PLASMA AND ELECTROLYTE CHANGES IN HUMANS FOLLOWING INGESTION OF MULTIPLE BOLUSES OF PICKLE JUICE ASSOCIATED WITH EXERCISE

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

By

Michael Anthony McKenney

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

Major Program:
Advanced Athletic Training

March 2013

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Plasma and electrolyte changes in humans following ingestion of multiple

boluses of pickle juice associated with exercise

By

Michael Anthony McKenney

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

James E. Deal, PhD

Julie A. Garden-Robinson, PhD, LRD

Yeong S. Rhee, PhD, RD

Approved by:

3-28-2013
Date

Margaret Fitzgerald
Department Chair

ABSTRACT

No experimental research has examined the effect of drinking multiple boluses of pickle juice (PJ) on the same day, nor has its ingestion been examined during exercise. Additionally there are fears that PJ supplementation can cause hyperkalemia. We determined the effect of ingesting single or multiple boluses of PJ on plasma variables before, during, and after exercise. On three days, subjects ingested 0, 1, or 2 boluses of PJ and biked vigorously for 60 minutes. Blood samples were collected pre-ingestion and 30, 65, 95, and 125 minutes post ingestion. The number of PJ boluses consumed did not affect $[Na^+]_p$, $[K^+]_p$, OSM_p, or changes in plasma volume over time. Ingesting up to 2 boluses of PJ and resuming exercise causes negligible changes in blood variables and will not increase $[Na^+]_p$ or cause hyperkalemia.

ACKNOWLEDGMENTS

Thank you to my advisor and mentor, Dr. Kevin Miller. I could not have completed this thesis without his guidance and instruction. Also, all of this would not be possible if it were not for his willingness to teach me new skills and to challenge me on a regular basis.

Thank you to the rest of my graduate committee, Drs. Deal, Garden-Robinson, and Rhee. Your insight, knowledge, and support made this possible. Additionally, I would like to thank Dr. Tucker for his help in designing my study.

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

DEDICATION

I dedicate this thesis to my loving wife and family who have supported me throughout this adventure.

I also want to dedicate this to my cat, Jeep. Meow.

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Introduction

Sodium (Na^+) is the primary electrolyte in sweat; normal sweat Na^+ concentrations can range from 0.5 to 2.3 $g \cdot L^{-1}$ (20 to 100 $mmol \cdot L^{-1}$).[1] Sodium losses range widely; some authors[2,3] have observed athletes lose 2.5 to 30 g of Na^+ in a single day of training. Large Na^+ losses can put athletes at risk of developing hyponatremia, an injury marked by a plasma Na^+ concentration ($[Na^+]_p$) less than 135 $mmol \cdot L^{-1}$. Moreover, Na^+ losses are thought to increase the risk of developing exercise-associated muscle cramps (EAMC).[4-7]

Several authors[5-8] have made Na^+ replacement recommendations for athletes. The National Athletic Trainers Association[6] recommends adding 0.3 to 0.7 g of Na^+ to every liter of rehydration drink to offset Na^+ losses due to sweating. The American College of Sports Medicine[8] recommends adding 1.2 g to 2.5 g of Na^+ to every liter of sports drink to treat EAMC. Bergeron[9] reported success treating EAMC by adding up to 6 $g \cdot L^{-1}$ of Na^+ to a sports drink. Other clinicians have experimented with different methods of replacing Na^+ including drinking chicken noodle soup[10,11] or pickle juice.[12,13]

Twenty five percent (92 of 370) of athletic trainers polled use pickle juice to treat EAMC.[13] However, some authors[12,14] caution against pickle juice ingestion. Fowkes-Godek et

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[*] The material in this chapter was co-authored by Michael McKenney, Kevin C. Miller, PhD, ATC, LAT, Jim E. Deal, PhD, Julie A. Garden-Robinson, PhD, LRD, and Yeong S. Rhee, PhD, RD. Michael McKenney had primary responsibility for collecting samples in the field and for interviewing users of the test system. Michael McKenney was the primary developer of the conclusions that are advanced here. Michael McKenney also drafted and revised all versions of this chapter. Kevin C. Miller, PhD, ATC, LAT, Jim E. Deal, PhD, Julie A. Garden-Robinson, PhD, LRD, and Yeong S. Rhee, PhD, RD served as proofreader and checked the math in the statistical analysis conducted by Michael McKenney.

al.[12] observed mild hyperkalemia, a plasma potassium concentration ($[K^+]_p$) greater than 5 $mmol \cdot L^{-1}$, when American football players supplemented their meals with pickle juice. Hyperkalemia is a concern because it is associated with cardiac abnormalities[15] and may contribute to the onset of fatigue.[16] Another possible concern is that drinking pickle juice may increase plasma osmolality (OSM_p) and $[Na^+]_p$ thereby rapidly expanding plasma volume, decreasing thirst, and impairing rehydration.[14] However, others observed no significant changes in plasma volume, OSM_p, or plasma electrolyte concentrations when euhydrated[17] or mildly hypohydrated[18-20] individuals ingested small volumes (~80 mL) of pickle juice. Furthermore, drinking pickle juice did not alter perceived thirst or the volume of water ingested *ad libitum* post-exercise.[19]

Prior examinations[17-21] of pickle juice's effects on the extracellular fluid space had three limitations. First, they[17-21] only provided a single bolus of pickle juice at one time, either pre-exercise[17,21] or post-exercise.[18-20] Anecdotally, some athletic trainers give athletes pickle juice multiple times over the course of an exercise session to treat or prevent EAMC (e.g. before a game and/or at halftime). Second, subjects did not exercise post-ingestion of pickle juice.[17-19,21] No study has examined the extracellular fluid space when subjects drink pickle juice and resume exercise. Since aldosterone increases during exercise, consuming a salty drink and resuming exercise may cause an increase in $[Na^+]_p$ or OSM_p. Finally, the effects of drinking pickle juice on the extracellular fluid space have not been measured after 60 minutes post-ingestion.

Therefore, the purpose of our study was twofold: (1) To investigate the effects of ingesting single and multiple boluses of pickle juice on $[Na^+]_p$, $[K^+]_p$, changes in plasma volume, and OSM_p up to 125 minutes post-ingestion, and (2) to determine what happens to these variables when subjects ingest pickle juice and resume exercise. Based on prior research,[17-21] we hypothesized drinking multiple small boluses of pickle juice would not significantly increase $[Na^+]_p$, $[K^+]_p$, OSM_p, or changes in plasma volume, nor would these variables be altered significantly with exercise.

Methods

Experimental Design

A crossover, 3 x 5 factorial with repeated measures on time design guided data collection. The independent variables were number of 1 $mL \cdot kg^{-1}$ body mass pickle juice boluses ingested (0, 1, or 2) and time (-5, 30, 65, 95, and 125 minutes post-ingestion). The dependent variables were $[Na^+]_p$ ($mmol \cdot L^{-1}$), changes in plasma volume (% change from pre-ingestion), OSM_p ($mOsmol \cdot kg^{-1}$ H_2O), and $[K^+]_p$ ($mmol \cdot L^{-1}$). Sodium and K^+ content changes were estimated using hematocrit, hemoglobin, and plasma electrolyte data.[22] Urine specific gravity was measured to ensure subjects began testing euhydrated (euhydrated = urine specific gravity < 1.02).[23]

Subjects

A convenience sample of 12 non-heat acclimated subjects volunteered for this study. Three volunteers discontinued testing on the first day due to difficulties associated with venipuncture (e.g. venous collapse and syncope). Nine physically active (20-60 minutes of vigorous activity on 3 or more days a week)[24] males with no self-reported history of heat illness (e.g. heat stroke, heat exhaustion, or heat syncope) completed testing (Table 1). Exclusion criteria included: self-reported blood or plasma donation 8 weeks prior to data collection, diabetes, anemia, food allergy to pickles, musculoskeletal, cardiovascular, blood borne, or neurological diseases, or history of lower extremity injury within the 12 months preceding data collection. All volunteers provided written informed consent prior to data collection. All procedures were approved by North Dakota State University's institutional review board.

Procedures

Subjects reported for testing, after fasting for 12 hours, at approximately the same time of day on three days. All subjects were instructed to refrain from strenuous activity for 48 hours prior to testing, to maintain a similar diet throughout the course of experimentation, and avoid caffeine and alcohol for 24 hours prior to testing.

Subjects reported to a laboratory, voided their bladders completely, and had their urine specific gravity measured with a refractometer (SUR-Ne; Atago USA Inc., Bellevue, WA). If hypohydrated (specific gravity > 1.02),[23] subjects were excused and rescheduled for another testing session at least 24 hours later. If euhydrated, they inserted a rectal thermistor (#401 probe, YSI; Advanced Instruments Inc., Norwood, MA) at least 10 cm past the anal sphincter and put on a heart rate monitor (Polar Electric Inc., Lake Success, NY). One forearm's antecubital region was cleaned with isopropyl alcohol and a sterile, 20-gauge venous catheter was inserted into a superficial vein. Subjects were weighed (body weight; BW_1) nude to the nearest tenth of a kilogram (DA-150, Denver Instrument, Bohemia, NY) and sat for 30 minutes to ensure equilibration of fluid compartments.[25] Body weight measurement one (BW_1) was used to calculate the ingested pickle juice volume ($1\ mL \cdot kg^{-1}$ BW, Table 1).

After the 30-minute rest period, a 5-mL blood sample was collected (-5 minutes). Subjects were weighed nude (BW_2), put on a sweat suit (hooded sweatshirt and sweat pants) and had 60 seconds to ingest either 0 or 1 bolus of chilled (~6ºC) pickle juice (Gedney Dill Pickles, M.A. Gedney Co, Chaska, MN). On the 0 bolus days, subjects rested for 60 seconds at all ingestion periods. Following pickle juice ingestion, subjects biked on a semi-recumbent cycle ergometer (846: Precor, Woodinville, MA) at 80% to 85% of their age-predicted maximum heart rate for 30 minutes in the heat (Table 1). After 30 minutes of exercise, a 5-mL blood sample was collected. On the 2 bolus days, subjects consumed another 1 $mL \cdot kg^{-1}$ body weight of chilled pickle juice. For the 0 or 1 bolus days, subjects rested for 60 seconds during this period. They resumed biking for another 30 minutes followed by a 5-minute cool down at a self-selected lower intensity.

A third, 5-mL blood sample was collected immediately after cool-down. Following the blood sample, subjects stood and exited the heat chamber, towel dried, removed their sweat suit, were weighed nude (BW_3), and voided their bladders completely. They were weighed nude again (BW_4) and removed the heart rate monitor and rectal thermistor. Subjects sat in a climate-controlled room (21°C) for an additional 60 minutes, during which blood samples were collected at 95 minutes post-ingestion (30 minutes post-exercise) and 125 minutes post-ingestion (60 minutes post-exercise).

Subjects were asked to report for their other testing days at least 48 hours later. Trials only differed by the number of boluses ingested (0, 1, or 2). The order of the number of boluses ingested was randomized and counterbalanced *a priori* using half of the possible order combinations.

Blood and Plasma Analysis

Whole blood was used to determine hematocrit and hemoglobin concentration. For hematocrit, blood was drawn into heparinized microcapillary tubes, centrifuged at 3000 rpm for 5 minutes, and read using a microcapillary reader (IEC 2201; Damon/IEC, Needham Heights, MA). Hemoglobin concentration was estimated using the cyanomethemoglobin technique.[17,21] Hematocrit and hemoglobin concentration were measured in triplicate immediately following sampling and averaged for statistical calculations. Changes in plasma volume were estimated by inserting hematocrit and hemoglobin data into the Dill and Costill[26] equation. Changes in K^+ and Na^+ content were estimated using Greenleaf et al.[22] equations.

The remaining whole blood was centrifuged at 3000 rpm for 15 minutes at 3ºC. Plasma was removed from the packed red cells, and plasma electrolyte concentrations were analyzed using an ion-selective electrode system (16; NOVA Biomedical, Waltham, MA). Plasma osmolality was determined by freezing-point depression osmometry (3D3; Advanced Instruments Inc., Norwood, MA). Plasma electrolyte concentrations and OSM_p were measured in duplicate and averaged for statistical analysis.

Statistical Analysis

Separate 3 x 5 repeated measures ANOVAs were used to determine the effects of ingesting multiple boluses of pickle juice on plasma variables over time. Shapiro-Wilk tests were used to assess normality. Mauchly's test was used to confirm sphericity. When sphericity was violated, Greenhouse-Geisser correction to degrees of freedom and *P*-values were applied. Tukey-Kramer multiple comparison tests were used to determine differences within each independent variable at each time-point. Due to the number of ANOVAs performed, a Bonferroni correction to alpha level was applied *a priori*. Significance was accepted when $P < 0.01$ (NCSS 2007, ver: 07.1.18, Kaysville, UT).

Results

Subjects reported compliance with all pre-testing instructions before each testing session. Subjects began testing similarly euhydrated, lost similar volumes of sweat, and were similarly hypohydrated post-exercise (Table 1). Subjects consumed 0, 1, or 2 boluses of pickle juice resulting in various quantities of Na^+ and K^+ ingested (Table 1). The composition of pickle juice can be found in Table 2.

We observed no interaction between the number of boluses ingested and time for $[Na^+]_p$ ($F_{8,64} = 2.2$, $P = 0.04$). Similarly, no differences in $[Na^+]_p$ were observed between the number of boluses ($F_{2,16} = 4.2$, $P = 0.04$). However, $[Na^+]_p$ did change over time ($F_{2,15} = 43.2$, $P < 0.01$, Figure 1). Pre-exercise $[Na^+]_p$ (-5 minutes) was less than all other times ($P < 0.01$).

We observed an interaction between number boluses consumed and time for plasma Na^+ content changes ($F_{8,64} = 3.2$, $P = 0.004$). Within 0-bolus and 1-bolus conditions, -5 minute Na^+ content was higher than 30 and 65 minutes ($P < 0.01$). Within 2-bolus, -5 minutes was greater than 30 minutes ($P < 0.01$). Within 0-bolus and 1-bolus conditions, 30 minutes was less than 95 minutes ($P < 0.01$). Within 2-bolus, 30 minutes was less than 65, 95, and 125 minutes ($P < 0.01$). Within 1-bolus and 2-bolus, 65 minutes was less than 95 minutes ($P < 0.01$). Between conditions, 0-bolus 95 minutes was less than 2-bolus 95 minutes ($P < 0.01$). Between conditions, 0-bolus 125 minutes was less than 2-bolus 125 minutes ($P < 0.01$).

We observed no interaction between number of boluses ingested and time ($F_{5,25} = 0.7$, $P = 0.54$) or a main effect of bolus for $[K^+]_p$ ($F_{2,16} = 1.8$, $P = 0.21$). However, $[K^+]_p$ changed over time ($F_{4,32} = 0.4$, $P < 0.01$, Figure 2). At -5 minutes, $[K^+]_p$ was lower than all other times ($P < 0.01$). At 30 minutes post-ingestion, $[K^+]_p$ was greater than all other times ($P < 0.01$). Estimated changes in K^+ content did not differ between bolus and time ($F_{8,64} = 2.4$, $P = 0.03$), bolus only ($F_{2,16}= 0.8$, $P = 0.47$), or time only ($F_{4,32} = 3.4$, $P = 0.02$).

We observed no significant interaction between number of boluses ingested and time ($F_{8,64} = 1.2$, $P = 0.32$), or main effect of bolus for changes in plasma volume ($F_{2,16} = 0.02$, $P = 0.98$). However, changes in plasma volume occurred over time ($F_{4,32} = 61.4$, $P < 0.01$, Figures 1

& 2). The -5 minute sample was greater than all other time points (*P*< 0.01). Changes in plasma volume were lower at 30 and 65 minutes than at -5 and 95 minutes; 95 minutes was greater than 125 minutes ($P < 0.01$).

We observed no interaction between bolus and time ($F_{8,64} = 2.1$, $P = 0.05$) or main effect of bolus ($F_{2,16} = 2.5$, $P = 0.12$) for OSM_p. Plasma osmolality did change over time ($F_{4,32} = 61.4$, $P < 0.01$, Figure 3). Plasma osmolality at -5 minutes was less than all other times ($P < 0.01$).

