet, conditioning program, or further examination for a more serious and underlying problem.

Materials and Methods

Experimental Design

A cross-sectional pre-post experimental design will guide data collection. The independent variable will be hydration status (euhydrated or 5% hypohydrated). The dependent variable will be flexor hallucis brevis cramp threshold frequency (Hz). Descriptive variables will be sweat sodium concentration $[Na^+]_{sw}$, sweat potassium concentration $[K^+]_{sw}$, sweat chloride concentration $[Cl^-]_{sw}$, sweat volume (L), sweat rate $(L*hr)^{-1}$, plasma osmolality ($mOsm*kg^{-1}$ H_20), plasma sodium concentration $[Na^+]_p$, plasma chloride concentration $[Cl^-]_p$, plasma potassium concentration $[K^+]_p$, hematocrit (% red blood cells), and hemoglobin concentration $(g*dL^{-1})$. Change in plasma volume will be calculated using hematocrit and hemoglobin data. Sweat volume will be estimated from body mass measurements.

Control variables will include cramp intensity of maximum voluntary isometric contraction (MVIC) (%), rectal temperature (ºC), heart rate (bpm), urine specific gravity, relative humidity (%), and temperature of the environmental heat chamber (°C). Rectal temperature will be monitored to ensure subject's core temperature does not exceed 39ºC. Relative humidity and temperature of the environmental chamber will be measured to ensure exercise testing occurs under similar conditions. Heart rate will be measured to ensure exercise intensity is similar between subjects. Urine specific gravity will be measured to determine hydration status prior to testing. Cramp EMG activity will be normalized to MVIC EMG activity to ensure the euhydrated and 5% hypohydrated cramps have a similar intensity.

Subjects

Sample size was estimated *a priori*[74](Appendix A). A convenience sample of 9 healthy male subjects, between the ages of 18 and 30 will be needed to achieve 80% power at an alpha level of 0.05. Subjects will be excluded from participating if: 1) they experienced injury to their legs that has affected their activities of daily living for longer than 7 days in the 6 months prior to data collection; 2) they self-report any cardiovascular, neurological, or blood borne diseases; 3) they do not have a self-reported history of muscle cramps in lower extremity within the 12 months prior to data collection; 4) a muscle cramp cannot be electrically induced in the flexor hallucis brevis on the familiarization day; 5) they are not between the ages of 18 and 30; 6) they are not physically active ($\geq$ 30 minutes of activity on all or most days per week)[75] ; 7) they are taking any medication that affect physiological responses (heart rate, sweat rate, core temperature, etc.) as well as any variables that will be measured. All medications will be evaluated prior to testing; 8) they have any history of cardiac events or cardiac incidents in their family; or 9) they have a history of heat illness. Subjects testing day will be rescheduled if: 1)they have eaten in the previous 6 hours; 2)they are not well rested. All procedures will be approved by the universities institutional review board and subjects will provide written informed consent prior to participating in the study.

Procedures

Subjects will report for one familiarization day and one testing day. Prior to the familiarization day, subjects will be asked to avoid exercise for 24 hours and arrive hydrated by drinking water prior to arriving at the laboratory. On the familiarization day, subjects will report to a temperature controlled laboratory (room 14 Bentson Bunker Field House) and provide

consent. Subject's leg dominance will be identified by asking the subject to kick an imaginary ball; the leg used to kick will be deemed the dominant leg. Subjects will lay supine on a padded treatment table and have their dominant leg prepped for EMG analysis using standard protocol.[76] Subjects' dominant medial ankle, lower leg, tibial tuberosity, and midbelly of the gastrocnemius will be shaved (if necessary), debrided with sandpaper (Norton sandwet 600 grit ultra-fine sandpaper), and cleaned with alcohol. Two EMG electrodes will be placed over the midbelly of the flexor hallucis brevis with center-to-center inter-electrode distance of 2 cm. A single ground EMG electrode will be placed over the ipsilateral tibial tuberosity. A single Ag-AgCl electrode will be placed over the midbelly of the gastrocnemius.

Subjects will be prepped for MVIC measures. The dominant big toe will be placed into a toe harness. To prevent movement of the hip and knee, 4-cm nylon strips will be tightened over subject's mid-thigh and shin to prevent movement of the hip and knee. The subject's ankle will be placed in a foam block with a foot pad angled at 120º to keep the foot in slight plantar flexion and prevent any excessive ankle inversion and eversion (Figure 1). The subjects will perform 20 practice , 2 second duration flexor hallucis brevis MVICs with 1 minute rest separating MVICS. Subjects will rest for 5 minutes and perform 3 consecutive MVICs which will be averaged and used to determine the intensity of the muscle cramp. To verify that subjects are using the correct muscles when performing MVIC, gastrocnemius muscle activity will be monitored with a biofeedback unit (Pathway TR-10C, Prometheus Group, Dover, NH). Gastrocnemius activity exceeding 8 mV will indicate a failed attempt. If subjects perform MVIC incorrectly, a 1 minute rest period will be given and the MVIC will be attempted again. Researchers have reported high MVIC intratester reliability using this method (ICC (3,3)>0.81).[77] Following MVIC, subjects

big toe and ankle will be removed from toe harness and foam block, respectively, and subjects will be prepped for cramp induction.

An 8-mm Ag-AgCl shielded electrode (EL258S; Biopac Systems) will be placed over the medial ankle distal to the medial malleolus where the tibial pulse is felt. An 8-cm square dispersive electrode will be placed over the lateral malleolus. To determine proper placement of the stimulating electrode, the tibial nerve will be stimulated two to four times with 1 ms electrical stimuli at 80 V (Grass S88 stimulator with SIU5 stimulus isolation unit, (Astro-Med Inc., West Warwick, RI). The proper location will be the site that causes the greatest hallux flexion. The stimulating electrodes will be secured with medical tape and an elastic wrap.

Subjects will be instructed to relax as much as possible during the cramp protocol. The cramp protocol consists of subjects will receiving 1 second of rest followed by 2 seconds of electrical stimulation consisting of two consecutive bursts (one burst per second without rest intervals between bursts) at a frequency of 4 Hz. The 2-second stimulation will be followed by 15 seconds of EMG activity to allow the primary investigator to confirm cramping. If a cramp is not induced, subjects will rest for 1 minute and stimulation frequency will be increased by 2 Hz. These procedures have been used previously and have high intratester (ICC (3,1) = 0.84), and intertester reliability (ICC (3,1) > 0.96).[77]

A muscle cramp will be defined as an involuntary contraction of the flexor hallucis brevis immediately following the end of the 2 second electrical stimuli. Muscle cramps must: 1) have an EMG activity $\geq$ 50% of MVIC EMG activity, 2) maintain this intensity for a minimum of 5 seconds, 3) be verified by subjects, and 4) display sustained involuntary flexion of the 1st metatarsal pharyngeal joint (MTP). If a muscle cramp does not spontaneously resolve after 5 seconds, the flexor hallucis brevis will be passively stretched by hyperextending the 1st MTP

joint until the cramp is alleviated. The stimulation frequency used to induce a cramp meeting these criteria will be deemed the subjects cramp threshold frequency. The electrode sites will be marked with a permanent marker for future testing sessions and removed. Subjects will be instructed to remark the sites if the marks fade. Subjects will be asked to report for their testing session at least 48 hours following the familiarization day. No data will be collected on the familiarization day.

Twelve hours prior to the testing session subjects will be asked; 1) to fast for 6 hours; 2) to drink water consistently; and 3) to avoid consuming any beverage other than water. Twenty-four hours prior to testing, subjects will be asked to avoid exercise. On the testing day, the following procedures will be followed (Appendix C). Subjects will provide a midstream urine sample. Urine specific gravity will be measured using a refractometer (Sur-ne, Atago VSA Inc., Bellevue WA). If hypohydrated (urine specific gravity > 1.012)[51], subjects will ingest 3 mL $\cdot$ kg^{-1} of deionized water, and urine specific gravity will be reassessed 30 minutes later. If euhydrated, subjects will lay supine on padded treatment table for 30 minutes for body fluid compartment equilibration.[78] The dominant leg will be prepped for EMG analysis and muscle cramp induction using the procedures used on the familiarization day. Subjects arm will be cleaned and sterilized using alcohol and beta dine. A small sterile catheter will be inserted into subjects arm and a 5 mL blood sample will be collected (euhydrated sample). Blood will be placed in a 6 mL vacutainer and placed on ice for later analysis. All blood samples will be collected by a trained phlebotomist who is a member of the research team. Subjects will perform 10 practice MVICs with 1 minute rest separating each MVIC. After 15 minutes rest, subject's euhydrated threshold frequency will be determined using the procedures described above. Upon cramp induction, the electrodes will be removed and subjects will be prepped for exercise.

Subjects will don a heart rate monitor (Polar Electric Inc., Lake Success, NY) and insert a rectal thermistor (Yellow Spring Instruments 4600, Advanced Industrial Systems Inc., Prospect, KY) at least 10 cm past the anal sphincter. Subjects will insert rectal thermistor in a storage room with a lockable door for privacy. Risk for irritation or injury from the insertion of the rectal thermistor is minimal. Subjects will take off clothes in storage room and exit wearing only a towel for body weight measurement. The laboratory will be locked at all times and signs will be posted on the outside of the door stating "Testing in progress, knock to enter." Both posterior mid forearms will be shaved, cleaned with deionized water and dried with a clean towel. Sterile sweat patches will be placed at these sites.

Subjects will enter an environmental chamber (temperature 38-40ºC; relative humidity 20%) and begin the first 30 minute bout of moderate intensity exercise (70% of heart rate max)**.**[75] All subjects will begin exercise with an upper body ergometer. Subjects will alternate between upper body cycle ergometry and non-dominant leg exercise every 30 minutes. Sweat patches will be removed after 20 minutes of exercise, placed into a clean test tube and analyzed for sweat electrolyte concentrations. Subjects will wear a sweatshirt to wear for the remainder of the exercise protocol. This will help increase sweat rate and decrease the amount of time spent exercising. At the conclusion of the first hour, subjects will exercise for 5 minutes at a self-selected lower intensity to cool down. Subjects will: 1) exit the environmental chamber, 2) remove their clothes, 3) towel dry, 4) attempt to void their bladders, and 5) have their body mass assessed. Subjects will dress and reenter the environmental chamber, rest for 10 minutes and resume exercising for another 1 hour bout. After the 2nd hour of exercise subjects will remove clothes, towel off, have their body mass assessed, and then resume exercise protocol if 5% hypohydration is not yet obtained. This protocol of 30 minutes of alternating exercises and body

mass measurements will continue until subjects lose 5% of their body mass. Upon 5% hypohydration, the heart rate monitor and rectal thermistor will be removed and subjects will lay supine for 30 minutes in a temperature controlled environment (25ºC, 20% relative humidity).

During the 30 minute rest period, the EMG and stimulation electrodes will be placed over the previously marked locations. At the end of the 30 minute rest period, a second 5 mL blood sample will be collected (5% hypohydrated sample). Subjects will perform 10 practice MVICs. Upon completion of the practice MVICs, subjects will rest for 5 minutes and perform 3 consecutive 2 second duration MVICs. Cramp threshold frequency will be reassessed. Following cramp induction, the electrodes will be removed and subjects will be excused. No fluids will be given during testing.

Cramp and MVIC EMG Procedures

Muscle action potentials of the flexor hallucis brevis will be sampled at 2000 Hz and filtered (band pass, low frequency = 10 Hz high frequency = 500 Hz) using MP 150 analog-to-digital system operated by Acqknowledge v. 3.7.3 software (Biopac systems; Santa Barbara CA). Disposable long term recording electrodes (EL 502-10; Biopac Systems) will collect EMG data from subject's dominant limb. Signals were amplified using the TEL 100C (gain set to 5000, Biopac Systems) from disposable, long-term recording electrodes (Biopac, EL502-10). Amplifier impedance was 2 mega ohms with a common mode rejection ratio of 11 dB and a signal to noise ratio of 0.75 dB.

Blood Analysis Procedures

Blood will be collected into 6 mL lithium heparin vacutainers (BD, Franklin Lakes, NJ). One mL of blood will be used to analyze hematocrit and hemoglobin concentration (0.5 mL for each). Hematocrit and hemoglobin concentration will be determined in triplicate immediately after sampling. The remaining 4 mL will be placed on ice until all blood samples have been collected.