Discussion

Previous authors[14] have cautioned against ingesting pickle juice because they fear it will increase OSM_p and $[Na^+]_p$, rapidly expand plasma volume, decrease thirst, and impair rehydration. We observed no significant changes in $[Na^+]_p$, OSM_p, or changes in plasma volume after 0, 1, or 2 boluses of pickle juice were ingested and subjects exercised. These results are consistent with, and extend, the results of other scientists[17-21] who provided pickle juice before or after exercise. In studies examining hypohydrated subjects,[18-20] drinking 1 $mL \cdot kg^{-1}$ body weight of pickle juice did not significantly alter $[Na^+]_p$, OSM_p, or changes in plasma volume up to an hour post-exercise. While we observed a decrease in change in plasma volume, drinking pickle juice did not exacerbate plasma shifts as there were no differences between conditions over time. Thus, the initial decrease in plasma volume is likely due to exercise-induced shifts in fluid between compartments. The gradual increase in plasma volume in all conditions occurring between 30-minutes and 95-minutes post-ingestion is likely due to OSM_p increasing causing water to shift into the extracellular fluid space. The decrease in plasma volume change occurring in all conditions between 95-minutes and 125-minutes post-ingestion, is likely due to urine production.[17]

Unlike $[Na^+]_p$ and OSM_p, there was a change in plasma Na^+ content when different amounts of pickle juice were ingested. Our subjects lost 1.1 L of sweat during exercise. Assuming an average sweat Na^+ concentration of 50 $mmol \cdot L^{-1}$,[1,7] our subjects would have lost 55 mmol of Na^+ (1.3 g). The decrease in plasma Na^+ content observed in the first 65-minutes post-ingestion is likely due to Na^+ loss via exercise-induced sweating. We observed a significant increase in plasma Na^+ content change from 30 to 65-minutes in the 2-bolus condition that was not observed in the 0 or 1 bolus conditions. The delayed increase in plasma Na^+ content change in the 0 and 1 bolus conditions was likely due to a smaller volume of fluid being in the stomach. Gastric emptying is delayed by low stomach volumes,[27,28] vigorous exercise,[29,30] high osmolality,[31] and low pH[32] of ingested beverages. In our study, the gastric emptying rates were likely low for all conditions because of the small volumes ingested and vigorous exercise. Miller et al.[21] observed a gastric emptying rate of approximately 2 mL per minute when rested, euhydrated subjects consumed a single ~150 mL bolus of pickle juice. In our study, plasma Na^+ content only increased in the 2 bolus condition from 30 to 65 minutes. Thus, the second bolus consumed could have increased gastric distension more than the 1 bolus condition thereby accounting for the earlier increase in plasma Na^+ content change. This hypothesis is supported by the observation that it took 90 minutes to return plasma Na^+ content to baseline in the 1 bolus condition. Therefore, when subjects drink 1 bolus of pickle juice and begin exercising, it will take 90 minutes to return plasma Na^+ content to baseline levels. When 2 boluses are ingested, plasma Na^+ content will return to baseline between 65 and 95 minutes. Therefore, if cramping is due to Na^+ loss[5,7] and athletes intend to replace Na^+ by drinking pickle juice,[13] they have to consume more than 162 mL of pickle juice. However, the prolonged delay of the increase in Na^+ content suggests drinking pickle juice to treat an acute cramp would be an ineffective strategy.

Another concern of ingesting pickle juice during exercise is the possible development of hyperkalemia[12] presumably because of the K^+ content in the juice. In preliminary work, Fowkes-Godek et al.[12] observed an increase in $[K^+]_p$ when American football players supplemented their diet with pickle juice over 9 consecutive days. Prior to supplementation, $[K^+]_p$ was 4.7 ± 0.3 $mmol \cdot L^{-1}$. Plasma K^+ concentration was significantly higher after 5 days of pickle juice supplementation (5.2 ± 0.1 $mmol \cdot L^{-1}$). Interestingly, after 9 days of supplementation, $[K^+]_p$ decreased to 4.9 ± 0.3 $mmol \cdot L^{-1}$ (author correspondence March 2013). Hyperkalemia is associated with cardiac abnormalities, but $[K^+]_p$ must be elevated to 6 to 7 $mmol \cdot L^{-1}$ for abnormalities to occur.[15] We observed a $[K^+]_p$ increase in our study that was not exacerbated by drinking any volume of pickle juice. Thus, the increase in $[K^+]_p$ is likely the result of K^+ being released into the blood stream from the exercising muscles.[33,34] Zarvosky et al.[33] observed subjects $[K^+]_p$ increased from ~4 $mmol \cdot L^{-1}$ to 5.3 $mmol \cdot L^{-1}$ after 5 minutes of vigorous cycling but returned to baseline values after 5 minutes of rest. Therefore, causing hyperkalemia is not a concern if athletes drink multiple boluses of pickle juice during exercise on one day. The lack of a control group in Fowkes-Godek et al.[12] prevents us from determining the effect of drinking pickle juice on $[K^+]_p$ over 9 consecutive days. The hyperkalemia observed[12] may be due to exercise-induced muscle damage as a result of pre-season conditioning drills. Additional research is needed to determine the effects of pickle juice supplementation on $[K^+]_p$ over consecutive days of training.

We acknowledge the limited external validity of our study. However, we tried to emulate certain conditions athletes might experience if they participate in competitive athletics (e.g. a break during exercise and resumption of activity). Furthermore, we emulated dosage and timing of pickle juice ingestion being used by athletic trainers.[13] Athletes will normally have longer breaks, consume additional foods and/or fluids in addition to pickle juice, have varying degrees of hydration, or exercise at varying intensities. Given these aspects would have confounded our results, we chose to control them in order to answer our research questions.

Conclusion

When subjects ingest multiple, small boluses of pickle juice there are no significant changes in $[Na^+]_p$, $[K^+]_p$, or OSM_p up to 125-minutes post-ingestion. Furthermore, the addition of exercise did not significantly alter plasma variables. However, ingesting 2 boluses will return plasma Na^+ content to normal 30 minutes faster than when 1 bolus is ingested. Additionally, hyperkalemia is not a concern when 1 or 2 boluses of pickle juice are ingested with a single exercise session. However, clinicians should continue to exercise caution when athletes ingest pickle juice over consecutive days until controlled experimental studies can address this concern.

Acknowledgments

We want to thank NDSU’s College of Human Development and EducationNDSU”s Department of Health, Nutrition, and Exercise Sciences, and NDSU’s Athletic Training Education Program for partially funding this research. We would also like to thank Dr. Jared Tucker for his contributions to the experimental design of this study.

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Table 1. Subject demographics and descriptive data

		0 Bolus	1 Bolus	2 Bolus
Age (y)	23 ± 4			
Height (cm)	180.9 ± 5.8			
BW_1 (kg)		80.7 ± 13.8	80.6 ± 13.3	80.6 ± 13.6
BW_2 (kg)		80.7 ± 13.8	80.6 ± 13.3	80.6 ± 13.6
BW_3 (kg)		79.5 ± 13.9	79.5 ± 13.5	79.5 ± 13.8
BW_4 (kg)		79.1 ± 13.9	79.1 ± 13.4	79.1 ± 13.6
Sweat Volume (L)[a]		1.2 ± 0.2	1.1 ± 0.3	1.1 ± 0.3
% Hypohydration[b]		2.1 ± 0.5	2.0 ± 0.6	1.9 ± 0.6
Pre-Exercise U_{sg}		1.01 ± 0.004	1.009 ± 0.005	1.009 ± 0.006
PJ Volume Ingested (mL)		0	81 ± 13	162 ± 27
Na^+ Content Ingested (g)		0	0.99 ± 0.16	1.97 ± 27.2
K^+ Content Ingested (g)		0	0.1 ± 0.02	0.2 ± 0.03
Heat Chamber Temp (°C)		37 ± 1	36 ± 2	37 ± 1
Heat Chamber rH (%)		18 ± 2	18 ± 2	17 ± 2

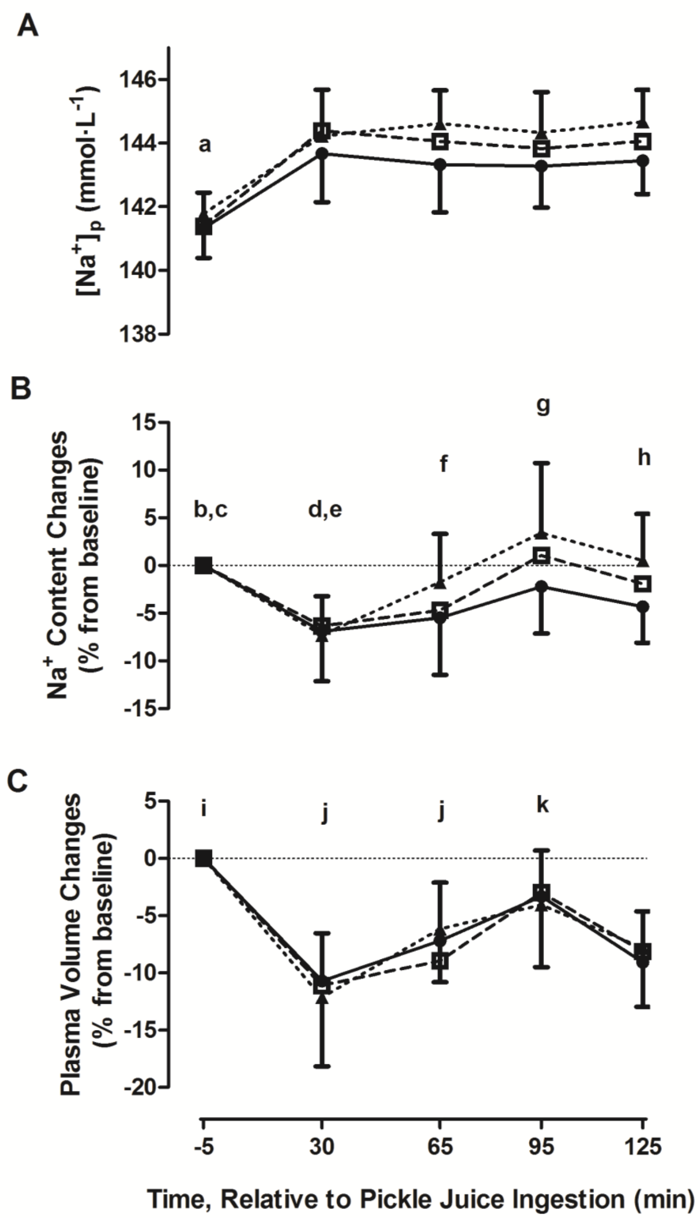
BW = body weight, U_{sg} = Urine specific gravity, PJ = pickle juice, Na^+ = sodium, K^+ = potassium, Temp = temperature, rH = relative humidity. [a] = Calculated by subtracting BW_3 from BW_2. [b] = Calculated by subtracting BW_4 from BW_2, dividing by BW_2, and multiplying by 100. Data are reported as means ± SD (n = 9).

Table 2. Pickle juice composition

OSM (mOsmol·kg^{-1} H_2O)	915 ± 0
pH	3.56 ± 0.02
Specific Gravity	1.018 ± 0
[Na^+] (mmol·L^{-1})	530 ± 14
[K^+] (mmol·L^{-1})	28.8 ± 0
[Cl^-] (m.mol·L^{-1})	344 ± 0
[Glucose] (mmol·L^{-1})	24.4 ± 0

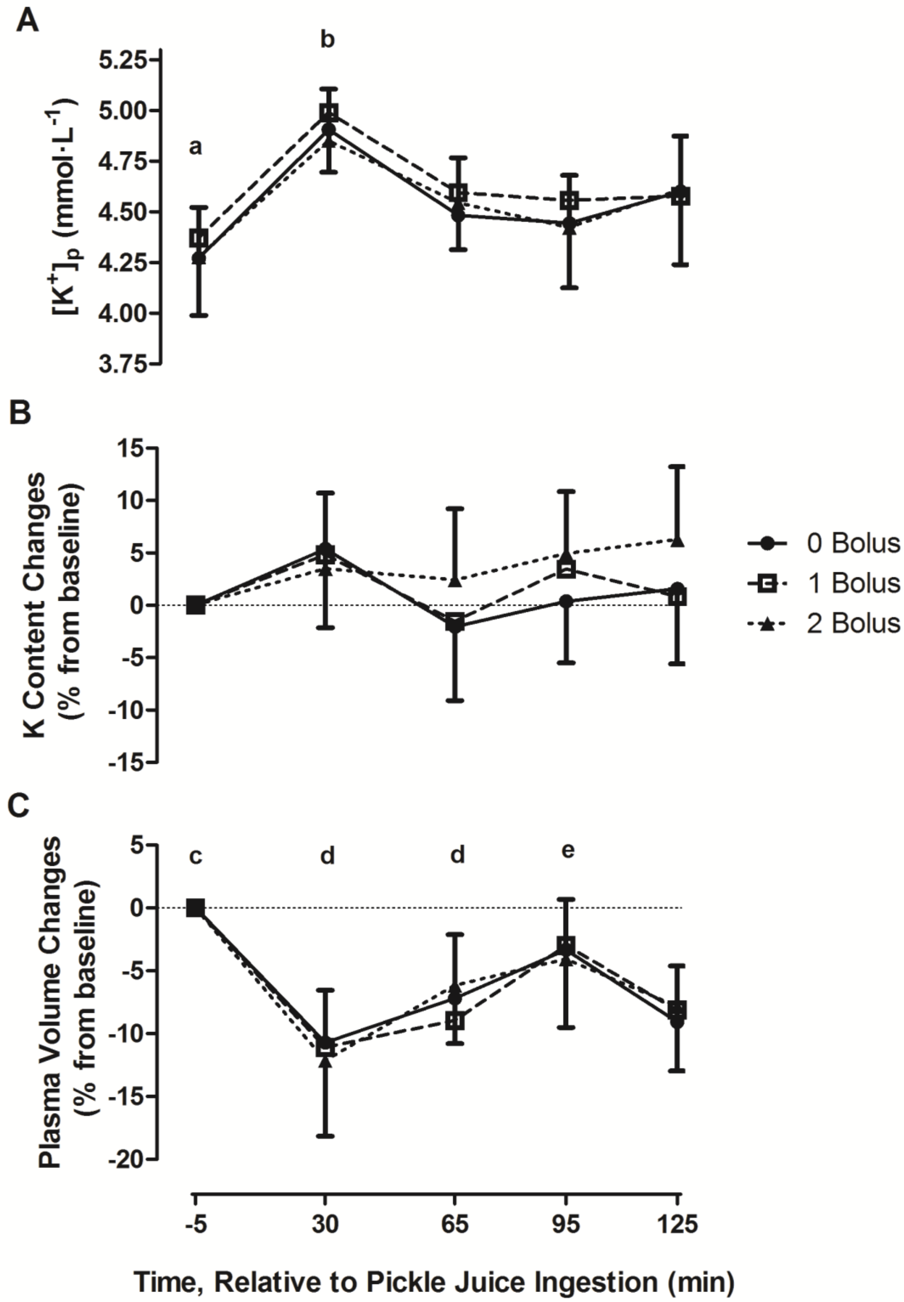
OSM = osmolality, [Na^+] = sodium concentration, [K^+] = potassium concentration, [Cl^-] = chloride concentration, [Glucose] = glucose concentration. Pickle juice was analyzed in duplicate. Data are means ± SD.

Figure 1. $[Na^+]_p$, Changes in Na^+ Content, Changes in Plasma Volume



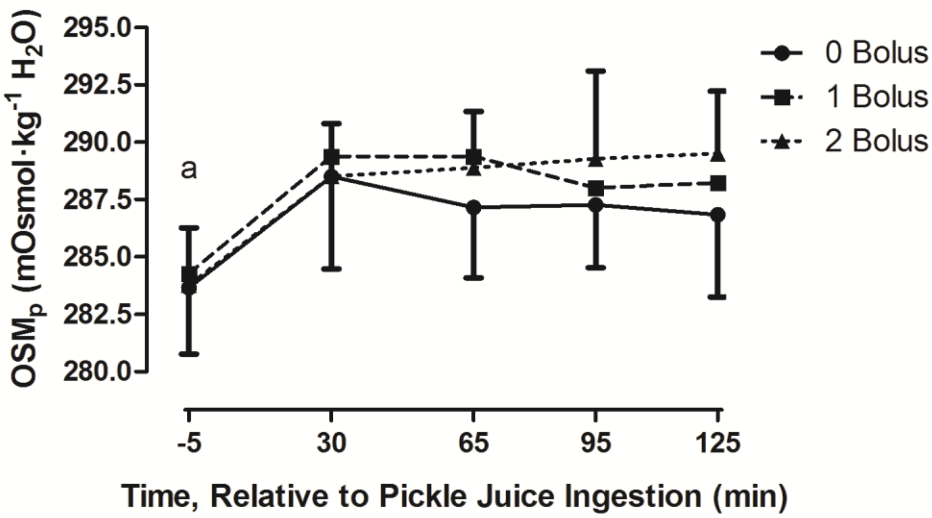
Plasma sodium concentration ($[Na^+]_p$, A), plasma sodium content changes (B), and changes in plasma volume (C) following ingestion of varying boluses of pickle juice (means ± SD). [a] = -5 < all other times. [b] = Within 0 and 1 bolus: -5 min > 30 and 65 min. [c] = Within 2 bolus: -5 min > 30 min. [d] = Within 0 and 1 bolus: 30 min < 95 min. [e] = Within 2 bolus: 30 min < 65, 95, 125 min post-ingestion. [f] = Within 1 bolus and 2 bolus: 65 min < 95 min. [g] = 0 bolus 95 min < 2 bolus 95 min. [h] = 0 bolus 125 min < 2 bolus 125 min. [i] = -5 min > 30, 65, 125 min. [j] = 30 and 65 min < -5 and 95 min. [k] = 95 min > 125 min. Significance accepted when $P < 0.01$ (n = 9).

Figure 2. $[K^+]_p$, Changes in K^+ Content, Changes in Plasma Volume



Plasma potassium concentration ($[K^+]_p$, A), plasma potassium content changes (B), and changes in plasma volume (C) following ingestion of varying boluses of pickle juice (means ± SD). [a] = -5 < 30 and 125 min. [b] = 30 min > all other times. [c] = -5 min > 30, 65, 125 min. [d] = 30 and 65 min < -5 and 95 min. [e] = 95 min > 125 min. Significance accepted when $P < 0.01$ (n = 9). Plasma volume change data are the same as in Figure 1 and are repeated here for convenience.