To determine hematocrit, blood will be drawn into heparinized microcapillary tubes and centrifuged at 3000 rpm (IEC Micro-MB; International Equipment Co., Needham Heights, MA) for 5 minutes. Hematocrit will be read using a microcapillary reader (model IEC 2201; Damon/IEC, Needham Heights, MA). To measure hemoglobin concentration, 20 µL of whole blood will be mixed with 5 mL of cyanomethemoglobin reagent. The hemoglobin concentration will be determined by reading the absorbance at 540 nm on spectrophotometer (iMark 3000; Bio-Rad, Hercules, CA) and using a hemoglobin standard curve. Percent change in plasma volume will be determined by inserting hematocrit and hemoglobin concentration data into the Dill and Costill equation.[79]

The remaining 4 mL of blood will be centrifuged at 3000 rpm at 3ºC for 15 minutes. Plasma will be removed from the centrifuged red blood cells and analyzed for $[Na^+]_p$, $[K^+]_p$, and $[Cl^-]_p$ with an ion selective electrode system (NOVA 16 electrolyte analyzer; NOVA Biomedical, Waltham, MA). Plasma osmolality will be determined in duplicate via freezing-point depression osmometry (model 3D3 Osmometer, Advanced instruments, Inc, Norwood, MA).

Sweat Analysis Procedures

Sweat patches will be collected after 20 minutes of exercise, placed in a clean test tube and centrifuged for 5 minutes at 5000 rpm. Sweat samples will then be analyzed in duplicate with an electrolyte analyzer (NOVA 16 electrolyte analyzer; NOVA Biomedical) for $[Na^+]_{sw}$, $[K^+]_{sw}$, $[Cl^-]_{sw}$. Sweat volume will be estimated by subtracting the final body measurement post exercise weight from subjects pre exercise weight measurement and correcting for urine produced. It will be assumed that 1 kg of body mass lost is equal to 1 L of fluid lost.

Statistical Analysis

A dependent t-test will be used to determine the difference in mean threshold frequency. The alpha level will be set at 0.05 for accepting significance. An intraclass correlation will be run to determine tester reliability for MVIC measures between familiarization and testing days (ICC [3,2]). All statistical analysis will be performed using Number Cruncher Statistical Software (NCSS 2007, Kaysville, UT)

References

1. Schwellnus M P, Derman E, Noakes T. Aetiology of skeletal muscle cramps during exercise: a novel hypothesis. *J Sports Sci.* 1997;15:177-185.
2. Maughan R, Leiper J. Limitations to fluid replacement during exercise. *Can J Appl Physiol.* 1999;24:173-187.
3. Nose H, Mack G W, Shi X R, Nadel E R. Shift in body fluid compartments after dehydrationin humans. *J Appl Physiol*. 1988; 65: 318-324.

4. Roeleveld K, Van-Engelen B G M, Stegeman D F. Possible mechanisms of muscle cramp from temporal and spatial surface EMG characteristics. *J Appl Physiol*. 2000; 88: 1698-1706.
5. Dumke C. Muscle cramps are not all created equal. *Athl Ther Tod.* 2003;8(3):42-43.
6. Maughan R J. Exercise-induced muscle cramps: a prospective biochemical study in marathon runners. *J Sports Sci*. 1986; 4: 31-34.
7. Cooper E R, Ferrara M S, Broglio S P. Exertional heat illness and environmental conditions during a single football season in the Southeast. *J Athl Train*. 2006; 41: 332-336.
8. Kantorowski P G, Hiller W D, Garrett W E. Cramping studies in 2600 endurance athletes. *Med Sci Sports Exerc*. 1990; 22: S104-S106.
9. Wagner T, Behnia N, Ancheta W-K, Shen R, Farrokhi S, Powers C. Strengthening and neuromuscular reeducation of the gluteus maximus in a triathlete with exercise-associated cramping of the hamstrings. *Journal of Orthopedic and Sports Physical Therapy*. 2010; 40:112-119.
10. Stone M, Edwards J, Stemmans C, Ingersoll C, Palmieri R, Krause B. Certified athletic trainers' perceptions of exercise-associated muscle cramps. *J Sport Rehab.* 2003;12:333-342
11. Eichner ER. The Role of Sodium in 'Heat Cramping. *Sports Med.* 2007;37:368-370.
12. Bergeron M F. Heat cramps: fluid and electrolyte challenges during tennis in the heat. *J Sci Med Sport.* 2003; 6: 19-27
13. Stofan J, Zachwieja J, Horswill C. Sweat and sodium losses in NCAA football players: a precursor to heat cramps? *Int J of Sports Nutr, Ex, and Metab.* 2005;15:641-652.

14. Bergeron M F. Heat cramps during tennis: a case report. *Int J Sports Nutr*. 1996; 6: 62-68.
15. Bergeron M F. Muscle cramps during exercise: is it fatigue or electrolyte deficit. *Am College of Sports Med*. 2008; 7: S50-S55.
16. Schwellnus M P. Muscle cramping in marathon: aetiology and risk factors. *Sports Med*. 2007; 37: 364-367.
17. Nicol J. Exercise associated muscle cramps in distance runners: the role of serum electrolytes both during and following an ultra-marathon and IEMG activity in the recovery phase post cramp. University of Capetown; Capetown South Africa, Sports Med. 1996.
18. Schwellnus M P. Cause of exercise associated muscle cramps: altered neuromuscular control, dehydration, or electrolyte depletion. *Br J Sports Med*. 2009; 43: 401-408.
19. Schwellnus M P. Skeletal muscle cramps during exercise. *Phys & Sportsmed.* 1999;27:109-115.
20. Miller K. Exercise-Associated muscle cramps: causes, treatment, and prevention. *Sports Health: A Multidisciplinary Approach.* 2010;2(4):279-283.
21. Miller K, Mack G W, Knight K L, Hopkins J T et al. Three percent hypohydration does not affect the threshold frequency of electrically induced cramps. *Med Sci Sports Exerc*. 2010; 42: 1-8.
22. Miller K C, Knight K L. Electrical stimulation cramp threshold frequency correlates well with the occurrence of skeletal muscle cramps. *Muscle Nerve*. 2009; 39: 364-368.
23. Maughan RJ, Shirreffs SM, Galloway DR, Leiper JB. Dehydration and fluid replacement in sport and exercise. *Sports Exerc Inj*. 1995; 1: 148 -153.

24. Fowkes-Godek S, Godek J, Bartolozzi A R. Hydration status in college football players during consecutive days of twice-a-day preseason practices. *Am J Sports Med*. 2005; 33: 843-851.

25. Talbot H T. Heat cramps. *Medicine*. 1935; 14: 323-376.

26. Armstrong L E, Casa D J, Millard-Stafford M. Exertional hear illness during training and competition: ACSM position stand. *Med. Sci. Sports Exerc*. 2007; 39: 556-572

27. MedicineNet. (1996-2010). "Electrolytes." Retrieved 11/5/10, from http://www.medicinenet.com/electrolytes/article.htm

28. Levin S. Investigating the cause of muscle cramps. *Phys Sports Med*. 1993; 21: 111-114.

29. Stamford B. Muscle cramps: untying the knots. *Phys Sports Med*. 1993; 21: 115-116.

30. Costill D L. Sweating: its composition and effects on body fluids. Ann N.Y. *Acad Sci*. 1977; 301: 160-174

31. Bergeron M F. Sodium: the forgotten nutrient. *Sports Sci Exch*. 2000; 13: 1-4

32. Bergeron M F. Exertional heat cramps: recovery and return to play. *J Sports Rehab*. 2007; 16: 190-196.

33. Schwellnus M, Nicol J, Laubscher R. Serum electrolyte concentrations and hydration status are not associated with exercise associated muscle cramping (EAMC) in distance runners. *Br J Sports Med*. 2004;38:488-492.

34. Sulzer N, Schwellnus M, Noakes T. Serum electrolytes in Ironman triathletes with exercise associated muscle cramping. *Med Sci Sports Exerc.* 2005;37(7):1081-1085.

35. Jung A, Bishop P, Al-Nawwas A, Dale R. Influence of hydration and electrolyte supplementation on incidence and time to onset of exercise-associated muscle cramps. *J Athl Train.* 2005;40:71-75.

36. Hutton R S, Nelson D L. Stretch sensitivity of golgi tendon organs in fatigued gastrocnemius muscle. *Med Sci Sports Exerc*. 1986; 18: 69-74.

37. Jansen P H P, Joosten E M G, Vingerhouts H M. Muscle cramp: main theories as to aetiology. *Eur Arch Psychiatr Sci*. 1990; 239: 337-342.

38. Miles M P, Clarkson M. Exercise induced muscle pain, soreness, and cramps. *J Sports Med Phys Fitness.* 1994; 34: 203-216.

39. Bentley S. Exercise induced muscle cramp. Proposed mechanisms and management. *Sports Med.* 1996;21:409-420.

40. Manjra S, Schwellnus M, Noakes T. Risk factors for exercise associated muscle cramping (EAMC) in marathoners. *Med Sci Sports Exerc.* 1996;28(5):S167.

41. Stone M B, Edwards J E. Exercise associated muscle cramps. *Athl Ther Today*. 2003; 8: 30-31.

42. Maddali S, Rodeo S A, Barnes R, Warren R F et al. Post exercise increase in nitric oxide in football players with muscle cramps. *Am J Sports Med*. 1998; 26: 820-824

43. Maquirriain J, Merello M. The athlete with muscular cramps: a clinical approach. *J Am Acad Orthop Surg.* 2007; 15: 425-431.

44. Dalbo V J, Roberts M D, Stout J R, Kerksick C M. Putting to rest the myth of creatine supplementation leading to muscle cramps and dehydration. *Br. J. Sports Med*. 2008; 42: 567-573.

45. Greenwood M, Kreider R, Greenwood L, Byars A. Cramping and injury incidence in collegiate football players are reduced by creatine supplementation. *J Athl Train.* 2003;38:216-219.

46. Volek J S, Mazzetti S A, Farquhar W B. Physiological responses to short term exercise in the heat after creatine loading. *Med Sci Sports Exerc*. 2001; 33: 1101-1108.

47. Bibley D L, Prabhakaran V M. Muscle cramps and magnesium deficiency: case reports. *Can Fam Physician*. 1996; 42: 1348-1351.

48. Fehlinger R, Mielke U, Fauk D, Seidel K. Rheographic indications for cerebral vasoconstriction after oral magnesium medication in tetanic patients, a double blind, placebo controlled study. *Magnesium*. 1986; 5: 60-65.

49. Parisi L, Pierelli F, Amabile G, Valente G et al. Muscle cramps: proposals for a new classification. *Acta Neurol Scand*. 2003; 107: 176-186

50. Mitchell J B, Phillips M D, Mercer S P, Baylies H L et al. Post exercise rehydration:effect of Na and volume on restoration of fluid spaces and cardiovascular function. *J Appl Physiol*. 2000; 89: 1302-1309.

51. Casa D, Armstrong L E, Montain S J, Rich B S E, Stone J A. National athletic trainers association position statement: fluid replacement for athletes. *J Athl. Train.* 2000;**35**: 212-224.

52. Sherwood L. *Human Physiology: From Cells to Systems.* 7th ed. Baltimore, MD: Cengage Learning.; 2008.

53. Sawka M, Burke L, Eichner E, Maughan R, Montain S, Stachenfeld N. American College of Sports Medicine position stand: exercise and fluid replacement. *Med Sci Sports Exerc.* 2007;39(2):377-390.

54. Benda C. Outwitting muscle cramps. *Phys Sportsmed*. 1989; 17: 173-178

55. Gaby A R. Nutritional interventions for muscle cramps. *Integ Med.* 2007; 6: 20-23

56. Chimera N J, Swanik K A, Swanik C B, Straub S J. Effects of plyometric training on muscle activation strategies and performance in female athletes. *J Athl Train*. 2004: 39: 24-31.

57. El-Sayed M S, Ali N, Ali Z E. Haemorheology in exercise and training. *Sports Med.* 2005; 35: 649-670.

58. McCutcheon L J, Geor R J. Muscular and metabolic responses to moderate intensity short term training. *Equine Veterinary Journal*. 1999; Suppl. 30: 311-317.

59. Bertolassi L, DeGrandia D, Bongiovanni L G, Zantette G P et al. The influence of muscular lengthening on cramps. *Annals of Neurology*. 1993; 33: 176-180.

60. Woodrup J. (2008). "Golgi Tendon Organ Inhibtion." Retrieved 11/5/10, from http://www.verticaljumping.com/golgi_tendon_organ.html

61. Everly G. (2007). "Physiology of Stress." Retrieved 11/5/2010 from http://www.jblearning.com/samples/0763740411/Ch%202_Seaward_Managing%20Stress_5e.pdf

62. McGee S R. Muscle cramps. *Arch Intern Med*. 1990; 150: 511-518.

63. Zeana C, Radu G. Magnesium in preventing muscular cramps in swimmers. *Magnes Res.* 1993;6:73.

64. Schindler R, Thöni H, Classen HG. The role of magnesium in the generation and therapy of benign muscle cramps. *Arzneimittel-Forschung*. 1998; 48: 161-166

65. Kargus A, Webber J. Muscle cramps: causes and treatment options. *J Am Chiro Assoc*. 2009; 14: 1-2.

66. Miller K, Knight K, Williams R. Athletic trainers' perceptions of pickle juice's effects on exercise associated muscle cramps. *Athl Ther Tod.* 2008;13(5):31-34.