Figure 3. OSM_p



Plasma osmolality following ingestion of varying boluses of pickle juice (means ± SD). [a] = -5 min < all other times. Significance accepted when $P < 0.01$ (n = 9).

APPENDIX A. PROSPECTUS

Introduction

Sodium (Na^+) is the primary electrolyte in sweat; normal sweat Na^+ concentrations can range from 0.46 to 2.3 $g \cdot L^{-1}$ (20 to 100 $mmol \cdot L^{-1}$).[1] Sodium losses ranging from 2.5 to 30 g have been reported in athletes after 4.5 hours practice in a single day.[2, 3] Large Na^+ losses can put athletes at risk of developing hyponatremia, an injury marked by a plasma Na^+ concentration ($[Na^+]_p$) less than 135 $mmol \cdot L^{-1}$. Moreover, electrolyte losses are thought to increase the risk of developing exercise-associated muscle cramps (EAMC).[4, 5, 6, 7]

Several authors[5, 7, 8] have made Na^+ replacement recommendations to treat EAMC. The National Athletic Trainers Association (NATA)[6] recommends adding 0.3 to 0.7 g of Na^+ to every liter of rehydration drink to offset Na^+ losses due to sweating. The American College of Sports Medicine (ACSM)[8] recommends adding 1.25 g to 2.5 g of Na^+ to every liter of sports drink to treat EAMC. Bergeron[5] reported success treating EAMC by adding up to 6 $g \cdot L^{-1}$ of Na^+ to a sports drink. Other clinicians have experimented with different methods of replacing Na^+ including drinking chicken noodle soup or pickle juice.[9, 10, 11, 12]

Twenty five percent (92 of 370) of athletic trainers polled use pickle juice to treat EAMC.[12] However, some authors[11, 14] caution against pickle juice ingestion. Fowkes-Godek et al[11] observed mild hyperkalemia, a plasma potassium concentration ($[K^+]_p$) greater than 5 $mmol \cdot L^{-1}$, when American football players supplemented their meals with pickle juice over five consecutive days. Hyperkalemia is a concern because it is associated with cardiac abnormalities and the onset of fatigue.[13] Dale et al[14] postulated that drinking pickle juice would increase plasma osmolality (OSM_p) and $[Na^+]_p$, thereby rapidly expanding plasma volume, decreasing thirst, and impairing rehydration.[14] However, others observed no significant changes in plasma volume, OSM_p, or plasma electrolyte concentrations when euhydrated[15,16] or mildly hypohydrated[17] individuals ingested small volumes of pickle juice (~80 mL). Furthermore, drinking pickle juice does not alter perceived thirst or the volume of water ingested *ad libitum* post-exercise (unpublished observations).

However, the preliminary examinations[15,16,17] of pickle juice's effects on the extracellular fluid space had three limitations. First, they[15,16,17] only provided a single, small bolus of pickle juice at one time, either pre-exercise or post-exercise. Anecdotally, some athletic trainers give athletes pickle juice multiple times over the course of an exercise session to treat EAMC. Second, they [15,16,17] did not allow the subjects to exercise after ingestion of pickle juice. No scientist has examined the extracellular fluid space when subjects drink pickle juice and resume exercise. Finally, the effects of drinking pickle juice on the extracellular fluid space have not been measured after 60 minutes post-ingestion. Therefore, the purpose of this study is to investigate the effects of ingesting multiple boluses of pickle juice on $[Na^+]_p$, $[K^+]_p$, changes in plasma volume, and OSM_p up to 125 minutes post-ingestion.

Research Questions

1. Do changes in plasma volume, $[Na^+]_p$, $[K^+]_p$, and OSM_p increase as the number of boluses of pickle juice ingested increases?
2. Are changes in plasma volume, $[Na^+]_p$, $[K^+]_p$, and OSM_p higher at 30, 65, 95 and 125 minutes post-ingestion of pickle juice compared to pre-ingestion?

Hypotheses

1. Drinking multiple boluses of pickle juice will not significantly increase $[Na^+]_p$, $[K^+]_p$, OSM_p, or changes in plasma volume.
2. Changes in plasma volume, $[Na^+]_p$, $[K^+]_p$, and OSM_p will not be higher at 30, 65, 95 and 125 minutes post-ingestion compared to pre-ingestion.

Assumptions

1. Athletes ingest 1 $mL \cdot kg^{-1}$ body mass of pickle juice during exercise.
2. Athletes are ingesting one or two boluses of pickle juice during an exercise session.
3. The 60 second break given to subjects after 30 minutes of exercise is representative of the time it takes to relieve an EAMC in a field setting.

Limitations

1. Only healthy males between the ages of 18 and 35 will be recruited.
2. Arginine vasopressin and aldosterone will not be measured.
3. Subjects' sodium losses and sweat volumes may vary.
4. Subject's diet will not be controlled or monitored.
5. Fitness ability and acclimatization status of subjects will not be measured.

Delimitations

1. Subjects will exercise on a treadmill for 60 minutes at 85-90% of age-predicted heart rate.
2. Subjects will be healthy, (i.e. no lower extremity orthopedic injury or blood borne diseases) between the ages of 18 and 35, have no food allergies, or history of heat illness (e.g. syncope, heat exhaustion, or heat stroke).
3. Subjects will drink 0, 1, or 2 boluses of pickle juice (1 $mL \cdot kg^{-1}$ body mass in each bolus).
4. Blood samples will be collected before ingestion (-0.5 minutes), 30, 65, 95, and 125 minutes post-ingestion.
5. Pickle juice will be strained from Vlasic dill pickles (Pinnacle Foods Corp, Cherry Hill, NJ).
6. Subjects will be euhydrated at the onset of experimentation.
7. Subjects will have 60 seconds to drink each bolus of pickle juice.

Definition of Terms

Age-predicted maximum heart rate: HR= ((220 – age) x 0.85)) and ((220 – age) x 0.9)) will be used to calculate the target heart rate range.

Arginine vasopressin: Hormone released from posterior pituitary gland that increases water reabsorption in response to an increase in plasma osmolality.

Aldosterone: Hormone that increases reabsorption of water and sodium in the kidneys.

Bolus: A volume of food or liquid.

Euhydration: A state of normal body water. In this study, a urine specific gravity of < 1.01 will be used to indicate subjects are well-hydrated.[6]

Exercise-associated muscle cramping: an involuntary, painful, spasmodic contraction of skeletal muscle associated with exercise.[18]

Hematocrit: The proportion of the blood that consists of packed red blood cells.

Hemoglobin: The oxygen-carrying pigment and predominant protein in the red blood cells.

Hyperkalemia: Plasma K^+ concentrations $> 5.0\ mmol \cdot L^{-1}$.

Hyponatremia: Plasma Na^+ concentration $< 135\ mmol \cdot L^{-1}$.

Pickle juice: A salty, acidic brine that will be strained from commercially available, whole dill pickles.

Plasma potassium concentration: The concentration of K^+ in the blood. Values range from $3.5\text{-}5.0\ mmol \cdot L^{-1}$.

Plasma sodium concentration: The concentration of Na^+ in the extracellular fluid. Normal values at rest range from $135\text{-}140\ mmol \cdot L^{-1}$.

Plasma: The volume of blood that is plasma.

Sweat rate: The volume of sweat lost in a given period of time ($L \cdot h^{-1}$).

Sweat sodium concentration: The amount of Na^+ in 1 L of subject's sweat. Normal values range from 20 to 100 $mmol \cdot L^{-1}$.[1]

Abbreviations

$[K^+]_p$: Plasma potassium concentration

$[Na^+]_p$: Plasma sodium concentration

$[Na^+]$: Sodium concentration

$[K^+]_{sw}$: Sweat potassium concentration

$[Na^+]_{sw}$: Sweat sodium concentration

ACSM: American College of Sports Medicine

AVP: Arginine vasopressin

EAMC: Exercise associated muscle cramp

Hb: Hemoglobin

Hct: Hematocrit

mmol: millimole

mOsm: milliosmole

NATA: National Athletic Trainers Association

OSM_p: Plasma osmolality

U_{sg}: Urine specific gravity

Literature Review

This literature review will discuss the effects of ingesting high Na^+ solutions, such as pickle juice, to treat exercise-associated muscle cramping (EAMC). This literature review will also discuss the effects of exercise on plasma Na^+ concentration, plasma K^+ concentration, plasma osmolality, and changes in plasma volume. The following is a list of topics that will be covered:

Databases and Keywords

Pickle Juice and EAMC Theory

  Dehydration Theory

  Electrolyte Loss and EAMC

  Athletic Trainer Perceptions of EAMC

  Limitations of Dehydration and Electrolyte Loss Theories

Effects of Exercise and Fluid Ingestion on Plasma Variables

  Sodium Losses from Sweating

  Exercise Associated Hyponatremia

  Changes in Plasma Osmolality

  Plasma Potassium Concentrations

Sodium Supplementation and Exercise

  Sodium Facilitated Hypervolemia

  Pickle Juice Ingestion Studies

Summary

Databases and Keywords Searched

The databases used to obtain research for this literature review: Sport discus (SPORTDiscus & EBSCO), and National Library of Medicine's Pubmed (Medline & EBSCO). Journal articles searched were between the years of 1965 and 2011.

Acidic brine
Athletes
Body water
Calcium
Carbohydrate
Dehydration
Exercise
Exercise associated muscle cramp
EAMC
Electrolytes
Electrolyte balance
Gastric emptying
Glucose
Fructose
Hyperkalemia
Hypervolemia
Hypertonic
Hypohydration
Hyponatremia
Hypotonic
Osmolality
Osmolarity
Pickle Juice
Plasma osmolality
Plasma potassium concentration
Plasma sodium concentration
Plasma variables
Plasma volume
Salt loss
Sodium
Sodium chloride
Serum sodium
Serum potassium
Soup
Sport drink
Supplementation
Sweat
Sweat concentration

Sweat Sodium	Water
Volume	Water Ingestion

Pickle Juice and EAMC Theory

Exercise associated muscle cramping (EAMC) is defined as an involuntary, painful, spasmodic contraction of skeletal muscle associated with exercise.[18] EAMC is common in both the athletic and active populations. In American football, 73% (102/139) reported experiencing EAMC, which translates to 3.07 cases for every 1000 participants.[19] Other types of athletes that commonly experience EAMC are triathletes, where 49% (216/433) report having an episode at some point during their training.[20] This prevalence demonstrates that EAMC does not affect one type of athlete, rather different types of athletes with varying levels of training and conditioning. In addition, there are also varying explanations for the cause of EAMC as well as additional treatment and prevention strategies.

Dehydration Theory. The dehydration and electrolyte imbalance theory states that EAMC occur after repeated bouts of exercise with significant electrolyte loss through sweating.[5] Bergeron[5] describes this process in detail. Through the process of sweating, athletes tend to lose more Na^+ and plasma then they replace through diet and hydration. As this happens, water leaves the interstitial space to maintain plasma volume and decrease plasma osmolality. The result of the described fluid movement is a contraction of the interstitial space, which increases the excitability of nerve endings and subsequently causes EAMC.[5, 21]

Electrolyte Loss and EAMC. Clinical observations made by athletic trainers suggest that athletes who continuously suffer from EAMC also tend to sweat more, leading to large Na^+ and body water losses.[7, 25] This leads some health care professional to term those individuals "Salty Sweaters".[5,7,22] This phenomenon has been observed in American football players at the

NCAA Division I level with a history of EAMC.[7] Stofan et al[7] reported that athletes with a history of EAMC sweat more than those without a history of cramping. In addition, the athletes with a history of EAMC had a higher sweat Na+ concentration ($[Na^+]_{sw}$) than non-crampers. Results such as these contribute to the theory that dehydration and electrolyte deficit causes cramping. However, this particular study is limited by the fact that none of the subjects actually experienced an EAMC during their study, so the authors were forced to assume there was a causal relationship between EAMC and dehydration or electrolyte loss. In addition, the authors could not quantify how much sweat constituted a "salty sweater."

Athletic Trainer Perception of EAMC. Seventy-two percent (717 of 997)[4] of athletic trainers believe that dehydration is the primary source of EAMC, with 20% (199 of 997) believing that electrolyte depletion is also a main cause.[4] In the National Football league, 70% of athletic trainers use hyperhydration with intravenous fluid with saline to prevent muscle cramps.[23] In addition, the dehydration and electrolyte theories are supported by both the ACSM[8] and the NATA.[6] To address body water depletion and associated Na^+ loss, fluid replacement is a strategy used by 75% of athletic trainers (748 of 997)[4] to treat and prevent EAMC in addition to electrolyte replacement (120 of 997)[4], which is often done through commercially available electrolyte-carbohydrate sports drinks or pickle juice.[12]

Limitations of Dehydration and Electrolyte Loss Theories. However, both the dehydration and electrolyte theories cannot stand up to scrutiny when tested in experimental conditions. Jung et al demonstrated that EAMC occurs in individuals that are hypohydrated or actively consuming electrolyte-carbohydrate beverages.[24] It has also been demonstrated that athletes with a reported history of EAMC are equally as dehydrated as athletes with no history of EAMC, following an ultra-distance road race[25] or Ironman triathlon.[26] Despite these findings, it is still recommended that athletes ingest fluids and Na^+ to prevent EAMC.[5,6,8] Even though the evidence to support the dehydration and electrolyte theories are not strong, 72% of athletic trainers believe dehydration to be the primary cause of EAMC[4], and that fluid replenishment and electrolyte supplementation are effective strategies in preventing EAMC.

Pickle juice is used by 25% of athletic trainers to treat EAMC.[12] Pickle juice has been used anecdotally in the past to treat EAMC[14], yet research and guidelines to support the use of pickle juice are scarce. The prevailing theory is that pickle juice relieves or prevents cramps by restoring serum Na^+ levels in the body.[12] While pickle juice has a high Na^+ content[15], it also contains a number of other ingredients that have effects on plasma variables and fluid balance such as glucose, magnesium, calcium, potassium, and acetic acid.[14,27,28] This makes it difficult to say that the Na^+ content in pickle juice is solely responsible for EAMC relief. Also, 95% of athletic trainers use other treatments in addition to pickle juice when treating EAMC[12], further complicating what method is effectively providing relief. There is also doubt if ingestion of electrolytes even treats acute EAMC.[16,17,25,26] It has been shown that pickle juice relieves electrically induced cramps faster than gastric emptying can occur[16], casting doubt on the theory that metabolic mechanisms are responsible for EAMC relief. Yet, as previously mentioned[12], the practice of administering pickle juice to replace electrolytes is still in use.

What is presently unknown about pickle juice or high Na^+ beverage ingestion, is how they affect plasma volume, plasma electrolyte concentrations when multiple boluses are ingested during an exercise session. In addition to pickle juice ingestion, it is also important to consider how plasma variables are affected during exercise.

Effects of Exercise and Fluid Ingestion on Plasma Variables

Sodium Losses from Sweating. During athletic activity or exercise, humans lose varying amounts of body water and Na^+ through sweat.[29] There are subsequent decreases in plasma volume and increases in OSM_p as a result.[30] A decrease in plasma volume will also cause a decrease in cardiac output and has been hypothesized to hurt the body's ability to dissipate heat, potentially leading to heat illness.[31] In addition to the potential for heat illness, high Na^+ losses can also contribute to hyponatremia.[2] Due to the potential negative impact of fluid imbalance, humans routinely ingest food and liquids to offset Na^+ losses and shifts in plasma volume during exercise.

Fowkes-Godek et al[32] investigated plasma variables of professional football players during pre-season training. Baseline values were recorded and subjects continued with their normal fluid replacement and dietary routines. At the conclusion of this study, the authors reported that $[Na^+]_p$ significantly dropped over a nine day period compared to baseline measurements.[32] The authors attribute those results to extremely high sweat rates and Na^+ losses they measured in another one of their studies evaluating sweat rates of three different groups of professional football players. In that study, Fowkes-Godek et al[2] found that lineman lost an average of 12.5g of Na^+ during two-a-day practices (one subject lost 30 g Na^+) and were ineffective at replacing Na+ to match the amounts that they lost.

Soccer players have also been shown to experience Na^+ losses during exercise. Maughan et al[33] measured fluid balance and plasma variables of soccer players during a 90 minute practice session that was one of two sessions in the same day. At the conclusion of this study, the subjects were shown to have lost up to 7.8 grams of Na^+. The authors also found that the subjects were inefficient at replacing fluids and Na^+ lost during exercise. This is important to note, because given the results of Fowkes-Godek[2] et al regarding American Football player's significant Na+ losses during two-a-day practices, it is reasonable to assume that the subjects in Maughan et al[32] would sustain additional losses during the second session as well.

Pahnke et al[34] presented data that also demonstrated a significant relationship between Na^+ losses and sweating with exercise. During an Ironman triathlon, the authors collected data from 46 athletes participating in the race. All subjects completed a pre-race sweat analysis, had baseline measurements taken on prior to the race, and were free to rehydrate and ingest foods freely throughout the race. All subjects averaged a 4% weight loss during the race, and $[Na^+]_p$ were lower in subjects with higher sweat rates, specifically males. Overall sweat losses in males averaged 16.9g during the race, which is greater than the data Godek et al[2] report for professional football players. Similar to Godek et al[2], Pahnke et al[34] found that male subjects were not adequately replacing Na^+ lost through sweat through their diet or ingesting fluids. However, there is disagreement in the literature regarding Na^+ losses in athletes, especially regarding endurance athletes competing in Ironman triathlons and other ultra-endurance events.