67. Williams R B, Conway D. Treatment of acute muscle cramps with pickle juice: a case report. *J Athl Train*. 2000; 35: S24.

68. Miller K, Mack G, Knight K. Electrolyte and plasma changes after ingestion of pickle juice, water, and a common carbohydrate-electrolyte solution. *J Athl Train.* 2009;44(5):454-461.

69. Williams R. Those Devilish Cramps. *Training and Condtioning*. 2000; 10: 1-2.

70. Mills K, Newham D, Edwards R. Severe muscle cramps relieved by transcutaneous nerve stimulation: A case report. *J Neurol Neurosurg Psychiatry*. 1982; **45:** 539-542.

71. Starkey C. (2004). *Therapeutic Modalities*. Philadelphia, F.A. Davis Company.

72. Layzar R B. The origin of muscle fasciculations and cramps. *Muscle Nerve*. 1994; 17: 1243-1249.

73. Natural-HomeRemedies.com (2010). "Natural Home Remedies for Stress." Retrieved 11/5/10, from http://www.natural-homeremedies.com/homeremedies_stress.htm

74. Vincent W. The t-Test. Comparing Means from Two Sets of Data Statistics in Kinesiology. Champaign, IL: *Human Kinetics*; 1995. Pgs 117-148.

75. Stone M, Edwards J, Babington J, Ingersoll C, Palmieri R. Reliability of an electrical method to induce a muscle cramp. *Muscle Nerve*. 2003; 27**:** 122-123.

76. Miller K., Knight K., Pain and soreness associated with percutaneous electrical stimulation muscle cramping protocol. *Muscle Nerve*. 2007; 36:711-714.

77. Hagan R D, Diaz F J, Horvath S M. Plasma volume changes with movement to supine and standing positions. *J Appl. Physiol.* 1978; 45:414-418.

78. Thompson W, Gordon N, Pescatello L, eds. ACSM Guidelines for Exercise Testing and Prescription. Eighth ed. Baltimore 2009: 18-39..

79. Dill D, Costill D. Calculation of percentage changes in volumes of blood, plasma, and red-cells in dehydration. *J Appl Physiol*. 1974; 37: 247-248.

APPENDIX B. ADDITIONAL METHODS

Table B 1. Sample Size Estimate.

$$n=\frac{2(SD)^2(Z\alpha +Z\,\beta)^2}{\Delta_2}$$

n= number of subjects needed
SD= cramp threshold frequency standard deviation assuming equal variance
Zα= z-score of alpha level 0.05
Zβ=z-score for 80% power
Δ= hypothesized difference in cramp threshold frequency between euhydrated and 5% hypohydrated trials

$$n=\frac{2(6)^2(1.95+0.80)^2}{8^2}$$

$$n=\frac{72\cdot 7.5625}{64}$$

$$=9$$

Table B 2. Experimental Timeline.

Minutes	Description
0	Informed Consent.
5	Urine Sample (euhydrated = u.s.g $\leq$ 1.02)
10	Supine for 30 minutes (prep for MVIC protocol).
40	Collect Blood Sample 1.
45	Perform 15 practice MVICs with 1 minute break between reps.
65	Rest for 5 minutes (Prep for muscle cramp induction of FHB).
70	Begin electrically induced muscle cramp protocol until muscle cramp induced (begin at 4 Hz and increase by 2 after 1 min rest).
90	Prep subject for exercise (attach HR monitor, insert rectal thermometer).
95	Measure nude body weight (BW1).
100	Prep arm for sweat patches.
105	Enter environmental chamber to begin exercise protocol One hr of exercise alternating between 15 minutes of upper arm cycling and non-dominant single leg cycling.
125	Remove sweat patches.
165	Five minute cool down at self-selected lower intensity.
170	Urine Sample 2.
175	Body weight measurement (BW2).
180	Re-enter environmental chamber to resume exercise protocol One hr of exercise alternating between 15 minutes of upper arm cycling and non-dominant single leg cycling.
240	Five minute cool down at self-selected lower intensity.

Table B 2. Experimental Timeline (Continued).

245	Thirty minute sauna period.
275	Body weight measurement (BW3).
280	Re-enter environmental chamber to resume exercise protocol One hr of exercise alternating between 15 minutes of upper arm cycling and non-dominant single leg cycling.
340	Five minute cool down at self-selected lower intensity.
345	Body weight measurement (BW4).
350	Lay supine for 30 minutes and prep for MVIC (or resume exercise protocol until 5% hypohydrated or too exhausted to continue).
380	Collect blood sample 2 (BS2).
385	Perform 15 practice MVICs with 1 minute break between reps.
405	Rest for 5 minutes (Prep for muscle cramp induction of FHB).
410	Begin electrically induced muscle cramp protocol until muscle cramp induced (begin at 4 Hz and increase by 2 after 1 min rest).
430	Excuse subject.

Table B 3. Data Collection Sheet.

Name:_____ Subject #: _____

Height (in):_____ Age (yrs):_____

Pre-Testing Questionnaire:	**Familiarization day**	**Testing Day**
1. Have you experienced any lower extremity injury within the last 6 months?	Yes No	
2. Do you have a prior history of muscle cramping in the lower extremities in the last 12 months?	Yes No	
3. Do you have any neurological, endocrine, neuromuscular or blood borne diseases?	Yes No	
4. Are you physically active (≥ 30 minutes of activity on ≥ 3 days per week)?	Yes No	
5. Do you have any history of any cardiac events or cardiac incidents in your family?	Yes No	Yes No
6. Do you have a history of heat illness (heat exhaustion, heat stroke, fainting in heat)?	Yes No	Yes No
7. Are you currently taking any medications?	Yes No	Yes No
8. Have you exercised strenuously within the last 24 hours?	Yes No	Yes No
9. Have you eaten within the last 6 hours?	Yes No	Yes No
10. Have you ingested at least 34 oz (1 L) of water in the previous 12 hours?	Yes No	Yes No
11. Have you avoided caffeine and alcohol in previous 12 hours?	Yes No	Yes No
12. Are you well rested?	Yes No	Yes No

DAY 1: FAMILIARIZATION

Dominant Leg: Right Left

MVC Practice Trials: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Table B 3. Data Collection Sheet (Continued).

5 mins rest.

MVIC: **AVG**

Mean EMG 1_____ Mean EMG 2_____ Mean EMG 3_____ _____

5 mins rest.

Prep for Muscle Cramp Induction

Cramp Threshold Frequency (cramp lasts >5 secs & ≥50% of MVC)? _____ Hz

Mean EMG of Cramp_____V **% of MVC**: _____%

Target EMG _____V

Visual Analog Scale of dominant leg fatigue: (0-none; 10-severely fatigued)

0 1 2 3 4 5 6 7 8 9 10

Testing Day

Urine Sample 1

Urine Specific Gravity (U.S.G.):_____

If u.s.g. > 1.012 *Drink 5 ml/kg in 5 min. Rest 30 mins.*

Begin Euhydrated Muscle Cramp Induction Protocol

Equilibrate for 30 mins (supine). Set-up EMG/Cramp Electrodes.

Take Blood Sample 1

Blood Sample 1 (euhydrated sample)

			AVG				AVG
Plasma Na	_____	_____	____	OSMp	_____	_____	____
Plasma K	_____	_____	____	Hct	_____	_____ ____	____
Plasma Cl	_____	_____	____	Hb	_____	_____	____
Plasma Protein ____							

MVC Practices: 1 2 3 4 5 6 7 8 9 10

Table B 3. Data Collection Sheet (Continued).

REST for 5 mins.

MVIC: **AVG**
Mean EMG 1_____ Mean EMG 2_____ Mean EMG 3_____ _____

Target EMG _____V

Prep for Muscle Cramp Induction

Cramp TF Euhydrated (cramp lasts >5 secs & ≥50% of MVC)?_____ Hz
Cramp Intensity: Mean EMG_____V **% of MVC**: _____%

Visual Analog Scale of dominant leg fatigue: (0-none; 10-severely fatigued)

0 1 2 3 4 5 6 7 8 9 10

Visual Analog Scale of whole body fatigue: (0-none; 10-severely fatigued)

0 1 2 3 4 5 6 7 8 9 10

Prep for exercise

Attach HR monitor and insert rectal thermometer

Measure Nude Body Weight

Body Weight 1: _____ kg **Target Weight**: _____ kg

Prep arms for sweat patches

Temperature _____(˚C) Relative Humidity _____(%)

Begin Exercise Protocol

(30 minutes alternating between upper arm cycling and non-dominant single leg biking)
5minute cool down after 1 hour

Remove Sweat Patches After 20 Min

Monitor Rectal Temperature Every 10 Min

Table B 3. Data Collection Sheet (Continued).

Rectal Temperature (˚C)

_____	_____	_____	_____	_____	_____
10	20	30	40	50	60

Symptoms Check Every 15 Min

Symptoms Check 1 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 2 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 3 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 4 : Nausea Headache Weakness Lightheadedness None

Remove Sweat Patches After 20 Min

			AVG
Sweat Na	_____	_____	_____
Sweat K	_____	_____	_____
Sweat Cl	_____	_____	_____

Urine Sample and Body Weight After 1 Hr of Exercise

Body Weight 2 _____(kg)

Urine Specific Gravity (U.S.G.):_____

10 Minute Break

Resume Exercise Protocol
(30 minutes alternating between upper arm cycling and non-dominant single leg biking)
5minute cool down after 1 hour

Temperature _____(˚C) Relative Humidity _____(%)

Rectal Temperature (˚C)

_____	_____	_____	_____	_____	_____
70	80	90	100	110	120

Table B 3. Data Collection Sheet (Continued).

Symptoms Check Every 15 Min

Symptoms Check 5 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 6: Nausea Headache Weakness Lightheadedness None

Symptoms Check 7 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 8 : Nausea Headache Weakness Lightheadedness None

Urine Sample and Body Weight After 1 Hr of Exercise

Body Weight 3 _____(kg)

Urine Specific Gravity (U.S.G.):_____

10 Minute Break

Resume Exercise Protocol
(30 minutes alternating between upper arm cycling and non-dominant single leg biking)
5minute cool down after 1 hour

Temperature _____(˚C) Relative Humidity _____(%)

Rectal Temperature (˚C)

_____	_____	_____	_____	_____	_____
130	140	150	160	170	180

Symptoms Check Every 15 Min

Symptoms Check 9 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 10 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 11 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 12 : Nausea Headache Weakness Lightheadedness None

Take Body Weight Measurement/Urine Sample

Table B 3. Data Collection Sheet (Continued).

Body Weight 4 _____(kg)

Urine Specific Gravity (U.S.G.):_____

Resume protocol if needed (if within 1 Kg begin taking body weight every 30 minutes)

Temperature _____(˚C) Relative Humidity _____(%)

Rectal Temperature (˚C)

_____	_____	_____	_____	_____	_____
190	200	210	220	230	240

Symptoms Check Every 15 Min

Symptoms Check 13 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 14 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 15 : Nausea Headache Weakness Lightheadedness None

Symptoms Check 16 : Nausea Headache Weakness Lightheadedness None

Take Body Weight Measurement/Urine Sample

Body Weight 5 _____(kg)

Urine Specific Gravity (U.S.G.):_____

Resume protocol if needed

Temperature _____(˚C) Relative Humidity _____(%)

Rectal Temperature (˚C)

_____	_____	_____
250	260	270

Symptoms Check Every 15 Min

Symptoms Check 1 : Nausea Headache Weakness Lightheadedness None

Table B 3. Data Collection Sheet (Continued).

Symptoms Check 2 : Nausea Headache Weakness Lightheadedness None

End Exercise Protocol

Take Body Weight Measurement

Body Weight 6 _____(kg)

Begin Hypohydrated Muscle Cramp Induction Protocol

Move out of Heat Chamber. Equilibrate for 30 mins (supine). Set-up EMG/Cramp Electrodes.

Take Blood Sample 2

			AVG				AVG
Plasma Na	_____	_____	____	OSMp	_____	_____	____
Plasma K	_____	_____	____	Hct	_____	_____	____
Plasma Cl	_____	_____	____	Hb	_____	_____	____
Plasma Protein	____						

MVC Practices: 1 2 3 4 5 6 7 8 9 10

REST for 5 mins.

MVC: **AVG**

Mean EMG 1_____ Mean EMG 2_____ Mean EMG 3_____ _____

Target EMG _____V

Cramp TF hypohydrated (cramp lasts >5 secs & ≥50% of MVC)?_____ Hz
Cramp Intensity: Mean EMG_____V **% of MVC**: _____%

Total Sweat Loss (from BW) _____ L Sweat Rate _____ L/hr
Total Exercise Duration _____ hrs

Total Urine Volume:_____

Table B 3. Data Collection Sheet (Continued).

Visual Analog Scale of dominant leg fatigue: (0-none; 10-severely fatigued)

0 1 2 3 4 5 6 7 8 9 10

Visual Analog Scale of whole body fatigue: (0-none; 10-severely fatigued)

0 1 2 3 4 5 6 7 8 9 10

Notes:

Table B 4. Statistical Analysis.

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1	5/4/2012 3:46:40 PM
Database		
Response	crampTF	

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: hydrationcondition	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	528	58.67			
B: hydrationcondition	1	20	20	3.00	0.12	0.34
AB	9	60	6.67			
S	0					
Total (Adjusted)	19	608				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: hydrationcondition	1	3.00	0.12	0.12	0.12	0.12
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: hydrationcondition	1	3.00	0.340745	0.340745	0.340745	0.340745
AB	9					
S	0					

Repeated Measures ANOVA Report

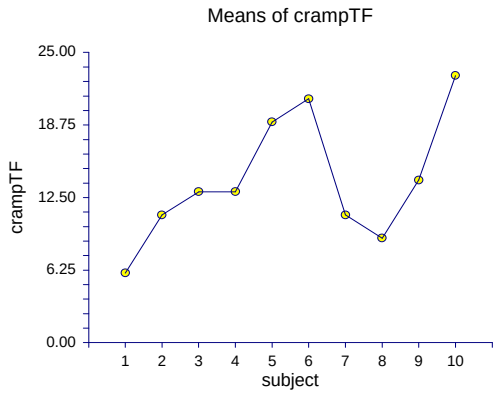
Page/Date/Time	2	5/4/2012 3:46:40 PM
Database		
Response		crampTF

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



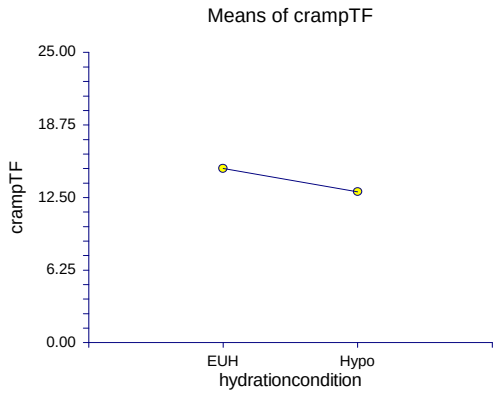
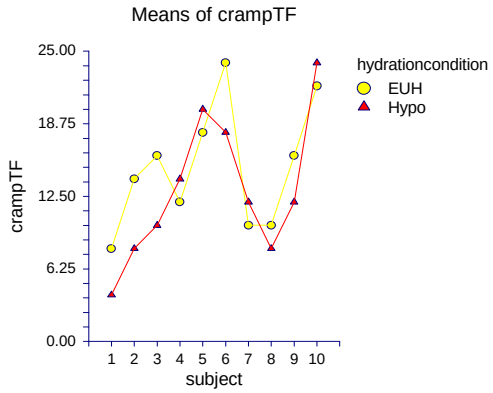
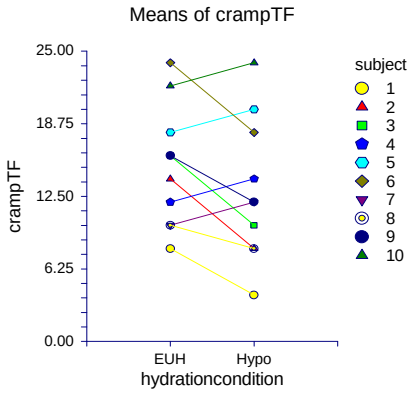


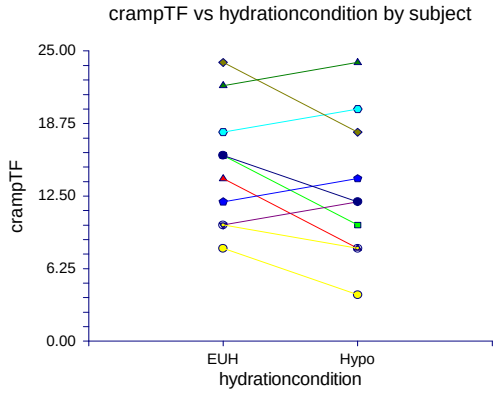
Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time 3 5/4/2012 3:46:40 PM
Database
Response crampTF



Tukey-Kramer Multiple-Comparison Test

Response: crampTF
Term B: hydrationcondition

Alpha=0.050 Error Term=AB DF=9 MSE=6.666667 Critical Value=3.1992

Group	Count	Mean	Different From Groups
Hypo	10	13	
EUH	10	15	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Table B 4. Statistical Analysis (Continued).

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1	5/4/2012 3:47:43 PM
Database		
Response	cramp_intensity	

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: hydrationcondition	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	53425.36	5936.151			
B: hydrationcondition	1	2356.991	2356.991	1.95	0.195921	0.239765
AB	9	10870.59	1207.844			
S	0					
Total (Adjusted)	19	66652.95				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: hydrationcondition	1	1.95	0.195921	0.195921	0.195921	0.195921
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: hydrationcondition	1	1.95	0.239765	0.239765	0.239765	0.239765
AB	9					
S	0					

Repeated Measures ANOVA Report

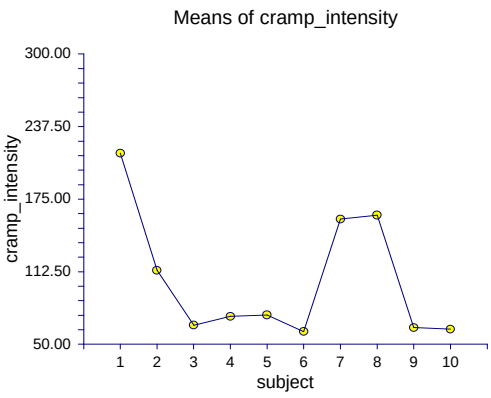
Page/Date/Time	2 5/4/2012 3:47:43 PM
Database	
Response	cramp_intensity

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



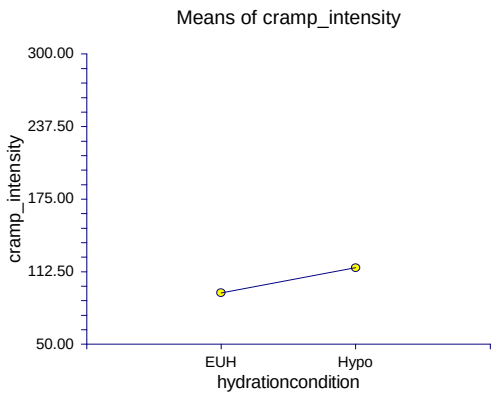
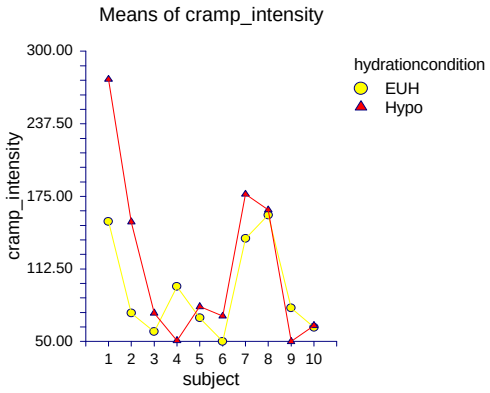
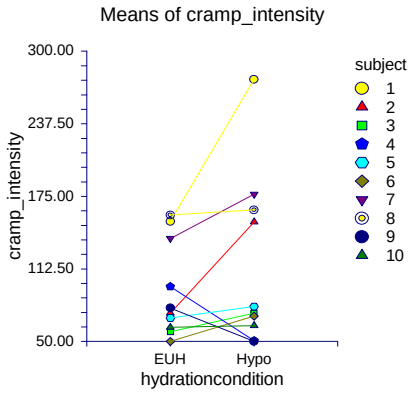


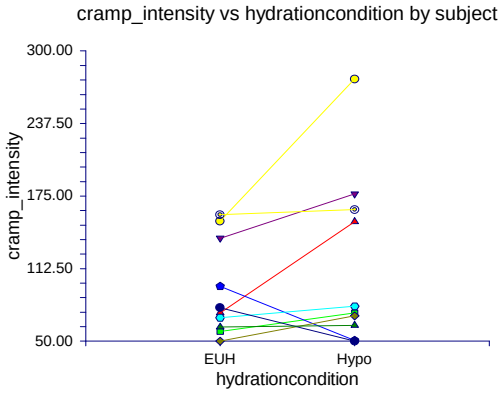
Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time 3 5/4/2012 3:47:43 PM
Database
Response cramp_intensity



Tukey-Kramer Multiple-Comparison Test

Response: cramp_intensity
Term B: hydrationcondition

Alpha=0.050 Error Term=AB DF=9 MSE=1207.844 Critical Value=3.1992

Group	Count	Mean	Different From Groups
EUH	10	94.22639	
Hypo	10	115.9381	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Table B 4. Statistical Analysis (Continued).

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1 5/4/2012 5:16:55 PM
Database	C:\Documents and Settings\Ke ... a\Fatigue of dominant leg.S0
Response	fatigue_of_DOM_leg

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: hydration_status	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	4.45	0.4944444			
B: hydration_status	1	0.45	0.45	1.98	0.193422	0.242141
AB	9	2.05	0.2277778			
S	0					
Total (Adjusted)	19	6.95				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: hydration_status	1	1.98	0.193422	0.193422	0.193422	0.193422
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: hydration_status	1	1.98	0.242141	0.242141	0.242141	0.242141
AB	9					
S	0					

Repeated Measures ANOVA Report

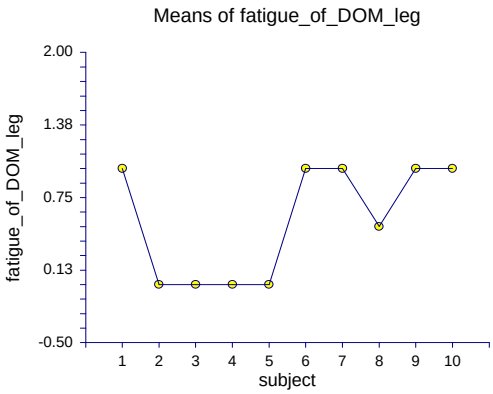
Page/Date/Time	2 5/4/2012 5:16:55 PM
Database	C:\Documents and Settings\Ke ... a\Fatigue of dominant leg.S0
Response	fatigue_of_DOM_leg

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



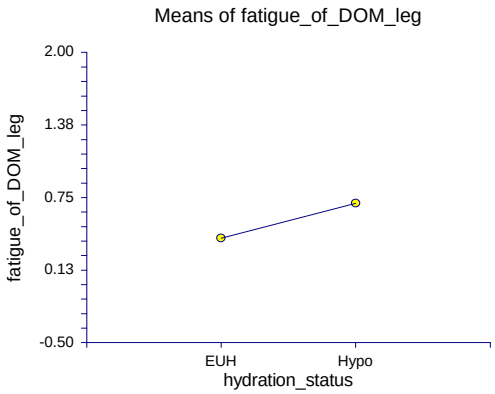
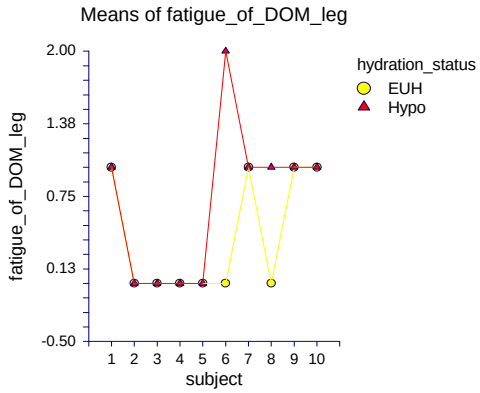
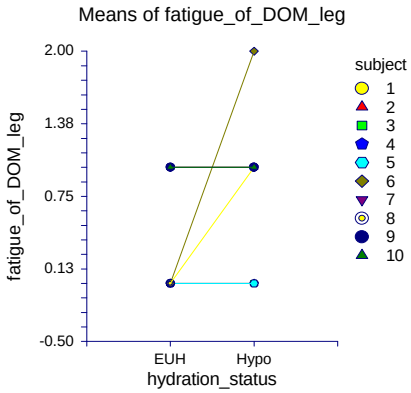


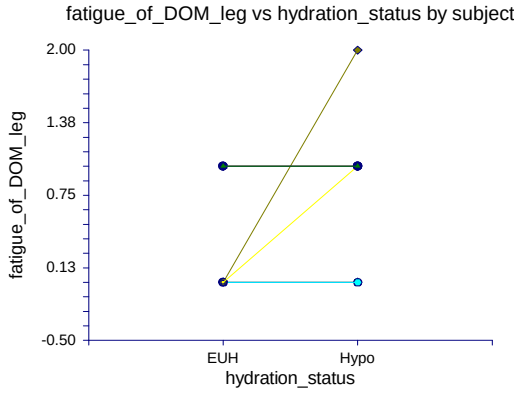
Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time	3 5/4/2012 5:16:55 PM
Database	C:\Documents and Settings\Ke ... a\Fatigue of dominant leg.S0
Response	fatigue_of_DOM_leg



Tukey-Kramer Multiple-Comparison Test

Response: fatigue_of_DOM_leg
Term B: hydration_status

Alpha=0.050 Error Term=AB DF=9 MSE=0.2277778 Critical Value=3.1992

Group	Count	Mean	Different From Groups
EUH	10	0.4	
Hypo	10	0.7	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Table B 4. Statistical Analysis (Continued).