In a recent study conducted by Hamouti et al[35], the authors evaluated $[Na^+]_{sw}$ in both trained and untrained subjects during exercise. All subjects completed the same aerobic workout at 40, 60, and 80% of their VO_{2max}. Aerobically trained subjects had higher $[Na^+]_{sw}$ than those that were untrained. Also, the results did not support the hypothesis that untrained individuals would have higher $[Na^+]_{sw}$ than those who were aerobically trained.[35] The trained group did not experience significant increases, but the concentrations were much greater than the untrained group at both 60 and 80% VO_2max trials (70 and 78 $mmol \cdot L^{-1}$ Na^+ compared to 50 and 54 $mmol \cdot L^{-1}$).[35] The higher Na^+ concentration in trained individuals suggests that the sweat losses in previous studies are more population specific than previously thought, considering the larger athletic population is not at a training level equivalent to professional football players[2], professional soccer[33],or ultra-endurance athletes.[25,36,37,38]

Hew-Butler et al[36] evaluated Na^+ concentrations of athletes competing in an ultra-distance road race. To address Na^+ losses, the experimental group was given Na^+ tablets to ingest ad libitum during the race. The results would be compared to a placebo group and an uncontrolled group participating in an unrelated study. At the conclusion of the race, even though the experimental group ingested Na^+ tablets throughout the race, there were no significant differences in $[Na^+]_p$ among all groups tested. $[Na^+]_p$ of all groups averaged 140.9 mmol·L^{-1}by the end of the race which is within normal limits, and doesn't support the hypothesis that large Na^+ loss through sweating will result in lower $[Na^+]_p$.[2, 33] Also, Hew-Butler et al[35] found that athletes were able to maintain plasma volume as well. Additional authors[25,26,38] evaluating Na^+ loss have also found results similar to Hew-Butler et al.[36] Additionally, in Hamouti et al[35], untrained subjects averaged sweat rates of 1.3L·h and trained subjects averaged 1.6 L·h while exercising in an environment at 36º C. All subjects in that study were able to maintain healthy $[Na^+]_p$ of greater than or equal to 140 mmol·L^{-1} in all trials despite their Na^+ losses.

Exercise Associated Hyponatremia. Hyponatremia is a major concern for athletes and healthcare professionals alike during events where Na^+ losses can be great. Exercise associated hyponatremia is defined as a $[Na^+]_p$ equal to or less than 135 mmol·L^{-1} (1mmol·L – 1mEq·L).[37,39,40,41] In a study conducted by Noakes et al[37], the authors retrospectively evaluated data from 2,135 athletic performances in order to identify the mechanisms that cause exercise associated hyponatremia. Three primary mechanisms are thought to contribute to hyponatremia, and they are overconsumption of fluids, anti-diuretic hormone abnormalities, and inability for subjects to utilize Na^+ stores in the body.[37]

Weight gain during activity is primarily caused by over-ingestion of fluids.[37] In addition to these fluid gains, they can also come from foods ingested *ad libitum* during competition.[35-38] Noakes et al[37] found that athletes who gain > 4% body weight gain during exercise have a 45% greater chance of developing exercise associated hyponatremia.[37] This is a result of ingesting more fluid due to an increased thirst during exercise, which is a result of an increase in OSM_p and an associated decrease in plasma volume.[42,43] The conclusions of Noakes et al[37] are similar to an earlier study conducted by Vrijens and Reher[40] where endurance athletes consumed plain water or a Na^+ containing sport drink every two minutes until exhaustion with the goal of replacing all fluid that was lost through sweat. At the conclusion of the study, Vrijens and Reher[40] found that ingesting plain water versus Na^+ containing sports drinks could dilute $[Na^+]_p$ and potentially cause a hyponatremic state. The authors[40] also concluded that overdrinking and hyponatremia were more likely in situations of high sweat losses, which Fowkes-Godek et al[2] witnessed with professional football players who sustained large Na^+ losses.

In addition to the above studies, Twerenbold et al[44] found that hyponatremia can occur in women who over-consume water during exercise. The authors conducted three trials where subjects ingested 1 L of liquid for every hour of exercise completed. The $[Na^+]$ varied between drinks, to include a plain water trial. When Na^+ drinks containing 680 mg·L were ingested, 46% (6 of 19) of subjects developed hyponatremia. Conversely, when plain water was ingested, 92% (12 of 13) of subjects developed hyponatremia, and of those 12 subjects, two developed severe hyponatremia (plasma Na+ < 125 $mmol \cdot L^{-1}$).[44] The results of this study are in agreement with Vrijens and Reher[40] in that plain water ingestion resulted in the most severe decrease in $[Na^+]_p$ by over dilution as a result of hypotonic beverage ingestion (> 130$mmol \cdot L^{-1}$).

In a study conducted by Steumpfle et al[45] in a cold weather environment, subjects did not develop hyponatremia but experienced decrease in $[Na^+]_p$ as a result of overdrinking.[45] Twerenbold et al[44] also demonstrated that even when Na^+ was added to drinks, hyponatremia still occurred in just under half of (6 of 19) subjects. Fowkes-Godek et al[2] caution against overdrinking to replace Na^+ losses. This is due to most athletes ingesting sports drinks that do not contain enough Na^+ to replace what was lost during exercise and are hypotonic in composition.[2] In addition to overdrinking, Noakes et al[37] concludes that there are more mechanisms that cause hyponatremia.

In Noakes et al[37], 70% (170/231) of subjects who finished with weight gain were able to maintain $[Na^+]_p$ within normal limits when they overdrank[37], casting doubt that there is only one cause of the condition. In addition to overdrinking, Noakes et al[37] conclude that there may also be abnormalities present with the release of anti-diuretic hormones such as arginine vasopressin (AVP) that can contribute to hyponatremia.[37,41] Vrijens and Rehrer[40] observed an overall decrease in plasma volume during exercise that could increase thirst stimulus and decrease free water clearance via urination.[40] As a result, $[Na^+]_p$ will continue to be diluted due to fluid retention.[40] This phenomenon was also observed in a study conducted by Hew-Butler et al[41] where AVP was elevated after an endurance race, and hypothesized that increasing fluid intake, while decreasing free water clearance, results in a $[Na^+]_p$ decrease.[41] In addition to overdrinking and AVP secretion abnormalities, Noakes et al[37] concluded that the inability to utilize Na^+ stores can also result in hyponatremia, but that there is not one single cause of a large decreases in $[Na^+]_p$ that result in the condition.[37] Additionally, it has been demonstrated the symptomatic exercise associated hyponatremia, while very serious, is relatively rare.

Knechtle et al[46] evaluated plasma variables in 145 ultra-marathon runners at a race in Switzerland. They found that only 4.8% (7 of 145) runners developed exercise associated hyponatremia by the end of a 100 km race, yet none of them were symptomatic.[46] Conversely, that means 95.2% of participants maintained $[Na^+]_p$ and plasma volume within normal limits (Normal $[Na^+]_p$ 135 to 140 mmol·L).[46] Additionally, plasma volume actually increased in some of the participants. The authors[46] also noted that many of the participants exceeded guidelines for drinking and consumed up to 1.34 L·h of fluid, yet still averaged a 2.4% weight loss by the end of the race. Knechtle et al[46] is similar to the results of Noakes et al[37] where they hypothesize that a 2% body weight loss may actually prevent exercise associated hyponatremia. This is strengthened by the fact that 138 of the athletes remained hydrated, which is similar to the results found by Hew-Butler et al.[38,46]

In a study conducted by Anastasou et al[31], subjects participated in moderate exercise to induce sweat loss and were randomly given beverages containing varying amounts of Na^+. Subjects were given enough fluids to replace what was lost as determined through body mass. Similar to previous authors[37,40,44], all subjects experienced a greater decline in $[Na^+]_p$ when drinks lacking Na^+ were ingested. When plain water or mineral water was ingested, mean $[Na^+]_p$ were 134.5 mmol·L^{-1} and 134.4 mmol·L^{-1} respectively.[31] Plasma Na^+ declines were least in beverages containing 19.9mmol·L^{-1} and 36.2 mmol·L^{-1} of Na+ (Gatorade brand drinks).[31] Plasma volume was also reduced by 2.5% in both water and mineral water trials.

The results of Anastasou et al[31]are similar to Fowkes-Godek et al[32] that evaluated plasma and Na^+ changes, because both sets of subjects had access to the same 19mmol·L^{-1} Gatorade solution. The subjects that drank the solution, or the one with greater Na^+ content, had $[Na^+]_p >$ 135mmol·L^{-1}. However, Fowkes-Godek et al[32] demonstrated that even though $[Na^+]_p$ remained relatively stable, plasma volume was 5% below baseline values by the third day of practice. These results are contradictory to Anastasou et al[31] likely because their trials were separated by one week at a minimum, whereas Fowkes-Godek et al[32] gathered data over nine consecutive days. This could possibly have allowed subjects to adequately replace Na^+ and fluid losses before each trial, making a comparison of plasma volume results between the studies more difficult.

Changes in Plasma Osmolality. OSM_p is also a concern when humans exercise, as it is affected by both Na^+ and body water loss. Nolte et al conducted a study evaluating plasma variable changes in soldiers marching over a distance of 25 km.[47] In addition to OSM_p, Nolte et al[47] also measure $[Na^+]_p$ and body mass changes. At the conclusion of data collection, the authors found that there were no significant changes in either plasma osmolality or plasma Na+ levels over the course of a 25km march. OSM_p averaged 300.6 $mOsm \cdot Kg^{-1}$ and $[Na^+]_p$ averaged 140 $mmol \cdot L^{-1}$, both of which were within normal limits, and not at risk of developing hyponatremia.[37]

The authors[47] willingly point out that these variables are maintained due to participants drinking large amounts of water during the trial. Even so, their results mirror other studies[35,38,46] where subjects were able to maintain normal $[Na^+]_p$ of > 135 $mmol \cdot L^{-1}$ when exercising in the heat and ingesting water *ad libitum*. Nolte et al[47] also presented results similar to previous studies where losses in body weight helped control $[Na^+]_p$.[47] Their subjects also ingested a significant amount of water *ad libitum* at a rate of 1264 ± 229 $mL \cdot h$. Without water loss through sweating, their subjects could have potentially diluted their $[Na^+]_p$ to create a hyponatremic state which has been observed in previous studies.[37,40,44,] In a study conducted by Maresh et al[48,] the authors evaluated plasma variables and their association with thirst when subjects exercised in the heat. Previous studies[36,38,41] have found that subjects were able to maintain $[Na^+]_{p,}$, plasma volume, and OSM_p when subjects exercised in the heat, lost large amounts of Na^+, and ingested water *ad libitum*. Maresh et al[48] removed the variable of *ad libitum* ingestion and restricted fluid for two of four trials. Subjects either began a trial hypohydrated or euhydrated, and were allowed to drink or were restricted from ingestion. Similar to previous studies[37,46,47,49], subjects that could ingest water *ad libitum* were able to maintain normal OSM_p (293 $mOsm \cdot kg^{-1}$) during exercise, even though they averaged a 3-4% decrease in body mass.[48] OSM_p increased to 307 mOsmol·kg when subjects were restricted from fluid intake and had an associated increase in thirst due to OSM_p rising above dipsogenic thirst threshold of 295 mOsmol·kg.[48] As plasma osmolality increased, an increase in secreted AVP was also present, which is in agreement with other studies.[37,40,41,49]

Plasma Potassium Concentrations. Previous authors[32] have found increases in $[K^+]_p$ levels associated with exercise. The increase in $[K^+]_p$ occurred in studies[11,32] where the authors supplemented professional football players with Na^+ to address significant losses associated with training. This rise in $[K^+]_p$ has been hypothesized to be the result of rhabdomyolysis from exercising in the heat or renal secretion due to Na^+ reabsorption[32], and potentially cause hyperkalemia.[11] However, the increases in $[K^+]_p$ of all subjects, on all days, remained within normal levels (3.5 to 5.0 $mmol \cdot L^{-1}$).[50] Shirreffs et al[30] also conducted a study that volume depleted subjects for the purpose of measuring rehydration. $[K^+]_p$ levels for their subjects remained within normal levels during exercise, which has been observed in additional studies.[25,31]

Zarvosky et al[51] performed a study where women cycled for repeated bouts of high intensity exercise to determine the relationships between plasma lactate, K^+, bicarbonate and pH levels with breathing. $[K^+]_p$ remained relatively constant, but elevated above normal levels of 3.5 to 5.0 $mmol \cdot L^{-1}$ [50], with an average of 5.2 $mmol \cdot L^{-1}$. Additionally, some K^+ levels were as high as 6.1 $mmol \cdot L^{-1}$. However, these results were not sustained and returned to baseline levels within 5 minutes of rest.[51] The authors concluded that the increases were a result of K^+ leaving exercising muscle and entering the blood stream.[51] An increase in $[K^+]_p$ were also observed by Hamouti et al[35] when exercise bouts were at 80% VO_{2Max} in both trained and untrained individuals. $[K^+]_p$ reached 5.3 $mmol \cdot L^{-1}$ in trained subjects and 5.6 $mmol \cdot L^{-1}$ in untrained subjects. In the two other trials completed at sub 80% $V0_{2max}$, $[K^+]_p$ remained within normal levels. However, despite the higher values in the one trial, $[K^+]_p$ stayed constant in all trials suggesting that $[K^+]_p$ remain relatively constant, or if there are increases or decreases that levels remain within normal limits.[35,51]

Sodium Supplementation and Exercise

As previously discussed, ingesting pickle juice before exercise is used by athletic trainers to treat EAMC by elevating electrolyte levels.[12] Besides its debated effectiveness in treating EAMC[25], this practice may unintentionally lead to physiological effects associated with fluid balance and plasma volume levels as a result of ingesting a concentrated Na^+ beverage.[14] Pickle juice contains levels of Na^+ that are far greater than any beverage previously described in the literature evaluating fluid balance.[2,29,34,52-56] However, some authors[56,57] have evaluated high Na^+ ingestion and its effects on exercise.

Ray et al[57] found that plasma volume levels restore to normal much faster with high Na^+ ingestion, than if water or simple carbohydrate solutions are ingested. Subjects completed a dehydration exercise protocol and drank Na^+ containing solutions immediately before rehydration. The two solutions highest in Na^+ content were chicken broth and chicken noodle soup. The other drinks in the trial were a carbohydrate-electrolyte solution and water. The chicken broth contained 110 $mmol \cdot L^{-1}$ of Na^+ and the chicken soup had a high Na^+ content 338 $mmol \cdot L^{-1}$. The highest amount of Na^+ previously tested by Shirreffs et al was 109$mmol \cdot L^{-1}$.[29] However, this study differs from earlier hydration studies in that they did not solely ingest the high Na^+ solutions for the duration of the rehydration period. Instead, they ingested 175 ml at the beginning of rehydration, then another 175 ml 20 minutes later for a total of 350ml (12 ounces). After the subjects ingested Na^+, they drank controlled amounts of water at 20 minute intervals for 2 hours, measured to replace the body water subjects lost during exercise.

The results of Ray et al[57] suggest that the addition of a high Na^+ ($>109\ mmol \cdot L^{-1}$) solution to post-exercise rehydration with water may be equally as effective as rehydration with a carbohydrate-electrolyte solution alone. Subjects also did not rehydrate to 150% of what they lost as recommend[6,29], but rather 100%. This contradicts Shirreffs et al[29] and suggests that with Na^+ supplementation, athletes may not have to drink 150% of what they lost in order to restore plasma volume and hydration levels. A possible weakness of this study is that the chicken noodle soup contained carbohydrates, potentially leading to the increased absorption rate of Na^+ and water in the small intestine.[27,28] Based on the contents of chicken noodle soup, the author's conclusion is made with the assumption that Na^+ was more influential in post-exercise hydration, rather than the additional ingredients in the soup.[57]

Na^+ supplementation pre-exercise has also been studied, though to a lesser degree than Na^+ intake after exercise. It has been demonstrated that Na^+ supplementation can aid in decreasing the amount of time needed to rehydrate after a bout of exercise[57], and the effects of hydrating with Na^+ containing beverages are well documented in the literature. Other authors[53-56] have evaluated Na^+ supplementation on the opposite end of the spectrum, focusing on pre-exercise ingestion and its physiological effects before and during exercise with the potential for performance increases.