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1 5/10/2012 3:31:15 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	Cramp_EMG__mV_

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: hydrationcondition	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	0.3071713	3.413014E-02			
B: hydrationcondition	1	7.575574E-04	7.575574E-04	0.07	0.790658	0.056940
AB	9	9.116139E-02	1.012904E-02			
S	0					
Total (Adjusted)	19	0.3990903				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: hydrationcondition	1	0.07	0.790658	0.790658	0.790658	0.790658
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: hydrationcondition	1	0.07	0.056940	0.056940	0.056940	0.056940
AB	9					
S	0					

Repeated Measures ANOVA Report

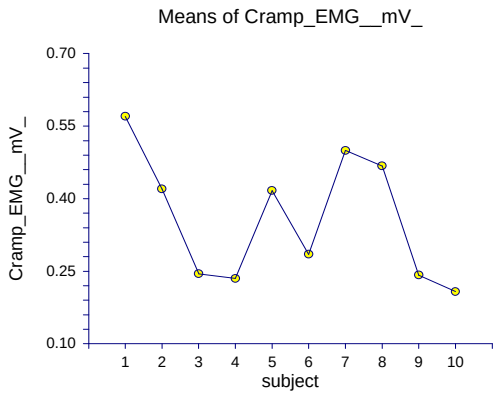
Page/Date/Time	2 5/10/2012 3:31:15 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	Cramp_EMG__mV_

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



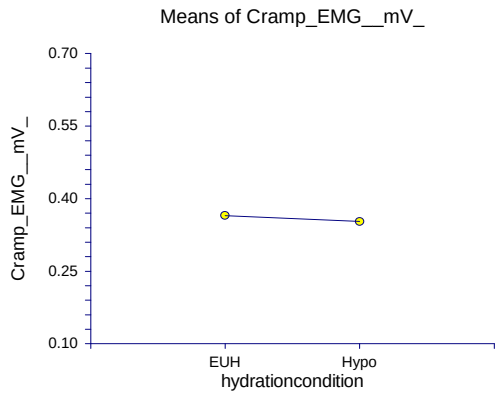
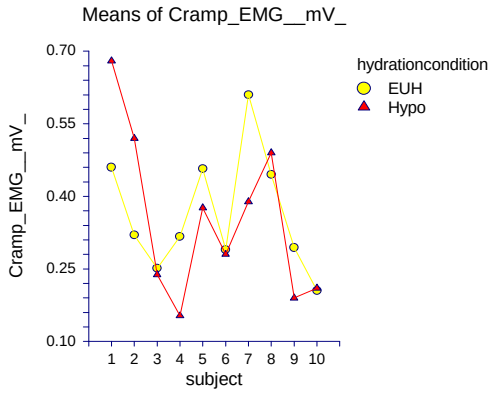
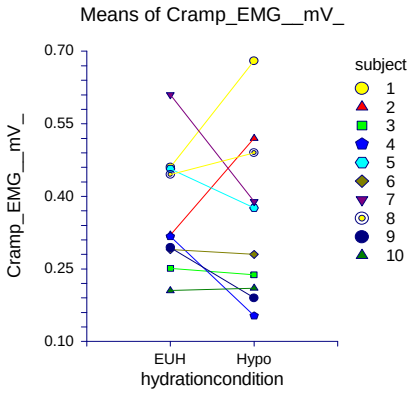


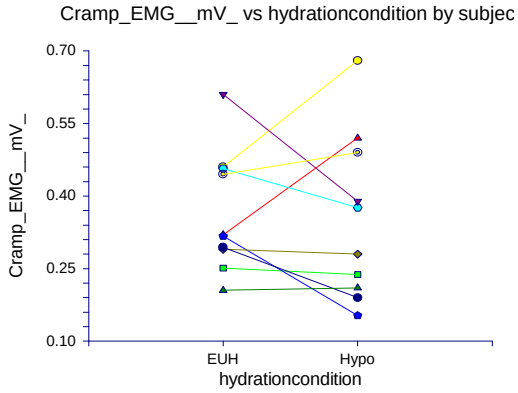
Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time	3 5/10/2012 3:31:15 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	Cramp_EMG__mV_



Tukey-Kramer Multiple-Comparison Test

Response: Cramp_EMG__mV_
Term B: hydrationcondition

Alpha=0.050 Error Term=AB DF=9 MSE=1.012904E-02 Critical Value=3.1992

Group	**Count**	**Mean**	**Different From Groups**
Hypo	10	0.352623	
EUH	10	0.364932	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Table B 4. Statistical Analysis (Continued).

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1 5/10/2012 3:31:50 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	Cramp_Intensity____

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: hydrationcondition	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	53425.36	5936.151			
B: hydrationcondition	1	2356.991	2356.991	1.95	0.195921	0.239765
AB	9	10870.59	1207.844			
S	0					
Total (Adjusted)	19	66652.95				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: hydrationcondition	1	1.95	0.195921	0.195921	0.195921	0.195921
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: hydrationcondition	1	1.95	0.239765	0.239765	0.239765	0.239765
AB	9					
S	0					

Repeated Measures ANOVA Report

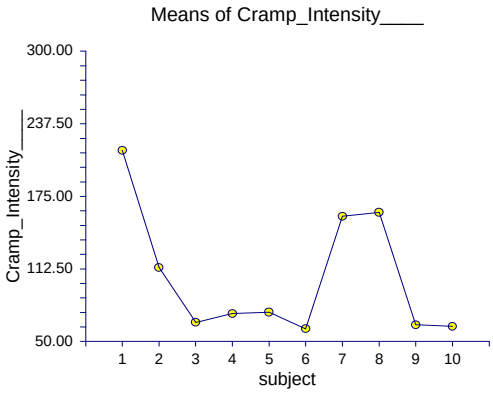
Page/Date/Time	2 5/10/2012 3:31:50 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	Cramp_Intensity____

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



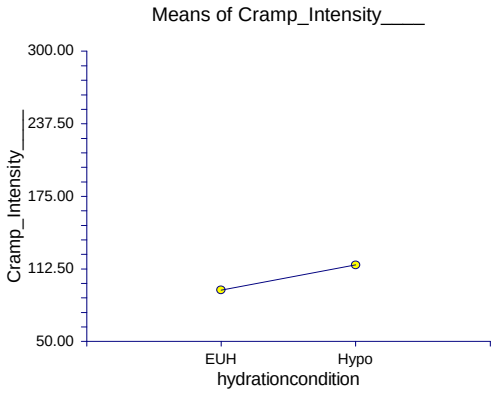
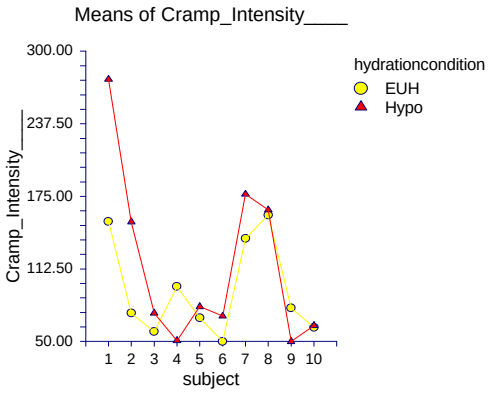
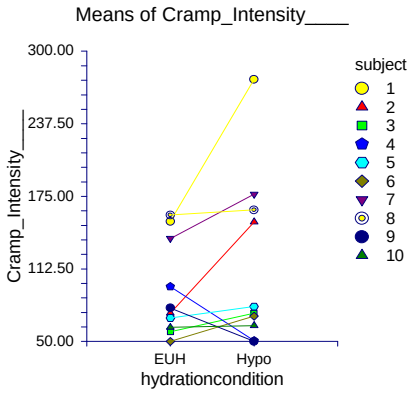


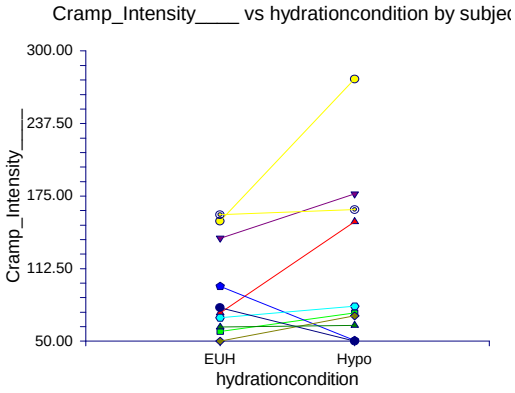
Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time	3 5/10/2012 3:31:50 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	Cramp_Intensity____



Tukey-Kramer Multiple-Comparison Test

Response: Cramp_Intensity____
Term B: hydrationcondition

Alpha=0.050 Error Term=AB DF=9 MSE=1207.844 Critical Value=3.1992

Group	Count	Mean	Different From Groups
EUH	10	94.22639	
Hypo	10	115.9381	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Table B 4. Statistical Analysis (Continued).

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1 5/10/2012 3:28:49 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	MVIC__mV_

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: hydrationcondition	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	0.1433376	0.0159264			
B: hydrationcondition	1	3.531361E-02	3.531361E-02	9.04	0.014814*	0.762768
AB	9	3.517467E-02	3.908297E-03			
S	0					
Total (Adjusted)	19	0.2138259				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: hydrationcondition	1	9.04	0.014814*	0.014814*	0.014814*	0.014814*
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: hydrationcondition	1	9.04	0.762768	0.762768	0.762768	0.762768
AB	9					
S	0					

Repeated Measures ANOVA Report

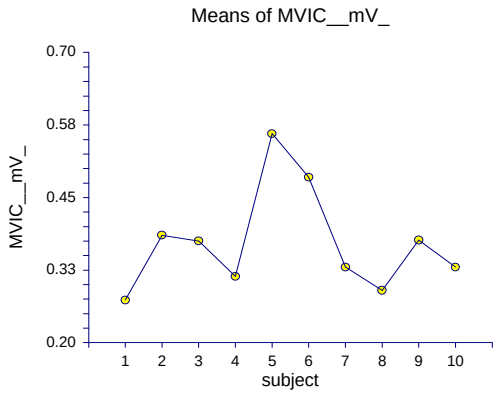
Page/Date/Time	2 5/10/2012 3:28:49 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	MVIC__mV_

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



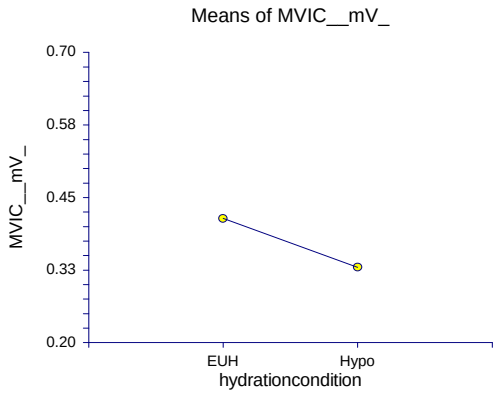
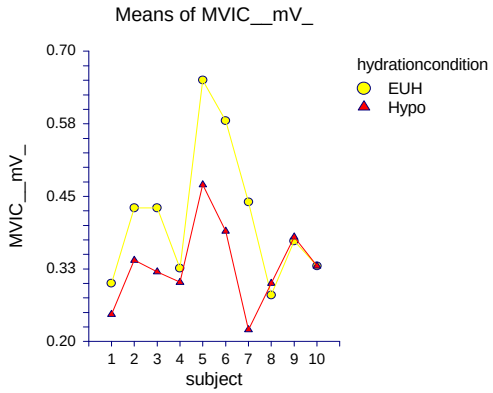
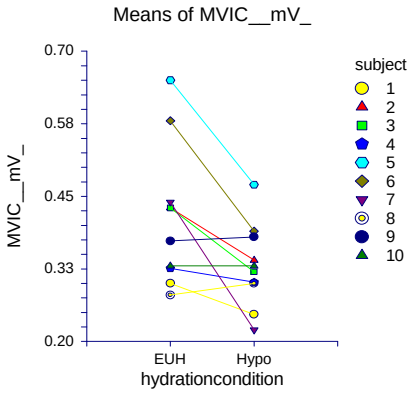


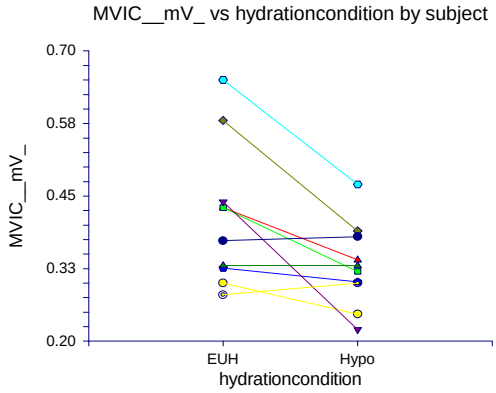
Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time	3 5/10/2012 3:28:49 PM
Database	C:\Documents and Settings\Ke ... ta\Cramp TF and Intensity.S0
Response	MVIC__mV_



Tukey-Kramer Multiple-Comparison Test

Response: MVIC__mV_
Term B: hydrationcondition

Alpha=0.050 Error Term=AB DF=9 MSE=3.908297E-03 Critical Value=3.1992

Group	Count	Mean	Different From Groups
Hypo	10	0.32986	EUH
EUH	10	0.4139	Hypo

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Table B 4. Statistical Analysis (Continued).

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1 5/4/2012 4:29:27 PM
Database	C:\Documents and Settings\Ke ... r reliability calculation.S0
Response	CrampTF

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: day	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	488.2	54.24445			
B: day	1	1.8	1.8	0.34	0.576312	0.081559
AB	9	48.2	5.355556			
S	0					
Total (Adjusted)	19	538.2				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: day	1	0.34	0.576312	0.576312	0.576312	0.576312
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: day	1	0.34	0.081559	0.081559	0.081559	0.081559
AB	9					
S	0					

Repeated Measures ANOVA Report

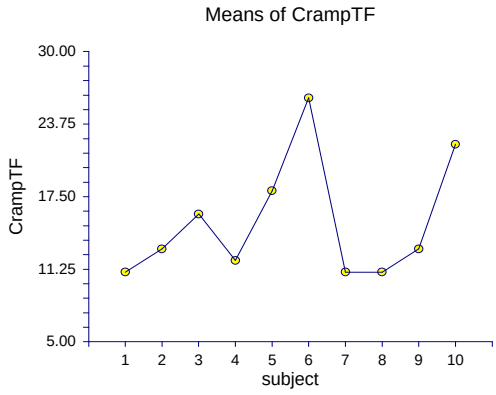
Page/Date/Time	2 5/4/2012 4:29:27 PM
Database	C:\Documents and Settings\Ke ... r reliability calculation.S0
Response	CrampTF

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



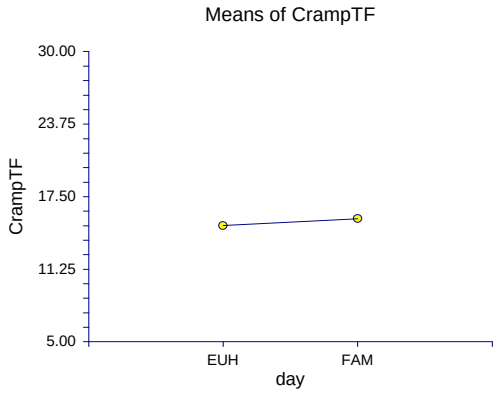
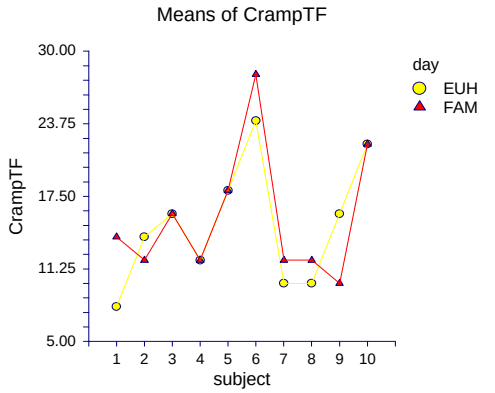
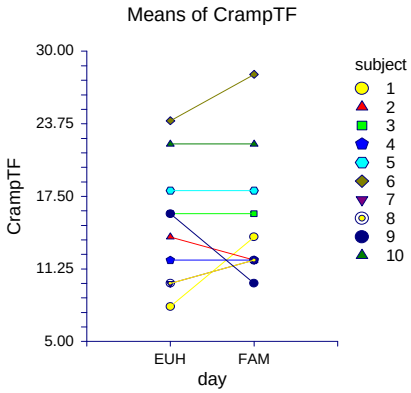


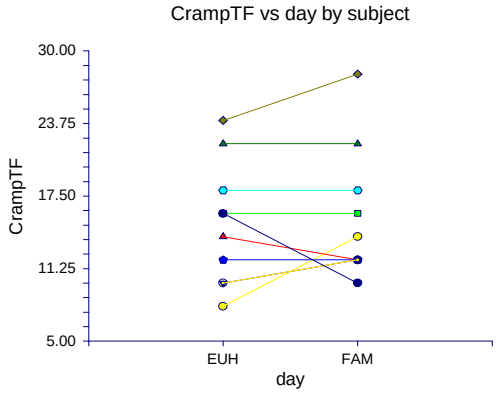
Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time	3 5/4/2012 4:29:27 PM
Database	C:\Documents and Settings\Ke ... r reliability calculation.S0
Response	CrampTF



Tukey-Kramer Multiple-Comparison Test

Response: CrampTF
Term B: day

Alpha=0.050 Error Term=AB DF=9 MSE=5.355556 Critical Value=3.1992

Group	Count	Mean	Different From Groups
EUH	10	15	
FAM	10	15.6	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Table B 4. Statistical Analysis (Continued).

Does serious or significant hypohydration affect cramp threshold frequency?

Repeated Measures ANOVA Report

Page/Date/Time	1 5/4/2012 4:30:13 PM
Database	C:\Documents and Settings\Ke ... r reliability calculation.S0
Response	MVIC_EMG

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: subject	9	No	S(AB)	S+bsA
B: day	1	Yes	AB	S+sAB+asB
AB	9	No	S(AB)	S+sAB
S(AB)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: subject	9	0.1905791	2.117546E-02			
B: day	1	2.50632E-04	2.50632E-04	0.05	0.835251	0.054247
AB	9	4.921509E-02	5.468343E-03			
S	0					
Total (Adjusted)	19	0.2400448				
Total	20					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	9					
B: day	1	0.05	0.835251	0.835251	0.835251	0.835251
AB	9					
S	0					

Table B 4. Statistical Analysis (Continued).

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Lower Bound Epsilon Power (Alpha=0.05)	Geisser Greenhouse Epsilon Power (Alpha=0.05)	Huynh Feldt Epsilon Power (Alpha=0.05)
A: subject	9					
B: day	1	0.05	0.054247	0.054247	0.054247	0.054247
AB	9					
S	0					

Repeated Measures ANOVA Report

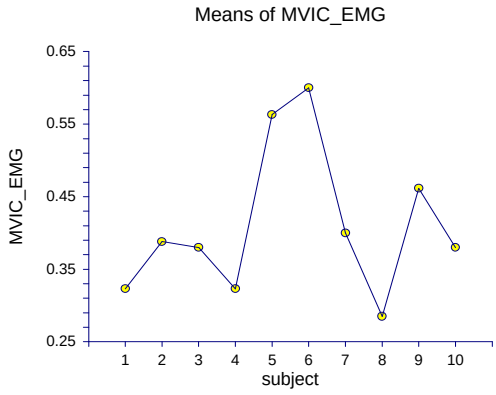
Page/Date/Time	2 5/4/2012 4:30:13 PM
Database	C:\Documents and Settings\Ke ... r reliability calculation.S0
Response	MVIC_EMG

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
AB	1.000000	1.000000	1.000000	1.000000	0.0	0.0	1.000000	Okay

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix
has compound symmetry.

Plots Section



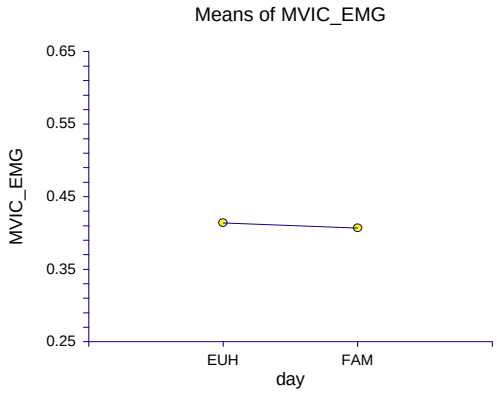
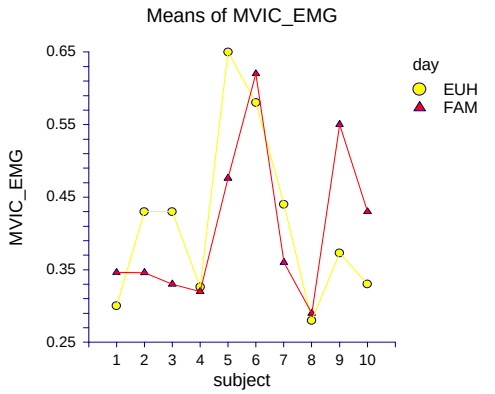
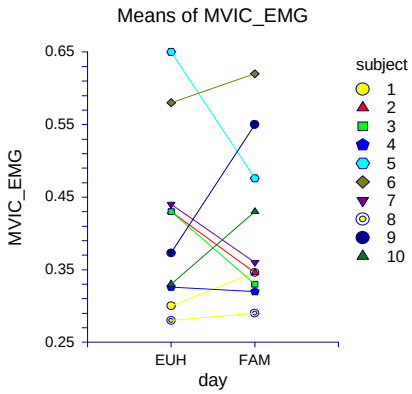


Table B 4. Statistical Analysis (Continued).





Repeated Measures ANOVA Report

Page/Date/Time	3 5/4/2012 4:30:13 PM
Database	C:\Documents and Settings\Ke ... r reliability calculation.S0
Response	MVIC_EMG

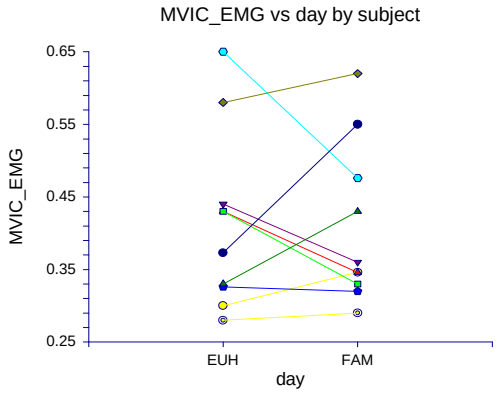


Table B 4. Statistical Analysis (Continued).

Tukey-Kramer Multiple-Comparison Test

Response: MVIC_EMG
Term B: day

Alpha=0.050 Error Term=AB DF=9 MSE=5.468343E-03 Critical Value=3.1992

Group	Count	Mean	Different From Groups
FAM	10	0.40682	
EUH	10	0.4139	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Figure B 1. Example of Toe and Ankle Harness for MVIC Procedures.

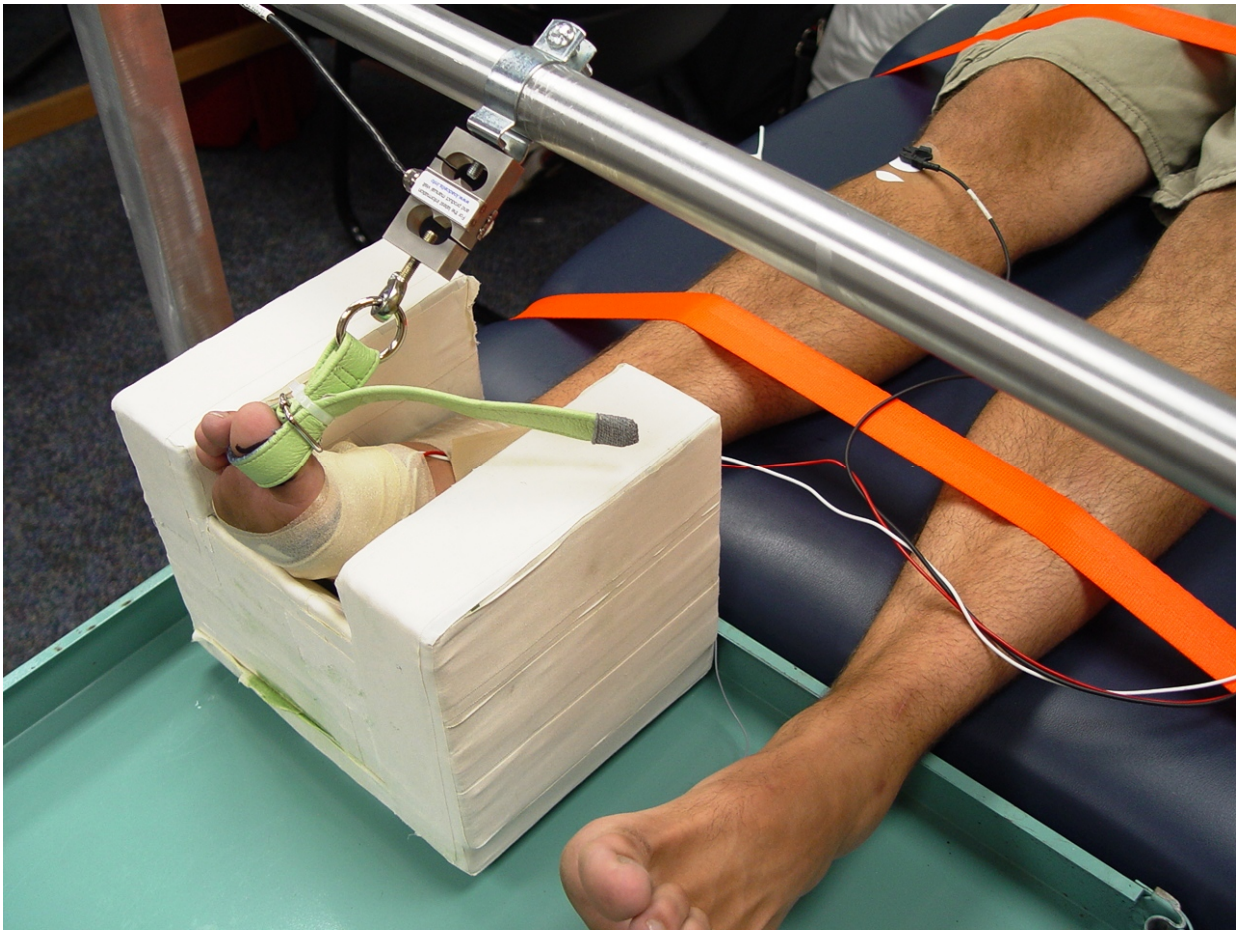


Figure B 2. Visual Analog Scale of Domiant Leg and Whole Body Fatigue.