Sodium Facilitated Hypervolemia. Greenleaf et al[52] demonstrated that a plasma expansion can occur with Na^+ supplementation in rested individuals.[52,55,56] Coles et al[55] investigated this claim on an active population, choosing to provide a concentrated Na^+ solution immediately before a 45 minute cycling workout that included 15 minute time-trial at its conclusion. The authors found that when Na^+ (10 $ml \cdot kg^{-1}$ body mass) was ingested before riding commenced, those subjects maintained a higher plasma volume than the placebo group for 30 minutes into the ride.[55] During the subsequent 15 minute time trial, the subjects who ingested Na^+ were able to travel 0.97km further than subjects who ingested the placebo. A strength of this study was that subjects sustained almost identical fluid losses of 1.7% body mass, suggesting it was likely the Na^+ ingestion that resulted in this performance increase.[55] Despite a significant performance increase, Coles et al[55] were not able to replicate the increase in plasma volume seen in Greenleaf et al[52] study.[55] However, a comparison between the two studies suggest that the amount of time Na^+ is ingested prior to exercise has an effect on overall plasma volume increase, which could potentially increase performance further.[55]

Like Coles et al[55], Sims et al[53] examined pre-exercise Na^+ supplementation and the potential effects on plasma volume. The justification for this study was also similar to Coles et al[55], where the authors were investigating the claims made by Greenleaf et al[52]. The differences in this study[53] versus Coles et al[52] were that two different Na^+ solutions were ingested as opposed to one. This test was also completed on trained men, who the authors assumed would be more hypervolemic pre exercise.[53] Also, instead of completing a timed test at the end of the exercise phase, subjects ran on a treadmill until complete exhaustion occurred. Sims et al reported that plasma volume before exercise increased 4.5% after Na^+ ingestion of 164 $mmol \cdot L^{-1}$ in water, and did not increase with the 10 $mmol \cdot L^{-1}$ Na^+ beverage (both in amounts of 10 $ml \cdot kg^{-1}$ body mass). Also, they had results similar to Coles et al[55] that demonstrated a performance increase with ingestion of a high Na^+ beverage, where subjects took 21 minutes longer on average to reach exhaustion. Sims et al[53] also reported increased urine loss with the low Na^+ beverage which confirms previous author's[29,37] concerns about hypotonic beverage consumption increasing urine output, subsequently leading to increased water loss and hyponatremia.

A limitation of both Coles et al[55] and Sims et al[53] is that they tested very specific populations at specific training levels. This would make it difficult to broadly apply their results to all athletes. Also, the Na^+ beverage in both studies contained a combination of Na^+ chloride and sodium citrate, making it difficult to say if one ingredient or both influenced hydration. The research conducted by Sims et al[53] initially only focused on trained men, which limits the external validity of their study. This is addressed in a subsequent study by Sims et al[54] that applied the same pre-exercise Na^+ ingestion protocol to women training in the heat. However, the exercise test performed by subjects was cycling instead of running, but they still exercised until exhaustion. Similar to the results of their previous research[53], high Na^+ ingestion pre-exercise led to increased plasma volume, and subjects were able to exercise 20 minutes longer to exhaustion on average compared to the low Na^+ (10 $mmol \cdot L^{-1}$) group.[54]

What is significant about both studies by Sims et al[53,54] is that they were able to observe the same performance and plasma increases in different populations while performing different forms of exercise. Like the previous study, differences were only observed when subjects ingested beverages containing 164 mmol·L^{-1} of Na^+. Due to hormonal differences, plasma volume in women is subject to changes in progesterone and estrogen, which fluctuate with the menstrual cycle.[54] Also, oral contraceptives may further affect hormone and plasma volume levels in women.[54] However, the authors found no significant differences between subjects who were taking oral contraceptives and those who were not. This particular study was conducted during the phase of menstruation when hormone levels were highest, so the authors cannot concluded that women will physiologically respond to the interventions in this study through all phases of the menstrual cycle. Additionally, similar to Coles et al[55], in both studies[53,54] the 164 mmol·L^{-1} Na^+ solution was a mixture of Na^+ chloride and Na^+ citrate. So it is unknown if these results are due to the two different types of Na^+ ingested in one solution or one of them independently.

As previously demonstrated by Ray et al[57], a concentrated Na^+ solution (> 109mmol·L^{-1}) ingested immediately post-exercise can decrease the time it takes to rehydrate and restore plasma volume.[57] However, the rate of water ingestion was fixed for the subjects post-exercise, as was also the case during exercise in other studies examining Na^+ supplementation.[53-55] As mentioned by Wemple et al[58], the addition of Na^+ to a beverage can increase *ad libitum* fluid ingestion during exercise. However, it is not known how high pre-exercise Na^+ ingestion effects ad libitum fluid ingestion during exercise.[56] Johansen et al[56] performed a study that examined *ad libitum* fluid ingestion during exercise after a pre-exercise Na^+ supplementation. The study examined the differences of *ad libitum* ingestion after drinking 355ml a high Na^+ solution (Chicken noodle soup 167 mmol·L^{-1} Na^+), carbohydrate electrolyte beverage (16 mmol·L^{-1} Na^+), or water. All subjects completed a 90 minute steady state cycling workout with a 5 minute performance test at the conclusion, and all were allowed to drink water *ad libitum* throughout the entire trial.

Ingesting chicken noodle soup prior to exercise maintained fluid balance during exercise by increasing ad libitum fluid ingestion.[56] Both the carbohydrate and water trials were ineffective at restoring fluid balance. The results of this study are significant because as the authors state, this is the first data set that supports the ACSM's position statement[8] that ingesting Na^+ pre-exercise may improve water intake and delay dehydration.[56] The authors also observed a decrease in urinary output with ingestion of higher Na^+ concentrations helping to retain fluid, which is in agreement with previous authors[53,54] Johansen et al[56] demonstrated a way to significantly improve fluid balance after ad libitum fluid and high Na^+ ingestion.

Pickle Juice Ingestion. The above studies investigating high Na^+ ingestion measured beverages that contained significantly less Na^+ than pickle juice (Pickle juice 415.2 $mmol \cdot L^{-1}$ Na^+).[15] In a study conducted by Miller et al[15], the authors evaluated changes in plasma variables in rested humans after pickle juice ingestion. Contrary to previous authors[52], Miller et al[15] did not observe any changes in plasma volume at 60 minutes post-ingestion of 1 $ml \cdot kg^{-1}$ body mass of pickle juice (mean 86.3 mL). This is in direct contradiction to Greenleaf et al[52] who experienced a 7.9% increase in plasma volume when rested subjects ingested a Na^+ containing beverage. However, the differences in these two studies[15,52] may have had to do with the differences in the osmolality of ingested beverages. Pickle juice had a much higher osmolality of 778 $mOsm \cdot kg^{-1}$ H_20 compared to the 253 $mOsm \cdot kg^{-1}$ H_20 solution ingested in Greenleaf et al.[52] It has been shown that beverages that are hypertonic leave the stomach slower,[59] to include pickle juice.[16] Also, Greenleaf et al[52] allowed for 90 minutes of rest while ingestion was occurring, and Miller et al[15] allowed 60 minutes of rest after a single bolus. So it is also possible that enough time had not elapsed for the effects of pickle juice ingestion to become apparent in Miller et al[15]. However, plasma variables have not been measured further than 60 minutes post pickle juice ingestion, so it is unknown if pickle juice could have similar effects up to 125 minutes post-ingestion.

Summary

A large number of athletic trainers administer pickle juice during or before exercise as a preventative measure for EAMC[12]. As Miller et al state[12], there is not only a lack of evidence to support this practice, but little evidence regarding physiological consequences once someone ingests such a concentrated sodium solution during exercise from either single or multiple boluses. The studies evaluating high sodium ingestion[52-58] reveal the possibility that athletic trainers may be causing a plasma volume expansion, but those conditions have not been studied using pickle juice. Also, there have been no studies conducted to evaluate the extended use of high sodium supplementation over more than 5 days[11] and the potential to develop health conditions associated with excessive Na^+ intake[14].

Health care professionals must continue to exercise caution when administering pickle juice as a supplement to replace electrolyte levels in athletes with the goal of preventing EAMC. More research needs to be conducted to determine the physiological effects on the body when multiple boluses of pickle juice are ingested.

Methods

Experimental Design

A crossover, 3 x 5 factorial with repeated measures on time design will guide data collection. The independent variables will be number of pickle juice boluses ingested (0, 1, or 2) and time (-0.5 minutes pre-ingestion, and 30, 65, 95, and 125 minutes post-ingestion). The dependent variables will be $[Na^+]_p$ ($mmol \cdot L^{-1}$), changes in plasma volume (% from pre-ingestion), OSM_p ($mOsmol \cdot kg^{-1}$ H_2O), and $[K^+]_p$ ($mmol \cdot L^{-1}$).

Subjects

Twelve healthy, physically active (20-60 minutes of vigorous activity on 3 or more days a week)[60] males between the ages of 18 and 35 with no self-reported history of heat illness (e.g. heat stroke, heat exhaustion, or heat syncope), diabetes, anemia, food allergy to pickles, musculoskeletal, cardiovascular, blood borne, or neurological diseases, or history of lower extremity injury within the 12 months preceding data collection will be recruited. All volunteers will provide written informed consent prior to data collection. All procedures will be evaluated and approved by North Dakota State University's institutional review board.

Procedures

Subjects will report for testing, at approximately the same time of day, on three days separated by at least 48 hours. All subjects will be instructed to refrain from strenuous activity for 48 hours prior to testing. Subjects will be asked to maintain a similar diet throughout the course of experimentation and to avoid caffeine and alcohol for 24 hours prior to testing. Subjects will report compliance of pre-testing instructions before each testing session.

Subjects will report to a laboratory, void their bladders completely, and have their urine specific gravity measured with a refractometer (SUR-Ne; Atago USA Inc., Bellevue, WA) to determine if subjects are euhydrated (specific gravity < 1.01).[6] If hypohydrated (specific gravity > 1.01),[6] subjects will be excused and rescheduled for another testing session at least 24 hours later. If euhydrated, they will insert a rectal thermistor (YSI; Advanced Instruments Inc., Norwood, MA) at least 10 cm past the anal sphincter and put on a heart rate monitor (Polar Electric Inc., Lake Success, NY). One forearm's antecubital region will be cleaned with isopropyl alcohol and a sterile, 20-guage venous catheter assembly will be inserted into a superficial vein. Subjects will be weighed (body weight; BW_1) nude to the nearest hundredth of a kilogram (DA-150, Denver Instrument, Bohemia, NY) and sit for 30 minutes to ensure equilibration of fluid compartments.[61] Body mass measurement one will be used to calculate each bolus' volume.

After the 30-minute rest period, a 5-mL blood sample will be collected (-0.5 minutes sample). Subjects will void their bladders and have 60 seconds to ingest 0, 1, or 2 boluses (1 $mL \cdot kg^{-1}$ body mass in each bolus) of chilled (~6º C) pickle juice (strained from whole dill pickles, Vlasic Pickles, Pinnacle Foods Corp., Cherry Hill, NJ). Subjects will be weighed nude (BW_2), put on a sweat suit (hooded sweatshirt and sweat pants), enter an environmental chamber (~38º C, 15% relative humidity), and bike on a semi-recumbent cycle ergometer (846: Precor, Woodinville, MA) at 85% to 90% of their age-predicted maximum heart rate for 30 minutes. After 30 minutes, a 5-mL blood sample will be collected. Subjects will rest for 60 seconds and, if on the 2 bolus trial, consume another bolus of chilled pickle juice (if on the 0 or 1 trials, subjects will rest during this period). They will resume biking for another 30 minutes. Exercise will be terminated if rectal temperature exceeds 39.5º C, subjects display any signs or symptoms of heat illness (e.g. nausea, light headedness, disorientation), or the subject wishes to stop.

After the 60-minute exercise bout, subjects will exercise at a self-selected lower intensity for 5 minutes to cool down. A third, 5-mL blood sample will be collected and subjects will exit the environmental chamber, towel dry, remove the sweat suit, be weighed nude (BW_3), and void their bladders. They will be weighed nude again (BW_4) and remove the heart rate monitor and rectal thermistor. They will sit and rest for 30 minutes for body compartment equilibration. Blood samples will be collected at 95 minutes post-ingestion (30 minutes post-exercise) and 125 minutes post-ingestion (60 minutes post-exercise). The catheter assembly will be flushed with 1 to 2 cc of 0.9% saline after each blood sample is collected to ensure line patency. Trials will only differ by the number of boluses ingested (0, 1, or 2). The order of the number of boluses ingested will be randomized and counterbalanced a priori.

Blood and Plasma Analysis

Whole blood will be used to determine hematocrit and hemoglobin concentration immediately post-sampling. For hematocrit, blood will be put into heparinized microcapillary tubes, centrifuged at 3000 rpm for 5 minutes, and read using a microcapillary reader (IEC 2201; Damon/IEC, Needham Heights, MA). Hemoglobin concentration will be estimated using the cyanomethemoglobin technique. Hematocrit and hemoglobin concentration will be measured in triplicate immediately following sampling and averaged for statistical calculations. Changes in plasma volume will be estimated by inserting hematocrit and hemoglobin data into the Dill and Costill equation.[62]

The remaining whole blood will be centrifuged at 3000 rpm for 15 minutes at 3º C. Plasma will be removed from the packed red cells, and plasma electrolyte concentrations will be analyzed using an ion selective electrode system (16; NOVA Biomedical, Waltham, MA). Plasma osmolality will be determined by freezing-point depression osmometry (3D3; Advanced Instruments Inc., Norwood, MA). Plasma electrolyte concentrations and OSM_p will be measured in duplicate and averaged for statistical analysis.

Statistical Analysis and Calculations

Separate repeated measures ANOVAs will be used to determine the effects of ingesting multiple boluses of pickle juice on changes in plasma volume, OSM_p, $[Na^+]_p$, and $[K^+]_p$ over time. Shapiro-Wilk tests will be used to assess normality. Mauchly's test will be used to assess sphericity. Tukey-Kramer multiple comparison tests will be used to determine differences within each dependent variable at each time-point. Significance will be accepted when $P < 0.05$ (NCSS 2007, ver: 07.1.18, Kaysville, UT).

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APPENDIX B. ADDITIONAL METHODS

Table B1. Sample Size Estimate

Subject sample estimated using Gpower statistical software 3.1

Statistical Test: ANOVA: Repeated Measures, Within factors

Type of Power Analysis: Compute required sample size – given α, power, and effect size

Input Parameters

Effect Size f: .46

α **err prob:** 0.05

Power: 0.8

Number of Groups: 3

Number of Measurements: 5

Corr Among Rep Measures: 0.50

Nonsphericity correction: 1

Sample Size $n = 9$

Table B2. Subject Order Randomizations

Latin Square

Subject #	Day 1	Day 2	Day 3
1	0	1	2
2	1	2	0
3	2	0	1
4	0	1	2
5	1	2	0
6	2	0	1
7	0	1	2
8	1	2	0
9	2	0	1

Table B3. Data Collection Sheet

Name:__________________________ Subject # ______ Age (yrs) ___________

Date: ___________________________ Height (in) ______ Day 1 2 3

Bolus group (Circle): B0 B1 B2

Pre-Testing Questionnaire:	**Answer**	**Decision**
1.)Have you ingested at least 34oz (1 L) of water in the previous 12 hours?	YES NO	Reschedule if 'NO'
2.)Have you exercised strenuously within The last 48 hours?	YES NO	Reschedule if 'YES'
3.)Are you well rested (≥ 8 Hours) ?	YES NO	Reschedule if 'NO'
4.)Do you have any neurological, cardiovascular, or blood borne diseases?	YES NO	Disqualify if 'YES'
5.)Have you eaten within the last 12 hours?	YES NO	Reschedule if 'YES'
7.)Have you had any alcohol or Caffeine in the last 24 hours?	YES NO	Reschedule if 'YES'
8.)Do you have a history of heat illness such as heat fainting, heat stroke, or heat exhaustion?	YES NO	Disqualify if 'YES'
9.) Are you allergic to any ingredients in pickle juice?	YES NO	Disqualify if 'YES'
<u>Day 2-3 Questions</u>		
1.)Have you ingested at least 34oz (1 L) of water in the previous 12 hours?	YES NO	Reschedule if 'NO'
2.)Have you exercised strenuously within The last 48 hours?	YES NO	Reschedule if 'YES'
3.)Has your diet been consistent since last session?	YES NO	Reschedule if 'NO'
4.)Are you well rested (≥ 8 Hours)?	YES NO	Reschedule if 'NO'
5.)Have you eaten within the last 12 hours?	YES NO	Reschedule if 'YES'
6.)Has your diet been consistent for the past 24 hours?	YES NO	Reschedule if 'NO'
7.)Have you had any alcohol or Caffeine in the last 24 hours?	YES NO	Reschedule if 'YES'

(continued)

Table B3. Data Collection Sheet (continued)

Obtain Urine Sample – Must be < 1.02

Urine Sample 1 Specific Gravity: _______________

Towel Weight: _______kg

Attach heart rate monitor, subject will insert rectal thermistor in private

Subject's Target HR (HR_{MAX} = 0.80 x (220-age) to (0.85 x (220-age))______________bpm

Subject remains nude with towel

Clean and prepare arm for venipuncture

Insert venous catheter into arm

BW_1 (Nude Body Weight): ____________________ kg

Calculated Pickle Juice Volume (- towel weight) ____________ml

Sit and rest for 30 minutes – NO SWEAT SUIT YET

Blood Sample 1 at 30 minutes (Pre-Ingestion):

			AVG					AVG
Plasma $[Na^+]$	_____	_____	__________	Hct	______	_____	_____	______
Plasma $[K^+]$	_____	_____	__________	[Hb]	______	_____	_____	______
				OSM_p	______	_____		______

BW_2 (Nude body weight): ____________________ kg

Put sweat suit on in private

(continued)

Table B3. Data Collection Sheet (continued)