Visual Analog Scale of dominant leg fatigue: (0-none; 10-severely fatigued)

0 1 2 3 4 5 6 7 8 9 10

Visual Analog Scale of whole body fatigue: (0-none; 10-severely fatigued)

0 1 2 3 4 5 6 7 8 9 10

Figure B 3. Institutional Review Board Approval Letter.

NDSU NORTH DAKOTA STATE UNIVERSITY

Institutional Review Board
Office of the Vice President for Research, Creative Activities and Technology Transfer
NDSU Dept. 4000
1735 NDSU Research Park Drive
Research 1, P.O. Box 6050
Fargo, ND 58108-6050

701.231.8995
Fax 701.231.8098
Federalwide Assurance #FWA00002439

January 10, 2012

Kevin C. Miller
Department of Health, Nutrition and Exercise Science
9A Bentson Bunker Fieldhouse

IRB Approval of Protocol **#HE12090, "The effect of 5% hypohydration on muscle cramp threshold frequency"**
Co-investigator(s) and research team: **Kyle Braulick**

Approval period: 1/10/2012 to 1/09/2013 **Continuing Review Report Due: 12/1/2012**

Research site(s): **NDSU** Funding agency: **n/a**
Review Type: ☐ Expedited category # ☒ Full Board
IRB approval is based on original submission, with revised: protocol and consent form (received 1/09/12).

Additional approval is required:

- prior to implementation of any proposed changes to the protocol (*Protocol Amendment Request Form*).
- for continuation of the project beyond the approval period (*Continuing Review/Completion Report Form*). A reminder is typically sent two months prior to the expiration date; timely submission of the report is your responsibility. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved prior to the expiration date.

A report is required for:

- any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence (*Report of Unanticipated Problem or Serious Adverse Event Form*).
- any significant new findings that may affect risks to participants.
- closure of the project (*Continuing Review/Completion Report Form*).

Research records are subject to random or directed audits at any time to verify compliance with IRB regulations and NDSU policies.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

Sincerely,

Teryl Grosz, MS, CIP
Manager, Human Research Protection Program

Last printed 1/10/2012 11:23:00 AM

NDSU is an EO/AA university.

Figure B 4. Institutional Review Board Consent to be a Research Subject.

NDSU North Dakota State University

Dept. of Health, Nutrition, and Exercise Sciences

PO Box 6050

Fargo, ND 58108-6050

701-231-5686

Title of Research Study: The effects of 5% hypohydration on muscle cramp threshold frequency.

This study is being conducted by: Kevin C. Miller, PhD, ATC, LAT, Kyle Braulick, ATC, Jay Albrecht, PhD, ATC, LAT, Jared Tucker, PhD, James Deal, PhD.

Why am I being asked to take part in this research study? You are being asked to volunteer in this study because you: (1) are a healthy, college-aged male age 18-30, (2) Have a prior history of muscle cramps in lower extremity in previous 12 months, (3) have no history of cardiovascular, neurological, or blood borne diseases, (4) have no food allergies, (5) have no history of heat related illnesses such as heat fainting, heat exhaustion, or heat stroke, (6) Are injury free in the lower extremity for 6 months, (7) are physically active ($\geq$ 30 minutes of activity on $\geq$ 3 days of the week, and (8) have no history of any cardiac events or cardiac incidents in your family. If you are known to be sensitive to exercise in the heat, you should not take part in this study.

What is the reason for doing the study? The purpose of this study is to determine moderate dehydration changes on cramp risk. This study may help people who suffer from muscle cramps.

What will I be asked to do? You will come to a laboratory (Room 14 Bentson Bunker Fieldhouse) on 2 days separated by at least 48 hours. We ask that you drink water prior to coming in each day, maintain a consistent diet, not eat for 6 hours prior to participating, and avoid exercising for 24 hours prior to each testing session. On the first day of testing (i.e., familiarization day), you will provide written consent by signing your name at the end of this form. We will then ask you questions about your health history (e.g., do you have a history of heat illness?, etc.) as well as questions to determine if you have followed the instructions we provided you prior to coming in for testing (e.g., drink water, fasted for 6 hours, etc.).

Figure B 4. Institutional Review Board Consent to be a Research Subject (Continued).

IF INSTRUCTIONS HAVE NOT BEEN FOLLOWED, THE TESTING DAY WILL BE RESCHEDULED AND YOU WILL BE EXCUSED.

You will then give us a urine sample. If you are not hydrated, you will be given a moderate amount of water to drink and provide another urine sample 30 minutes later. If you are hydrated, you will not be given this extra water to drink. You will then kick an imaginary ball so we can determine which leg is your dominant leg. The leg used to "kick the ball" will be considered your dominant leg. This leg will be prepped for the cramp protocol. This preparation may involve us shaving the hair from a small portion of your knee, gently scrubbing your foot with light sand paper to remove dead skin, and cleaning your foot with alcohol. Small electrodes will be put under your big toe, under your knee cap, and over your calf muscle. Your big toe will be placed into a toe harness. Nylon strips will be tightened over your legs to prevent movement of your hips and knees and your ankle will be placed in a foam block.

You will perform 20 practice maximal contractions with a small muscle in your big toe. You will have 1 minute of rest between contractions. After the practice contractions, you will rest for 5 minutes and then perform three more contractions. These last three contractions will be recorded. If you perform one of these contractions incorrectly, you will rest for 1 minute and try again. Once all the contractions are done, your big toe and ankle will be removed from toe harness and foam block, respectively, and you will be prepped for cramp induction.

A small electrode will be placed on the inside of your ankle. A large sponge electrode will be placed on the outside of your ankle. Your nerve will then be electrically stimulated to see if we can get the big toe to contract. This process is generally painless. Upon proper placement, we will secure the electrodes to your ankle with medical tape and an elastic wrap.

We will then attempt to cramp your big toe muscle. You must relax as much as possible at this time. You will receive 2 seconds of electrical stimulation at a low frequency (4 Hz). This means your toe will be stimulated four times each second. Since the stimulation lasts 2 seconds, you will receive 8 shocks on the first cramp attempt. If you do not cramp, you will rest for 1 minute and stimulation frequency will be increased by 2 Hz. This process of increasing frequency by 2 Hz will continue until you cramp or until a frequency of 46 Hz is achieved. If we can get your big toe to cramp, we will verify cramping for 5 seconds and then stretch your toe until the cramp is gone. We will then remove the electrodes and mark the sites with a permanent marker. You will then be asked to come back to the lab two days later for the testing day.

On the testing day, you will provide a urine sample. If you are not hydrated, we will give you water and check your hydration again after 30 minutes like on the familiarization day. If hydrated, you will not be given this water and simply will lay on your back on a padded table for 30 minutes. Your leg will be prepped like on the familiarization day. However, in addition to prepping your leg, your right forearm will also be prepared for blood sampling.

Figure B 4. Institutional Review Board Consent to be a Research Subject (Continued).

A trained phlebotomist (person experienced in taking blood samples) will clean your arm with alcohol twice to remove any dirt or contaminants from the needle stick site. We will use universal precautions (eg, wear gloves, use alcohol to sterilize your arm) when dealing with your blood. This makes the risk of infection very small. A sterile needle with a catheter (i.e., a flexible hollow tube) over it will then be inserted into a vein in your forearm. After the needle enters your vein, we will slide the catheter over the needle and into the vein. The needle will then be taken out of your arm. By removing the needle you can move your arm more comfortably and do not have to worry about the needle hurting you. We will then collect a 5 mL blood sample.

You will perform 10 practice maximal contractions with your big toe with 1 minute of rest separating each contraction. You will rest for 5 minutes and then perform 3 more maximum contractions. You will rest for 15 minutes and we will attempt to cramp your big toe using the procedures followed on the familiarization day. After cramping, you will be prepped for exercise.

A heart rate monitor will be placed around your chest. You will enter the storage room that has a lockable door for you to insert a rectal thermometer so we can make sure your temperature does not reach dangerous levels (40ºC or 104°F). You will be weighed nude. The laboratory will be locked at all times and signs posted on the outside of the door stating "Testing in progress knock to enter" to ensure your privacy. You will then put on sweatpants and a t-shirt. A small portion of your forearms will be shaved, washed with water, and a sterile sweat patch (gauze) will be put on your skin.

You will then exercise for 1 hour in a heat chamber (small room surrounded by plastic). The heat chamber's temperature will be 100-104°F (38-40°C). The exercise will consist of you performing 15 minutes of upper arm biking. You will then bike for 15 minutes with your non-dominant leg only. This alternating of exercise will continue until you lose 5% of your body mass. After 20 minutes the sweat patches will be removed and you will be given a sweatshirt to wear. This is to get you to sweat more and help decrease the amount of time you spend exercising.

At the end of the 1 hour bout of exercise you will exit the heat chamber, void your bladder, and be weighed nude. If you are not 5% dehydrated, you will put on the sweat pants, t-shirt, and sweatshift and re-enter the heat chamber. This protocol of exercising and measurements will continue every hour until you complete two hours of exercise. From this point on, body weight will be taken every 30 minutes.

Once you have lost 5% of your body weight, you will lay on a padded table for 30 minutes. A 5 mL blood sample will be collected and we will prepare your legs for cramping. Upon cramping, 1) your data will be recorded, 2) you will be relieved of the cramp by having the

big toe stretched, 3) the equipment will be removed and 4) you will be given your compensation for the study and be excused.

The total volume of blood collected for the entire experiment is very small (10 mL or a third of an ounce). We do not anticipate you having any negative consequences in your everyday activities as a result of you donating this volume of blood.

Where is the study going to take place, and how long will it take? You will report to Room 14 in the Bentson Bunker Fieldhouse each day. The familiarization day will last about 1 hr. The testing day will last 6-8 hours depending on how much you sweat.

What are the risks and discomforts?

The main risk is you developing a heat related illness such as heat fainting, or heat exhaustion. This is risk is small because you will only be exercising at a moderate intensity, and we will monitor your body temperature during exercise to make sure it does not exceed safe levels (i.e., >104°F). Regardless, if you have a history of heat related illnesses, you should not participate in this study. A symptoms check will be done every 15 minutes during exercise to help prevent occurrences of heat related illnesses. Possible symptoms you may experience include fatigue, dizziness, faintness, headache, nausea, and increased body temperature.

Should a medical emergency arise, the primary investigator will provide emergency care since he is a certified and licensed athletic trainer. Such care will likely involve removing you from the heat chamber and having you drink cool liquids while ice packs are placed under your arms, legs, and head. Further information on the treatment of heat illnesses can be provided to you by the primary investigator, an expert in the care and prevention of heat illnesses, if you wish.

Another risk that you could develop is an infection at the site where we insert the catheter to take your blood. This risk will be near zero because universal precautions will be taken when handling your blood or touching you. These precautions include: the investigator will wear non-latex gloves at all times, alcohol will be used to disinfect and clean your arm, and you will be taught the signs of an infection (e.g., redness, swelling, increase in body temperature, pussy discharge, pain) and what to do if you suspect an infection has occurred (see a physician immediately).

Finally, the cramp protocol is painful for some people. Our experience is that most people tolerate this protocol well and only experience mild to moderate discomfort or pain (previous work we have done shows that the average pain experienced ranges from 11-13 on a scale of 1-100 where 100 is very painful). We have also shown that the amount of pain you experience with the protocol decreases the more times you do it.