***************DRINK**************

B1 and B2 DRINK first bolus of pickle juice: 60 seconds to finish **B0 NO DRINK**

Temperature in Chamber: START _______°C _______%RH

Enter environmental chamber and begin exercise on semi-recumbent bike at 85-90% of max HR for 30 minutes

Signs and Symptoms of Heat Illness _____ Rectal Temp 10 min _____

_____ Rectal Temp 20 min_____

_____ Rectal Temp 30 min______

STOP exercising at 30 minutes

Blood Sample 2 at 30 minutes of exercise:

			AVG					AVG
Plasma [Na^+]	______	______	___________	Hct	_______	______	______	______
Plasma [K^+]	______	______	___________	[Hb]	_______	______	______	______
				OSM_p	_______	______		______

***************DRINK*************

B2 drink second bolus: 60 seconds! **B0 and B1 rest for 60 seconds**

Resume exercise for 30 minutes

Signs and Symptoms of Heat Illness _____ Rectal Temp 10 min _____

(continued)

Table B3. Data Collection Sheet (continued)

_____ Rectal Temp 20 min_____

_____ Rectal Temp 30 min_____

5 minute cool down after 60 total minutes of exercise- REMAIN SEATED

Blood Sample 3 at 60 minutes exercise:

			AVG					AVG
Plasma [Na^+]	______	_____	___________	Hct	______	_____	_____	______
Plasma [K^+]	______	_____	___________	[Hb]	______	_____	_____	______
				OSM_p	______	_____		

Temperature in Chamber END: _______°C _______%RH

Stand up and exit environmental chamber and towel dry, remove sweat suit

BW_3 (Nude body weight): ____________________ kg

Void bladder completely

Urine Volume______

BW_4 (Nude Body Weight): ____________________ kg

Remove HR Monitor and Rectal Thermistor
Sit and rest for 30 minutes

Blood Sample 4 (30 minutes Post-exercise):

			AVG					AVG
Plasma [Na^+]	______	____	___________	Hct	______	_____	_____	_____

(continued)

Table B3. Data Collection Sheet (continued)

Plasma $[K^+]$	_____	_____	__________	[Hb]	______	_____	_____	____
				OSM_p	______	_____		____

Blood Sample 5 (60 minutes Post-exercise):

			AVG					AVG
Plasma $[Na^+]$	______	_____	__________	Hct	______	_____	_____	_____
Plasma $[K^+]$	_____	_____	__________	[Hb]	______	_____	_____	____
				OSM_p	______	_____		____

SCHEDULE NEXT SESSION! **Date:______ Time:________**

Testing is complete: Remove catheter and excuse subject for the day

Table B4. Experimental Timeline

Overall Time	Post Ingestion Time	Procedure
0	-	Urine Sample 1 Specific Gravity, calculate HR, insert rectal thermistor probe, don HR monitor, prep arm for venipuncture
5		Insert catheter into arm
10		BW_1 (Nude)
15		Sit and rest for 30 minutes, calculate pickle juice ingestion
35		Blood sample 1
35	0	Void bladder, ingestion 1, $BW_{2,}$ put on sweat suit, enter heat chamber, begin 60 minute exercise period at 85- 90% max HR
65	30	Pause exercise, blood sample 2, Ingestion 2, resume after 60 seconds
95	60	Begin cool-down
100	65	Blood sample 3, exit heat chamber, BW_3, void bladder, BW_4, remove HR monitor and rectal thermistor, remove sweat suit.
100	65	Sit and rest for 30 minutes
130	95	Blood sample 4
160	125	Blood sample 5, remove catheter, excuse subject

BW – Body weight measurement (1, 2, 3, 4)
HR – Heart Rate

Table B5. Statistical Analysis

1. The effects of ingesting single and multiple boluses of pickle juice on $[Na^+]_p$, $[K^+]_p$, changes in plasma volume, and OSM_p up to 125 minutes post-ingestion

$[Na^+]_p$

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.01)
A: subject	8	93.05926	11.63241			
B: bolus	2	18.85926	9.429629	4.15	0.035377	0.359246
AB	16	36.37407	2.27338			
C: time	4	135.2333	33.80833	43.22	0.000000*	1.000000
AC	32	25.03333	0.7822917			
BC	8	3.9	0.4875	2.15	0.043775	0.581712
ABC	64	14.53333	0.2270833			
S	0					
Total (Adjusted)	134	326.9926				
Total	135					

* Term significant at alpha = 0.01

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	Regular F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: subject	8					
B: bolus	2	4.15	0.035377	0.076071	0.048915	0.038319
AB	16					
C: time	4	43.22	0.000000*	0.000174*	0.000001*	0.000000*
AC	32					
BC	8	2.15	0.043775	0.181033	0.096593	0.043775
ABC	64					
S	0					

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

Source Term	DF	F-Ratio	Regular Power (Alpha=0.01)	Lower Bound Epsilon Power (Alpha=0.01)	Geisser Greenhouse Epsilon Power (Alpha=0.01)	Huynh Feldt Epsilon Power (Alpha=0.01)
A: subject	8					
B: bolus	2	4.15	0.359246	0.174571	0.280583	0.339935
AB	16					
C: time	4	43.22	1.000000	0.993848	0.999999	1.000000
AC	32					
BC	8	2.15	0.581712	0.082397	0.309988	0.581712
ABC	64					
S	0					

(continued)

Table B5. Statistical Analysis (continued)

Covariance Matrix Circularity Section

Source Term		**Lower Bound Epsilon Circularity?**	**Geisser Greenhouse Epsilon**	**Huynh Feldt Epsilon**	**Mauchly Test Statistic**	**Chi2 Value**	**DF**	**Covariance ProbMatrix Level**
AB	0.500000	0.787446	0.947379	0.730071	2.2	2.00.332489		Okay
AC	0.250000	0.475056	0.618957	0.059676	18.1	9.00.034172		Violated
ABC	0.125000	0.507199	1.000000	0.000257	42.4	35.00.183055		Okay

$[K^+]_p$

Analysis of Variance Table

Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.01)**
A: subject	8	3.729667	0.4662083			
B: bolus	2	0.1763333	8.816667E-02	1.75	0.206136	0.118109
AB	16	0.808	0.0505			
C: time	4	5.360741	1.340185	20.43	0.000000*	0.999999
AC	32	2.099593	6.561227E-02			
BC	8	0.1212593	1.515741E-02	0.74	0.657641	0.125800
ABC	64	1.314407	2.053761E-02			
S	0					
Total (Adjusted)	134	13.61				
Total	135					

* Term significant at alpha = 0.01

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	8					
B: bolus	2	1.75	0.206136	0.222934	0.208258	0.206136
AB	16					
C: time	4	20.43	0.000000*	0.001951*	0.000009*	0.000000*
AC	32					
BC	8	0.74	0.657641	0.415291	0.543710	0.606592
ABC	64					
S	0					

(continued)

Table B5. Statistical Analysis (continued)

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

Source Term	DF	F-Ratio	Regular Power (Alpha=0.01)	Lower Bound Epsilon Power (Alpha=0.01)	Geisser Greenhouse Epsilon Power (Alpha=0.01)	Huynh Feldt Epsilon Power
	(Alpha=0.01)					
A: subject	8					
B: bolus	2	1.75	0.118109	0.066338	0.112829	0.118109
AB	16					
C: time	4	20.43	0.999999	0.835879	0.998677	
	0.999985					
AC	32					
BC	8	0.74	0.125800	0.030826	0.059525	
	0.088804					
ABC	64					
S	0					

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	0.500000	0.950276	1.000000	0.947675	0.4	2.0	0.828528	Okay
AC	0.250000	0.592612	0.858596	0.160512	11.7	9.0	0.228452	Okay
ABC	0.125000	0.388369	0.663251	0.000013	57.8	35.0	0.009039	Violated

OSM$_p$

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power
	(Alpha=0.01)					
A: subject	8	830.7	103.8375			
B: bolus	2	46.03333	23.01667	2.45	0.117661	0.182856
AB	16	150.1	9.38125			
C: time	4	445.1963	111.2991	61.40	0.000000*	1.000000
AC	32	58.0037	1.812616			
BC	8	35.55926	4.444907	2.10	0.048852	0.566172
ABC	64	135.6407	2.119387			
S	0					
Total (Adjusted)	134	1701.233				
Total	135					

* Term significant at alpha = 0.01

(continued)

Table B5. Statistical Analysis (continued)

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	8					
B: bolus	2	2.45	0.117661	0.155899	0.128129	0.117661
AB	16					
C: time	4	61.40	0.000000*	0.000051*	0.000000*	0.000000*
AC	32					
BC	8	2.10	0.048852	0.185594	0.101331	0.048852
ABC	64					
S	0					

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

Source Term	DF	F-Ratio	Regular Power (Alpha=0.01)	Lower Bound Epsilon Power (Alpha=0.01)	Geisser Greenhouse Epsilon Power (Alpha=0.01)	Huynh Feldt Epsilon Power (Alpha=0.01)
A: subject	8					
B: bolus	2	2.45	0.182856	0.095322	0.156637	0.182856
AB	16					
C: time	4	61.40	1.000000	0.999687	1.000000	1.000000
AC	32					
BC 8	2.10	0.566172	0.080360	0.306487	0.566172	
ABC	64					
S	0					

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	0.500000	0.854232	1.000000	0.829358	1.3	2.0	0.519513	Okay
AC	0.250000	0.537214	0.740868	0.074810	16.6	9.0	0.054710	Okay
ABC	0.125000	0.517929	1.000000	0.005757	26.4	35.0	0.850984	Okay

(continued)

Table B5. Statistical Analysis (continued)

Tukey-Kramer Multiple-Comparison Test

Response: OSMp
Term BC: bolus,time

Alpha=0.010 Error Term=ABC DF=64 MSE=2.119387 Critical Value=5.7629

Group	Count	Mean	Different From Groups
0,pre	9	283.6667	(0,125post), (0,immpost), (0,95post) (1,95post), (1,125post), (2,during) (0,during), (2,immpost), (2,95post) (1,immpost), (1,during), (2,125post)
2,pre	9	283.7778	(0,125post), (0,immpost), (0,95post) (1,95post), (1,125post), (2,during) (0,during), (2,immpost), (2,95post) (1,immpost), (1,during), (2,125post)
1,pre	9	284.2778	(0,immpost), (0,95post), (1,95post) (1,125post), (2,during), (0,during) (2,immpost), (2,95post), (1,immpost) (1,during), (2,125post)
0,125post	9	286.8333	(0,pre), (2,pre)
0,immpost	9	287.1667	(0,pre), (2,pre), (1,pre)
0,95post	9	287.2778	(0,pre), (2,pre), (1,pre)
1,95post	9	288	(0,pre), (2,pre), (1,pre)
1,125post	9	288.2222	(0,pre), (2,pre), (1,pre)
2,during	9	288.5	(0,pre), (2,pre), (1,pre)
0,during	9	288.5	(0,pre), (2,pre), (1,pre)
2,immpost	9	288.8889	(0,pre), (2,pre), (1,pre)
2,95post	9	289.2778	(0,pre), (2,pre), (1,pre)
1,immpost	9	289.3889	(0,pre), (2,pre), (1,pre)
1,during	9	289.3889	(0,pre), (2,pre), (1,pre)
2,125post	9	289.5	(0,pre), (2,pre), (1,pre)

Changes in Plasma Volume

Analysis of Variance Table

Source Term	Sum of DF (Alpha=0.01)	Mean Squares	Square	Prob F-Ratio	Power Level	
A: subject	8	917.2271		114.6534		
B: bolus	2	1.019981		0.5099907	0.02	0.984699
	0.010544					
AB	16	528.7032		33.04395		
C: time	4	2107.839		526.9597	31.48	0.000000*
	1.000000					
AC	32	535.671	16.73972			
BC	8	57.03751		7.129688	1.18	0.324268
	0.255912					
ABC		64	386.2772	6.035581		
S		0				
Total (Adjusted)		134	4533.774			
Total		135				

* Term significant at alpha = 0.01

(continued)

Table B5. Statistical Analysis (continued)

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	8					
B: bolus	2	0.02	0.984699	0.904196	0.945804	0.958816
AB	16					
C: time	4	31.48	0.000000*	0.000504*	0.000000*	0.000000*
AC	32					
BC	8	1.18	0.324268	0.308756	0.338050	0.328172
ABC	64					
S	0					

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

Source Term	DF	F-Ratio	Regular Power (Alpha=0.01)	Lower Bound Epsilon Power (Alpha=0.01)	Geisser Greenhouse Epsilon Power (Alpha=0.01)	Huynh Feldt Epsilon Power (Alpha=0.01)
A: subject	8					
B: bolus	2	0.02	0.010544	0.010385	0.010442	0.010467
AB	16					
C: time	4	31.48	1.000000	0.963564	1.000000	1.000000
AC	32					
BC	8	1.18	0.255912	0.045520	0.121232	0.225803
ABC	64					

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	0.500000	0.650812	0.725148	0.463459	5.4	2.0	0.067770	Okay
AC	0.250000	0.681359	1.000000	0.295800	7.8	9.0	0.552795	Okay
ABC	0.125000	0.454516	0.880102	0.000332	41.1	35.0	0.222181	Okay

(continued)

Table B5. Statistical Analysis (continued)

Tukey-Kramer Multiple-Comparison Test

Response: PlasmaVolume
Term BC: bolus,time
Alpha=0.010 Error Term=ABC DF=64 MSE=6.035581 Critical Value=5.7629

Group	Count	Mean	Different From Groups
2,during	9	-12.13367	(0,immpost), (2,immpost), (2,95post) (0,95post), (1,95post), (2,pre), (0,pre) (1,pre)
1,during	9	-11.09177	(2,immpost), (2,95post), (0,95post) (1,95post), (2,pre), (0,pre), (1,pre)
0,during	9	-10.70505	(2,95post), (0,95post), (1,95post), (2,pre) (0,pre), (1,pre)
0,125post	9	-9.067203	(2,95post), (0,95post), (1,95post), (2,pre) (0,pre), (1,pre)
1,immpost	9	-8.936328	(2,95post), (0,95post), (1,95post), (2,pre) (0,pre), (1,pre)
1,125post	9	-8.123158	(0,95post), (1,95post), (2,pre), (0,pre) (1,pre)
2,125post	9	-7.825338	(1,95post), (2,pre), (0,pre), (1,pre)
0,immpost	9	-7.184781	(2,during), (2,pre), (0,pre), (1,pre)
2,immpost	9	-6.169334	(2,during), (1,during), (2,pre), (0,pre) (1,pre)
2,95post	9	-4.073045	(2,during), (1,during), (0,during) (0,125post), (1,immpost)
0,95post	9	-3.320457	(2,during), (1,during), (0,during) (0,125post), (1,immpost), (1,125post)
1,95post	9	-3.007767	(2,during), (1,during), (0,during) (0,125post), (1,immpost), (1,125post) (2,125post)
2,pre	9	-2.442491E-15	(2,during), (1,during), (0,during) (0,125post), (1,immpost), (1,125post) (2,125post), (0,immpost), (2,immpost)
0,pre	9	-9.992007E-16	(2,during), (1,during), (0,during) (0,125post), (1,immpost), (1,125post) (2,125post), (0,immpost), (2,immpost)
1,pre	9	-4.440892E-16	(2,during), (1,during), (0,during) (0,125post), (1,immpost), (1,125post) (2,125post), (0,immpost), (2,immpost)

Changes in Plasma Na^+ Content

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.01)
A: subject	8	774.0774	96.75967			
B: bolus	2	171.3478	85.67388	2.67	0.100166	0.203800
AB	16	514.1803	32.13627			
C: time	4	1031.11	257.7774	17.82	0.000000*	0.999987
AC	32	462.8061	14.46269			
BC	8	150.4612	18.80764	3.21	0.003994*	0.835770
ABC	64	375.2411	5.863143			
S	0					
Total (Adjusted)	134	3479.223				
Total	135					

* Term significant at alpha = 0.01

(continued)

Table B5. Statistical Analysis (continued)

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	8					
B: bolus	2	2.67	0.100166	0.141159	0.105532	0.100166
AB	16					
C: time	4	17.82	0.000000*	0.002909*	0.000013*	0.000000*
AC	32					
BC	8	3.21	0.003994*	0.111065	0.033618	0.008829*
ABC	64					
S	0					

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

Source Term	DF	F-Ratio	Regular Power (Alpha=0.01)	Lower Bound Epsilon Power (Alpha=0.01)	Geisser Greenhouse Epsilon Power (Alpha=0.01)	Huynh Feldt Epsilon Power (Alpha=0.01)
A: subject	8					
B: bolus	2	2.67	0.203800	0.104575	0.188629	0.203800
AB	16					
C: time	4	17.82	0.999987	0.774152	0.997490	0.999973
AC	32					
BC	8	3.21	0.835770	0.129156	0.442538	0.727290
ABC	64					
S	0					

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	0.500000	0.925826	1.000000	0.919883	0.6	2.0	0.746560	Okay
AC	0.250000	0.634179	0.953203	0.196808	10.4	9.0	0.316773	Okay
ABC	0.125000	0.426414	0.781863	0.000400	40.1	35.0	0.254370	Okay

(continued)

Table B5. Statistical Analysis (continued)