Figure B 4. Institutional Review Board Consent to be a Research Subject (Continued).

What are the benefits to me? You are not likely to gain any benefit from being in this research study. However, if you are a student, you may gain some benefit by seeing how experimental research is performed.

What are the benefits to other people? Many people believe muscle cramps are caused by dehydration and electrolyte deficiency. This research will provide evidence to support or refute this theory.

Do I have to take part in the study? Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

What will it cost me to participate? There is no monetary cost to you. This study will require about 7-9 hours of your time.

Who will see the information that I give? We will keep private all research records that identify you. When we write about the study, we will write about the combined information from all subjects that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private. We will make every effort to prevent anyone who is not on the research team from knowing your information or even that you gave us information. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key.

Can my taking part in the study end early? If you fail to show up to all sessions you may be removed from the study and may not receive your monetary compensation.

Will I receive any compensation for taking part in this study? You will receive $40 for your time upon completion of the study. If you excuse yourself from the study at any time you will be compensated with 5 dollars for every hour you participated.

What happens if I am injured because of this research? If you receive an injury in the course of taking part in the research, you should contact Dr. Margaret Fitzgerald, chair of the department of Health, Nutrition, and Exercise Sciences, at the following phone number (701) 231-5590. Treatment for the injury will be available including first aid, emergency treatment and follow-up care as needed. Payment for this treatment must be provided by you and your third party payer (such as health insurance or Medicare). This does not mean that you are releasing or waiving any legal right you might have against the researcher or NDSU as a result of your participation in this research.

Figure B 4. Institutional Review Board Consent to be a Research Subject (Continued).

What if I have questions?

Before you decide whether to accept this invitation to take part in the research study, please ask any questions that might come to mind now. Later, if you have any questions about the study, you can contact the researcher, Kyle Braulick at (507)-276-6102 or kyle.braulick@my.ndsu.edu.

What are my rights as a research participant?

You have rights as a participant in research. If you have questions about your rights, complaints about this research, or wish to notify someone about any research related injuries you incur as a result of this study, you may talk to the researcher or contact the NDSU Human Research Protection Program by:

- Telephone: 701.231.8908
- Email: ndsu.irb@ndsu.edu
- Mail: NDSU HRPP Office, NDSU Dept. 4000, PO Box 6050, Fargo, ND 58108-6050.

The role of the Human Research Protection Program is to see that your rights are protected in this research; more information about your rights can be found at: www.ndsu.edu/research/irb .

Figure B 4. Institutional Review Board Consent to be a Research Subject (Continued).

Documentation of Informed Consent:

You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You may request a copy of this informed consent if you wish to have one for your records.

Your signature Date

Your printed name

Signature of researcher explaining study Date

Printed name of researcher explaining study

Figure B 5. Institutional Biosafety Committee Approval Letter.

NDSU NORTH DAKOTA STATE UNIVERSITY

701.231.8114
Fax 701.231.8098

Institutional Biosafety Committee
Office of the Vice President for Research, Creative Activities and Technology Transfer
NDSU Dept. 4000
1735 NDSU Research Park Drive
Research 1, P.O. Box 6050
Fargo, ND 58108-6050

March 20, 2012

Dr. Kevin Miller
Dept. of Health, Nutrition & Exercise Science
BBFH

Re: IBC Project **#B12016: "Laboratory research performed in Room 14 BBFH"**

Approval Date: **March 20, 2012**

Co-Investigators and research team: Kevin Miller, Scott Allen, Kyle Braulick, Jarett Peikert, Mike McKenney

The project referenced above has been reviewed and accepted under the categorization of "***human blood and tissue***" by the Institutional Biosafety Committee (IBC). A copy of the *IBC Protocol Form* is being forwarded to you with the committee approval.

No further reporting to the NDSU IBC is required for this project unless there are unexpected events concerning exposure or containment of the agent(s) involved, or you decide to make a change in the project. Although, no further reporting is necessary an annual update will be sent to you to help track and monitor the work over the course of the project. If you decide to make changes, please notify the NDSU IBC before any change is implemented.

Thank you for complying with NDSU IBC procedures, and best wishes for success with your project.

NDSU, Institutional Biosafety Committee

APPENDIX C. ADDITIONAL RESULTS

Table C 1. Subject Demographics.

Subject	Age	Ht(cm)	Pre-Ex Wt (kg)	Post-Ex (kg)	%H	Duration in Heat Chamber (hr)
1	25	182.9	93.20	88.48	5.06	4.5
2	19	188.0	79.37	75.38	5.03	4.0
3	24	188.0	106.59	102.20	4.12	4.0
4	25	180.3	68.97	65.49	5.04	4.8
5	29	182.9	83.27	80.02	4.00	4.5
6	24	177.8	85.62	82.12	4.09	4.0
7	20	193.0	79.15	75.62	4.46	3.5
8	23	180.3	71.45	67.66	5.30	2.5
9	30	188.0	96.13	91.31	5.01	4.0
10	18	180.3	83.83	80.01	4.56	2.5

H=Hypohydration

Table C 2. Subject Familiarization MVIC and Cramp Variables.

Subject	TF(Hz)	MVIC EMG (μV)	Cramp EMG (μV)	Cramp Intensity (% of MVIC)
1	14	0.173	0.138	79.45
2	12	0.173	0.347	200.58
3	16	0.165	0.095	57.50
4	12	0.160	0.138	86.16
5	18	0.238	0.155	65.13
6	28	0.310	0.175	56.45
7	12	0.180	0.215	119.44
8	12	0.145	0.105	72.41
9	10	0.275	0.140	50.91
10	22	0.215	0.230	106.98

TF=Threshold Frequency, MVIC=Maximum Voluntary Isometric Contractions, EMG=Electromyography

Table C 3. Subject Testing Day Euhydrated MVIC and Cramp Variables.

Subject	TF(Hz)	MVIC EMG (μV)	Cramp EMG (μV)	Cramp Intensity (% of MVIC)
1	8	0.150	0.230	153.33
2	14	0.215	0.160	74.42
3	16	0.215	0.126	58.41
4	12	0.163	0.159	97.24
5	18	0.325	0.229	70.31
6	24	0.290	0.145	50.00
7	10	0.220	0.305	138.64
8	10	0.140	0.223	158.93
9	16	0.187	0.147	78.82
10	22	0.165	0.103	62.17

TF=Threshold Frequency, MVIC=Maximum Voluntary Isometric Contraction, EMG=Electromyography

Table C 4. Subject Testing Day Hypohydrated MVIC and Cramp Variables.

Subject	TF(Hz)	MVIC EMG (µV)	Cramp EMG (µV)	Cramp Intensity (% of MVIC)
1	4	0.123	0.340	275.75
2	8	0.170	0.260	152.94
3	10	0.160	0.119	74.37
4	14	0.151	0.077	50.66
5	20	0.235	0.188	80.00
6	18	0.195	0.140	71.79
7	12	0.110	0.195	176.82
8	8	0.150	0.245	163.33
9	12	0.190	0.095	50.00
10	24	0.165	0.105	63.71

TF=Threshold Frequency, MVIC=Maximum Voluntary Isometric Contraction, EMG=Electromyography

Table C 5. Subject Blood Variables.

Subject	E/H	Hct (%)	Hb (abs)	Hb (g/dl)	Plasma Vol. Change	OSMp
1	E	44.0	0.22	15.7	0	286.5
2	E	46.2	0.23	16.8	0	288.0
3	E	40.1	0.21	14.9	0	282.5
4	E	38.3	0.20	14.2	0	290.5
5	E	50.1	0.24	17.2	0	299.0
6	E	47.1	0.23	16.5	0	284.0
7	E	45.0	0.23	16.5	0	288.0
8	E	48.3	0.23	16.8	0	273.5
9	E	40.6	0.20	14.9	0	287.5
10	E	45.8	0.22	16.1	0	289.5
1	H	45.6	0.25	18.0	-15.4	299.5
2	H	47.8	0.25	17.8	-8.6	301.0
3	H	42.7	0.23	16.5	-13.8	297.5
4	H	41.3	0.23	16.4	-17.6	304.0
5	H	51.7	0.26	18.6	-10.3	308.5
6	H	48.5	0.24	17.5	-8.4	298.0
7	H	46.1	0.23	16.7	-3.0	300.0
8	H	52.1	0.27	19.3	-19.3	292.5
9	H	43.5	0.22	16.3	-12.9	307.0
10	H	48.3	0.23	16.9	-9.2	301.5

E=Euhydrated, H=Hypohydrated, Hct=Hematocrit, Hb=Hemoglobin, OSMp=Plasma Osmolality

Table C 6. Subject Plasma Electrolytes.

Subject	E/H	Plasma [Na^+]	Plasma [K^+]	Plasma [Cl^-]
1	E	142.0	4.8	104.5
2	E	144.0	4.7	104.5
3	E	139.5	5.1	104.0
4	E	143.0	4.7	107.5
5	E	147.0	5.7	107.0
6	E	140.0	4.9	103.0
7	E	142.0	4.8	108.0
8	E	135.5	4.1	100.5
9	E	143.0	4.7	105.5
10	E	143.0	5.5	104.5
1	H	148.0	5.3	107.0
2	H	150.5	5.2	108.0
3	H	148.0	5.2	110.0
4	H	151.0	4.9	111.0
5	H	152.0	5.8	112.0
6	H	148.0	4.8	108.0
7	H	149.5	4.4	113.0
8	H	147.0	4.7	106.0
9	H	152.0	4.8	110.0
10	H	149.0	5.0	107.5

E=Euhydrated, H=Hypohydrated, [Na^+]=Sodium, [K^+]=Potassium, [Cl^-]=Chloride

Table C 7. Subject Sweat Data.

Subject	Total Fluid Lost (L)	Sweat Volume (L)	Sweat Rate (L/h)	$[Na^+]_{sw}$ (mmol)	$[K^+]_{sw}$ (mmol)	Total Na^+ Lost (g)	Total K^+ Lost (g)
1	4.72	3.86	0.86	68.62	5.29	6.09	0.80
2	3.99	2.97	0.74	□	□	□	□
3	4.39	2.95	0.74	31.57	4.25	2.14	0.49
4	3.48	2.94	0.62	68.62	4.05	4.64	0.47
5	3.25	2.85	0.63	45.82	4.57	3.01	0.51
6	3.5	3.20	0.80	34.42	3.94	2.53	0.49
7	3.53	2.73	0.77	41.26	5.50	2.59	0.59
8	3.79	2.79	1.12	60.64	5.09	3.89	0.56
9	4.82	4.32	1.08	48.67	4.98	4.84	0.84
10	3.82	3.44	1.38	79.00	3.94	6.25	0.53

$[Na^+]$sw=Sweat Sodium Concentration, $[K^+]_{sw}$=Sweat Potassium Concentration.

□=Error Due to Technical Difficulties

Table C 8. Subject Urine Data.

Subject	Usg Pre-Ex	Usg During	Usg Post-Ex (If too exhausted to continue)	Total (mL)	Weight (kg)
1	1.005	1.009	H	855	0.86
2	1.004	¥	H	1015	1.02
3	1.005	¥		1430	1.44
4	1.003	1.007	H	535	0.54
5	1.008	¥	1.031	397	0.40
6	1.004	1.013	1.031	295	0.30
7	1.003	1.008	1.030	795	0.80
8	1.004	1.003	H	995	1.00
9	1.004	1.010	H	495	0.50
10	1.008	1.008	1.034	375	0.38

Usg=Urine Specific Gravity

=urine specific gravity was not measured and subject did not achieve 5% hypohydration

¥=urine specific gravity was not measured

H=subject reached 5% hypohydration

Table C 9. Subject Dominant Leg and Whole Body Fatigue (Visual Analog Scale [0-10]).

Subject	Familiarization Day Leg Fatigue	Pre-Ex Leg Fatigue	Post-Ex Leg Fatigue	Post-Ex Whole Body Fatigue
1	1	1	1	10
2	1	0	0	10
3	0	0	0	10
4	0	0	0	10
5	0	0	0	9
6	0	0	2	9
7	1	1	1	10
8	0	0	1	3
9	1	1	1	5
10	1	1	1	10

Table C 10. Environmental Heat Chamber Temperature and Relative Humidity.

Subject	Temp Start (°C)	Temp End (°C)	R.Humidity Start (%)	R. Humidity End (%)
1	38	38	16	16
2	36	39	16	20
3	40	38	16	23
4	36	38	20	20
5	37	41	20	25
6	38	38	26	22
7	42	39	16	16
8	39	40	16	16
9	40	41	16	16
10	41	42	16	16

APPENDIX D. RECOMMENDATIONS FOR FUTURE RESEARCH

Table D 1. Recommendations for Future Research.

1. Determine if hypohydration without central or peripheral fatigue will decrease cramp threshold frequency.
2. Determine if fatigue, while remaining euhydrated affects cramp threshold frequency.
3. Determine the effect of multiple nerve blocks on cramp threshold frequency.