Tukey-Kramer Multiple-Comparison Test
Response: NaContent
Term BC: bolus,time
Alpha=0.010 Error Term=ABC DF=64 MSE=5.863143 Critical Value=5.7629

Group	Count	Mean	Different From Groups
2,during	9	-7.284406	(0,95post), (1,125post), (2,immpost) (0,pre), (2,pre), (1,pre), (2,125post) (1,95post), (2,95post)
0,during	9	-6.904151	(0,95post), (1,125post), (2,immpost) (0,pre), (2,pre), (1,pre), (2,125post) (1,95post), (2,95post)
1,during	9	-6.334053	(0,pre), (2,pre), (1,pre), (2,125post) (1,95post), (2,95post)
0,immpost	9	-5.499087	(0,pre), (2,pre), (1,pre), (2,125post) (1,95post), (2,95post)
1,immpost	9	-4.713258	(0,pre), (2,pre), (1,pre), (2,125post) (1,95post), (2,95post)
0,125post	9	-4.32328	(2,125post), (1,95post), (2,95post)
0,95post	9	-2.19878	(2,during), (0,during), (2,95post)
1,125post	9	-1.931798	(2,during), (0,during), (2,95post)
2,immpost	9	-1.775138	(2,during), (0,during), (2,95post)
0,pre	9	-3.885781E-16	(2,during), (0,during), (1,during) (0,immpost), (1,immpost)
2,pre	9	-1.110223E-16	(2,during), (0,during), (1,during) (0,immpost), (1,immpost)
1,pre	9	2.109424E-15	(2,during), (0,during), (1,during) (0,immpost), (1,immpost)
2,125post	9	0.5181576	(2,during), (0,during), (1,during) (0,immpost), (1,immpost), (0,125post)
1,95post	9	1.035398	(2,during), (0,during), (1,during) (0,immpost), (1,immpost), (0,125post)
2,95post	9	3.413815	(2,during), (0,during), (1,during) (0,immpost), (1,immpost), (0,125post) (0,95post), (1,125post), (2,impost)

Changes in Plasma K^+ Content

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.01)
A: subject	8	624.7302	78.09128			
B: bolus	2	141.7141	70.85703	0.78	0.473037	0.047615
AB	16	1444.73	90.29565			
C: time	4	484.003	121.0007	3.42	0.019541	0.550900
AC	32	1133.013	35.40667			
BC	8	236.32	29.54	2.38	0.025818	0.651805
ABC	64	793.3354	12.39587			
S	0					
Total (Adjusted)	134	4857.847				
Total	135					

* Term significant at alpha = 0.01

(continued)

Table B5. Statistical Analysis (continued)

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		8					
B: bolus		2	0.78	0.473037	0.401543	0.465455	0.473037
AB	16						
C: time	4	3.42	0.019541	0.101689	0.063941	0.047996	
AC	32						
BC	8	2.38	0.025818	0.161235	0.077072	0.029482	
ABC	64						
S	0						

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

Source Term	**DF**	**F-Ratio**	**Regular Power (Alpha=0.01)**	**Lower Bound Epsilon Power (Alpha=0.01)**	**Geisser Greenhouse Epsilon Power (Alpha=0.01)**	**Huynh Feldt Epsilon Power (Alpha=0.01)**	
A: subject		8					
B: bolus		2	0.78	0.047615	0.032301	0.045487	0.047615
AB	16						
C: time	4	3.42	0.550900	0.139011	0.256598	0.330911	
AC	32						
BC	8	2.38	0.651805	0.092308	0.330379	0.618575	
ABC	64						
S	0						

Covariance Matrix Circularity Section

Source Term	**Lower Bound Epsilon Circularity?**	**Geisser Greenhouse Epsilon**	**Huynh Feldt Epsilon**	**Mauchly Test Statistic**	**Chi2 Value**	**Covariance ProbMatrix DFLevel**	
AB	0.500000	0.928231	1.000000	0.922682	0.6	2.00.754541	Okay
AC	0.250000	0.457222	0.585794	0.081868	16.1	9.00.065667	Okay
ABC	0.125000	0.468660	0.933475	0.000229	43.0	35.00.167162	Okay

(continued)

Table B5. Statistical Analysis (continued)

Tukey-Kramer Multiple-Comparison Test

Response: KContent
Term BC: bolus,time

Alpha=0.010 Error Term=ABC DF=64 MSE=12.39587 Critical Value=5.7629

Group	Count	Mean	Different From Groups
0,immpost	9	-2.034463	(1,during), (2,95post), (0,during) (2,125post)
1,immpost	9	-1.53969	(0,during), (2,125post)
1,pre	9	-1.554312E-15	
2,pre	9	-1.554312E-15	
0,pre	9	-4.440892E-16	
0,95post	9	0.3756406	
1,125post	9	0.8220208	
0,125post	9	1.577631	
2,immpost	9	2.411689	
1,95post	9	3.447363	
2,during	9	3.496103	
1,during	9	4.831219	(0,immpost)
2,95post	9	4.928869	(0,immpost)
0,during	9	5.372877	(0,immpost), (1,immpost)
2,125post	9	6.277623	(0,immpost), (1,immpost)

Figure B1. $[Na^+]_p$, Changes in Na^+ Content, Changes in Plasma Volume

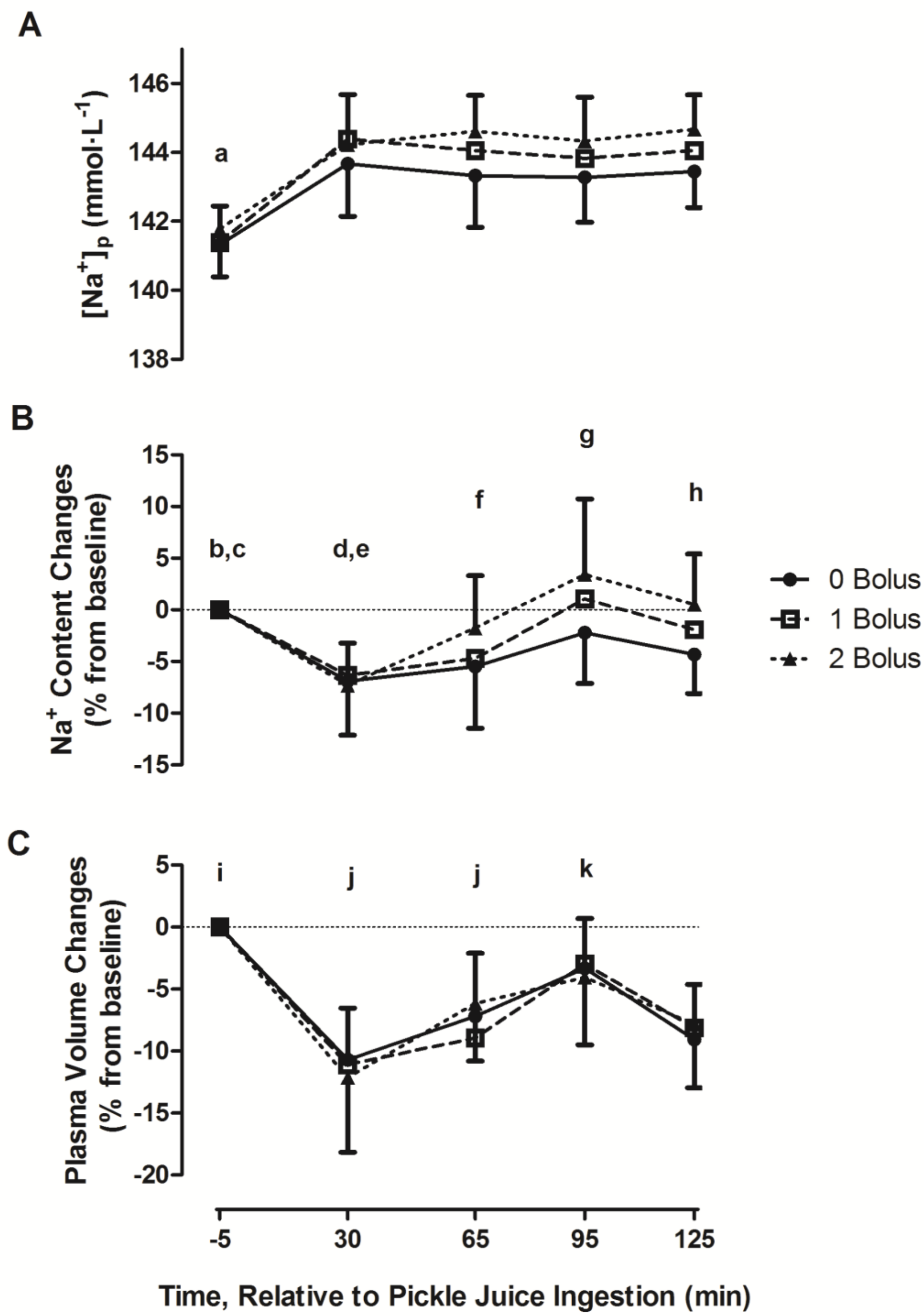

Figure B2. $[K^+]_p$, Changes in K^+ Content, Changes in Plasma Volume

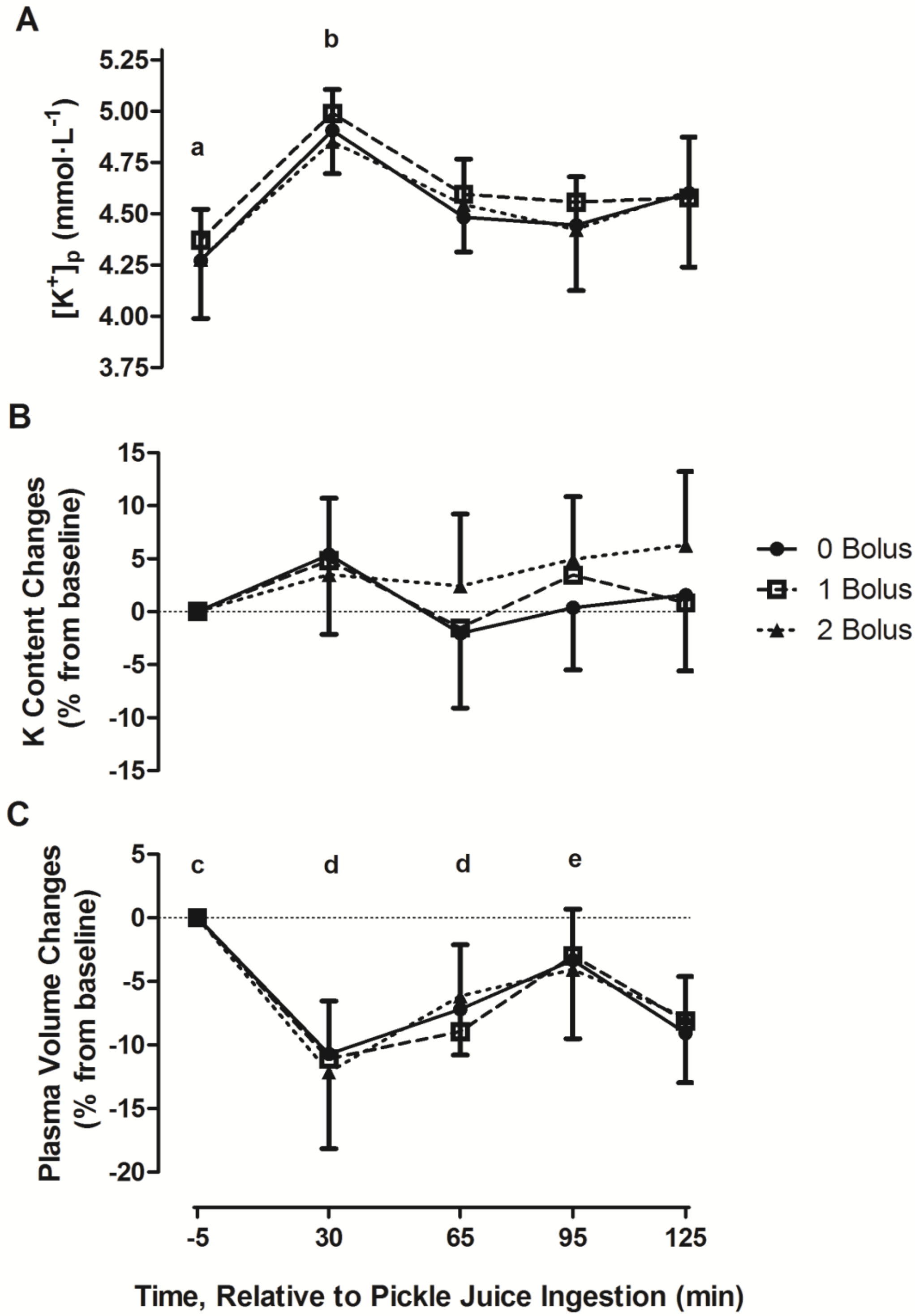

Figure B3. OSM_p

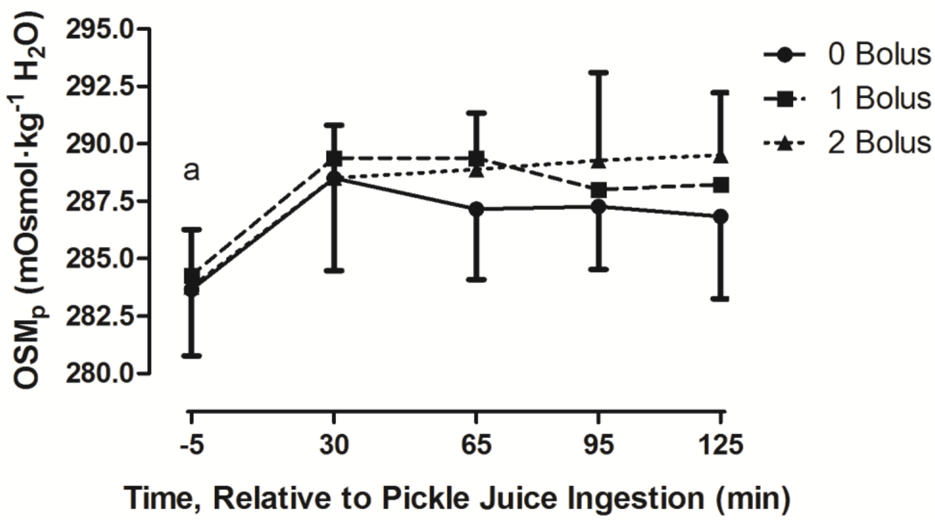

Figure B4. Institutional Review Board Approval Letter

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

June 20, 2012

Kevin C. Miller
Department of Health, Nutrition & Exercise Sciences
BBFH

IRB Approval of Protocol **#HE12214**, **"THe effect of drinking multiple boluses of pickle juice on plasma variables"**
Co-investigator(s) and research team: **Michael A. McKenney, Jared Tucker, Julie Garden-Robinson, Jim Deal**

Approval period: June 20, 2012 to June 19, 2013 **Continuing Review Report Due: May 1, 2013**

Research site(s): **NDSU** Funding agency: **n/a**
Review Type: Full Board, meeting date – June 8, 2012
Risk Level: A minor increase over minimal risk
IRB approval is based on original submission, with revised: protocol, consent, and data collection sheet (received 6/20/2012).

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,

Kristy Shirley

Kristy Shirley, CIP
Research Compliance Administrator

Last printed 6/20/2012 11:39:00 AM

NDSU is an EO/AA university.

Figure B5. Institutional Review Board Amendment

Institutional Review Board ...for the protection of human participants in research

North Dakota State University
Sponsored Programs Administration
1735 NDSU Research Park Drive
NDSU Dept #4000
PO Box 6050
Fargo, ND 58108-6050 231-8995(ph) 231-8098(fax)

RECEIVED
SEP 04 2012
INSTITUTIONAL REVIEW BOARD

Protocol Amendment Request Form

Changes to approved research may not be initiated without prior IRB review and approval, except where necessary to eliminate apparent immediate hazards to participants. Reference*:* SOP 7.5 Protocol Amendments.

Examples of changes requiring IRB review include, but are not limited to changes in: investigators or research team members, purpose/scope of research, recruitment procedures, compensation scheme, participant population, research setting, interventions involving participants, data collection procedures, or surveys, measures or other data forms.

Protocol Information:

Protocol #: **HE12214** Title: **The effect of drinking multiple boluses of pickle juice on plasma variables**

Review category: ☐ Exempt ☒ Expedited ☐ Full board

Principal investigator: **Kevin C. Miller, PhD, ATC** Email address: **kevin.c.miller@ndsu.edu**
Dept: **HNES**

Co-investigator: **Mike McKenney, ATC** Email address: **michael.mckenney@my.ndsu.edu**
Dept: **HNES**

Principal investigator signature, Date: Kevin Miller via email 9/4/12

Description of proposed changes:

1. Date of proposed implementation of change(s)*: **9-4-12**
   ** Cannot be implemented prior to IRB approval unless the IRB Chair has determined that the change is necessary to eliminate apparent immediate hazards to participants.*

2. Describe proposed change(s), including justification:
   Dr. Jared Tucker left NDSU and was on the current protocol and informed consent document. Dr. Yeong Rhee will replace Dr. Tucker on this project.
   7/2/11 – MH

3. Will the change involve a change in principal or co- investigator?
   ☒ No
   ☐ Yes: *Include an Investigator's Assurance (last page of protocol form), signed by the new PI or co-investigator.*

Protocol Amendment Request Form
NDSU Institutional Review Board
Form revised Feb 2011

Note: If the change is limited to addition/change in research team members, skip the rest of this form.

4. Will the change(s) increase any risks, or present new risks *(physical, economic, psychological, or sociological)* to participants?
   ☐ No
   ☐ Yes: *In the appropriate section of the protocol form, describe new or altered risks and how they will be minimized.*

5. Does the proposed change involve the addition of a vulnerable group of participants?
   Children: ☐ no ☐ yes – include the *Children in Research* attachment form
   Prisoners: ☐ no ☐ yes – include the *Prisoners in Research* attachment form
   Cognitively impaired individuals: ☐ no ☐ yes*
   Economically or educationally disadvantaged individuals: ☐ no ☐ yes*

   **Provide additional information where applicable in the revised protocol form.*

6. Does the proposed change involve a request to waive some or all the elements of informed consent or documentation of consent?
   ☐ no
   ☐ yes – include the *Informed Consent Waiver or Alteration Request* attachment form

7. Does the proposed change involve a new research site?
   ☐ no
   ☐ yes – include a letter of permission/cooperation, IRB approval, or grant application or contract

If information in your previously approved protocol has changed, or additional information is being added, incorporate the changes into relevant section(s) of the protocol. Highlight (e.g. print and highlight the hard copy, or indicate changes using all caps, asterisks, etc) the changed section(s) and attach a copy of the revised protocol to this form. *(If the changes are limited to addition/change in research team members, a revised protocol form is not needed.)*

Impact for Participants (future, current, or prior):

1. Will the change(s) alter information on previously approved versions of the recruitment materials, informed consent, or other documents, or require new documents?
   ☐ No
   ☐ Yes - attach revised/new document(s)

2. Could the change(s) affect the willingness of *currently* enrolled participants to continue in the research?
   ☐ No
   ☐ Yes - describe procedures that will be used to inform current participants, and re-consent, if necessary:

3. Will the change(s) have any impact to *previously* enrolled participants?

☐ No
☐ Yes - describe impact, and any procedures that will be taken to protect the rights and welfare of participants:

- - - - - - - - - - -FOR IRB OFFICE USE ONLY - - - - - - - - -

Request is: ☒ **Approved** ☐ **Not Approved** **Review:** ☐**Exempt, category#:** ___ ☒**Expedited method, ~~category #~~** ___ ☐**Convened meeting, date:** ___ minor change.	
IRB Signature: Kristy Shuley	**Date:** 9/4/12
Comments:	

<u>Protocols previously declared exempt:</u> (Allow 5 working days) If the proposed change does not alter the exemption status, the change may be administratively reviewed by qualified IRB staff, chair, or designee. If the change(s) would alter this status, Expedited or Full Board review will be required.

<u>Protocols previously reviewed by the expedited method:</u> (Allow 10 working days) Most changes may also be reviewed by the expedited method, unless the change would increase risks to more than minimal, and/or alter the eligibility of the project for expedited review.

<u>Protocols previously reviewed by the full board:</u> Minor changes (not involving more than minimal risks, or not significantly altering the research goals or design) may be reviewed by the expedited method (allow 10 working days). Those changes determined by the IRB to be more than minor will require review by the full board (due 10 working days prior to next scheduled meeting).

Figure B6. Institutional Review Board Consent to be a Research Subject

Institutional Review Board
North Dakota State University
PROTOCOL #: HE12214
APPROVED: 9/4/12
EXPIRES: 10/9/13

NDSU North Dakota State University
Department of Health, Nutrition, and Exercise Sciences
PO Box 6050
Fargo, ND 58108-6050
701-730-6249

Title of Research Study: The effect of drinking multiple boluses of pickle juice on plasma variables

This study is being conducted by: Kevin C. Miller, PhD, ATC; Michael A. McKenney, ATC, NASM-CES; Yeong Rhee, PhD, RD; Julie Garden-Robinson, PhD, LRD; and Jim Deal, PhD.

Why am I being asked to take part in this research study? You are being asked to volunteer for this study because you: (1) are a healthy male (18-35), (2) have no food allergies to pickles or pickle juice, (3) have no history of cardiovascular, neurological, or any blood borne diseases, (4) do not have a history of heat related illnesses (ex: fainting, heat stroke, or heat exhaustion), (5) have not sustained a lower extremity injury within the last12 months, (6) have not been diagnosed or a family history of anemia, (7) and can sustain exercise at 85-90% of max heart rate for one hour with a brief break at 30 minutes. Should you have a known sensitivity to pickles or exercise in the heat, you should not participate in this study.

What is the reason for doing the study? The purpose of this study is to determine what happens when you drink pickle juice multiple times during exercise, and what happens to your blood sodium and potassium levels.

What will I be asked to do? You will come to room 14 in the Bentson Bunker Fieldhouse on 3 days separated by at least 48 hours. We request that you drink water prior to each testing session, maintain a consistent diet, and avoid alcohol, caffeine, or strenuous exercise for 24 hours prior to your testing sessions. You must also bring a hooded sweatshirt and sweat pants to each testing day.

On the first day of testing, you will provide written consent by signing your name at the end of this form. We will then ask you questions about your health. First, you will empty your bladder into a graduated cylinder. Your forearm will be cleaned with alcohol and a trained phlebotomist (an individual trained in taking blood) will insert a needle in your arm, and a catheter will be put in place. The catheter is a flexible tube that is very small, and will remain in your arm so the needle may be removed. The catheter allows small amounts of blood to be drawn during your testing session without the repeated use of needles. Once the needle is removed, you will be able to move freely without fear of being stuck by the needle. Then, you will put on a heart rate monitor and insert a rectal thermometer. Once all equipment is on, you will be weighed nude behind a towel (and for all body weight measurements). You will then sit in a chair for 30 minutes. You will drink pickle juice 0, 1, or 2 times throughout testing depending on the testing day. On the days you drink, you will drink 1 mL of pickle juice for every kg of your body weight.

After the 30 minute rest period, we will take a 5 mL (¼ oz) blood sample. You will stand up and empty your bladder into a graduated cylinder. If it is a day where you drink pickle juice, you will now drink pickle juice. Then, you will be weighed and enter into a heat chamber to exercise. You will bike at a high intensity for 30 minutes on a semi-recumbent bike. You will stop at 30 minutes and a 5-mL blood sample will be taken. If you are assigned 2 drinks for your testing session, you will have 60 seconds to ingest your second drink at this time.

Once you have finished drinking, you will bike for another 30 minutes on the semi-recumbent bike. Once the 60 minute exercise period has concluded, you will complete a 5-minute cool down at a self-selected pace. When you finish the cool down, another 5-mL blood sample will be taken before you stand up. Then you will stand up, exit the heat chamber, and be weighed. You will then empty your bladder and have one final weight measurement. After the final weight, you will remove the heart rate monitor and rectal thermometer. You will then sit for 30 minutes and have two more 5-mL blood samples taken at 30 and 60 minutes post-exercise. You will complete the entire exercise protocol every session. If at any time during the study your temperature exceeds 39.5°C, any signs and symptoms of heat illness become evident, or you wish to stop, testing will end for the day.

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 for each testing session. Each session will last from 2.5-3 hours. Total participation for this study will be 7.5-9 hours over three days.

What are the risks and discomforts? (1) You could develop an infection at the site where your blood is drawn. However, due to the universal precautions that will be in use for handling blood or coming in contact with you, your risk will be near zero. The precautions include: using non-latex gloves at all times, alcohol will be utilized to disinfect all injection sites, and sterile equipment will be used during every testing session. Catheters will be used and disposed of after every session. You will also be informed of the signs of infection which include: redness, swelling, increase in body temperature, pus discharge, and pain. You will also be taught what to do if an infection occurs. (2) You could potentially develop a heat related condition to include fainting, heat exhaustion, or heat stroke. Your risk of these conditions is minimal due to the short duration you will be in a hot environment. Also, your core temperature will be continuously monitored to make sure it is within safe levels (Below 103°F). Should you have a history of heat related illness, you should not participate in this study. If a medical emergency should occur, the primary investigator will provide emergency care since he is a certified and licensed athletic trainer. Likely course of action will include being removed from the hot environment and being given cool liquids wile ice packs are placed under your arms, legs and head. (3) You could possibly have a cardiovascular event. If you have an unknown heart condition, exercising at a high intensity may place additional stress on your heart. If you have a family history of cardiac events, you should not participate in this study. To minimize this risk, we have very specific exclusion criteria for this study that are associated with cardiovascular events. (4) You may develop nausea, upset stomach, vomiting, or become uncomfortable following either blood sampling or drinking of pickle juice. If you are known to have a sensitivity to any food or food ingredient, or have had violent allergic reactions to drugs, chemicals, or food ingredients, you should not take part in this study.

What are the benefits to me? You are not expected to get any benefit from being in this research study.

What are the benefits to other people? Approximately 25% of athletic trainers use pickle juice to treat muscle cramps at various times during sport participation. However, there is little research evaluating the effects of drinking multiple boluses of pickle juice during these athletic

Institutional Review Board
North Dakota State University
PROTOCOL #: HE12214
APPROVED: 9/4/12
EXPIRES: 6/19/13

activities. This study can potentially provide more information about pickle juice ingestion during exercise.

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 between 2.5-3 hours of your time on 3 separate days. Total time spent will be between 7.5-9 hours.

What are the alternatives to being in this research study? Instead of being in this research study, you can choose not to participate.

Who will see the information that I give? We will keep private all research records that identify you. Your information will be combined with information from other people taking part in the study. When we write about the study, we will write about the combined information 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 that you gave us information, or what that information is. 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. Or if you are not able to complete the exercise phase of the testing, you will be removed from the study.

Will I receive any compensation for taking part in this study? Yes, you will be compensated $30 for your time.

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 Dr. Margret 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.

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 researchers, Michael A McKenney at 701-730-6249 and michael.mckenney@ndsu.edu or Dr. Kevin C. Miller at 701-231-5686 and kevin.C.miller@ndsu.edu.

Institutional Review Board
North Dakota State University
PROTOCOL #: HE12214
APPROVED: 9/4/12
EXPIRES: 6/19/13

What are my rights as a research participant?
You have rights as a participant in research. If you have questions about your rights, or complaints about this research [may add, "or to report a research-related injury" if applicable], 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
.

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 will be given a copy of this consent form to keep.

____________________________________ ________________
Your signature Date

____
Your printed name

____________________________________ ________________
Signature of researcher explaining study Date

____
Printed name of researcher explaining study

Institutional Review Board
North Dakota State University
PROTOCOL #: HE12214
APPROVED: 9/4/12
EXPIRES: 6/19/13

Revised April 2012

Figure B7. 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 C1. Blood Data

Subject #	Fluid	Time	$[K^+]_p$	$[Na^+]_p$	Hct	Hb g/dl	Plasma Volume	OSM_p
1	No PJ	Pre	4.8	142.5	43.33	15.72506	0	286.5
1	No PJ	During	5	146	44.33	17.16058	-9.98222	292
1	No PJ	immed post	4.45	146	44	16.23601	-4.29209	289.5
1	No PJ	90 min post	4.6	145.5	44.66	16.23601	-5.42007	290
1	No PJ	120 min post	4.6	145	45	16.86861	-9.52628	287.5
1	1 dose	Pre	4.65	142	42.33	14.80049	0	285.5
1	1 dose	During	5	145	42.66	15.65207	-5.98178	289
1	1 dose	immed post	4.7	146	44	16.33333	-12.0088	291
1	1 dose	90 min post	4.9	145.5	41.33	14.67883	2.57715	291
1	1 dose	120 min post	4.9	145.5	43.33	16.382	-11.2205	290
1	2 doses	Pre	4.5	142	40.66	15.95062	0	282
1	2 doses	During	5.05	145	43.33	16.91358	-9.93675	290
1	2 doses	immed post	4.6	146	42.33	16.88889	-8.2135	291
1	2 doses	90 min post	4.5	145.5	41.25	16.88889	-6.49459	296
1	2 doses	120 min post	4.6	146	42.33	16.91358	-8.34749	293
2	No PJ	Pre	4.1	142	42.33	15.48148	0	283.5
2	No PJ	During	5	144	44.66	17.1358	-13.3044	283.5
2	No PJ	immed post	4.5	143.5	44	16.19753	-7.1885	283.5
2	No PJ	90 min post	4.35	143	43	15.62963	-2.09864	285
2	No PJ	120 min post	4.5	143	43.33	16.71605	-8.99146	284
2	1 dose	Pre	4.2	142	41.83	15.21411	0	283
2	1 dose	During	5.2	144	43	15.94404	-6.49732	289.5
2	1 dose	immed post	4.6	143	42.33	15.6764	-3.78314	288.5
2	1 dose	90 min post	4.4	143	41.33	15.18978	1.021106	287.5
2	1 dose	120 min post	4.3	143.5	41.83	15.84672	-3.99202	287
2	2 doses	Pre	4.2	141	41	14.54321	0	283
2	2 doses	During	5.2	144.5	42.8	16.98765	-17.0014	290.5
2	2 doses	immed post	4.6	143.5	41.33	15.40741	-6.13692	288
2	2 doses	90 min post	4.5	143	41.33	15.7037	-7.90793	288
2	2 doses	120 min post	4.6	144	40.66	16.37037	-10.6494	287
3	No PJ	Pre	4.15	139.5	39	15.98519	0	281
3	No PJ	During	4.6	142	43.5	18.03457	-17.9024	286.5
3	No PJ	immed post	4.4	142	43.66	17.24444	-14.3839	287.5
3	No PJ	90 min post	4.3	142	42	15.96049	-4.77094	286
3	No PJ	120 min post	4.6	143	42.5	17.61481	-14.4584	287.5
3	1 dose	Pre	4.4	142	38.66	15.0963	0	284.5

(continued)

Table C1. Blood Data (continued)

Subject #	Fluid	Time	$[K^+]_p$	$[Na^+]_p$	Hct	Hb g/dl	Plasma Volume	OSM_p
3	1 dose	During	4.9	145	41.16	16.77531	-13.6766	290.5
3	1 dose	immed post	4.6	144.5	40.25	15.56543	-5.52794	288.5
3	1 dose	90 min post	4.6	144	39.33	15.88642	-6.01153	288
3	1 dose	120 min post	4.75	145	40.08	15.88642	-7.17341	289.5
3	2 doses	Pre	4.1	141.5	39.33	13.12099	0	285
3	2 doses	During	5	145	42.33	16.18272	-22.929	289
3	2 doses	immed post	4.65	144	38.9	15.46667	-14.5648	288.5
3	2 doses	90 min post	4.5	144	38.33	14.70123	-9.27799	288.5
3	2 doses	120 min post	4.7	144	40.08	15.6642	-17.2713	288.5
4	No PJ	Pre	4	141	47.33	17.06326	0	281
4	No PJ	During	4.7	143	49.66	18.15815	-10.1868	287
4	No PJ	immed post	4.7	142.5	50	18.20681	-11.0318	286
4	No PJ	90 min post	4.4	143	48.66	17.3309	-4.03045	286
4	No PJ	120 min post	4.8	143	49.33	18.20681	-9.83963	286
4	1 dose	pre	4.6	142	46.16	16.60097	0	287
4	1 dose	during	5.1	145	49.33	17.3309	-9.85155	290.5
4	1 dose	immed post	4.7	145	49.33	17.98783	-13.1439	290
4	1 dose	90 min post	4.6	145	47.33	16.96594	-4.27751	288.5
4	1 dose	120 min post	4.7	144.5	47.83	17.13625	-6.12856	287
4	2 doses	pre	4.1	143	46.16	16.84428	0	286.5
4	2 doses	during	4.5	146	48.66	17.72019	-9.35688	290.5
4	2 doses	immed post	4.45	146	47.66	17.18491	-4.71297	291
4	2 doses	90 min post	4.5	146	46	17.23358	-1.96847	291.5
4	2 doses	120 min post	4.7	146	46.33	17.6472	-4.85123	291
5	No PJ	pre	4.25	140.5	40.66	15.58025	0	283.5
5	No PJ	during	4.8	144	43.16	16.17284	-7.72276	290
5	No PJ	immed post	4.2	143	41.66	15.45679	-0.89994	286.5
5	No PJ	90 min post	4.1	143	40.33	15.1358	3.508825	287.5
5	No PJ	120 min post	4.2	143	41	15.55556	-0.41515	287
5	1 dose	pre	4	140	41.33	14.83951	0	279
5	1 dose	during	4.7	143	44	15.77778	-10.227	284
5	1 dose	immed post	4.1	143	44.33	15.80247	-10.8955	285
5	1 dose	90 min post	4.1	142	41.66	14.81481	-0.39674	283.5
5	1 dose	120 min post	4.4	142	42.33	14.83951	-1.70445	285.5
5	2 doses	pre	3.9	141	41.33	14.79012	0	284.5
5	2 doses	during	4.7	144	43.16	16.07407	-10.8577	286.5
5	2 doses	immed post	4.1	144	41.66	15.03704	-2.19527	287.5
5	2 doses	90 min post	4	143	39.66	15.25926	-0.31552	285
5	2 doses	120 min post	4.2	144	40.16	15.35802	-1.77729	287

(continued)

Table C1. Blood Data (continued)

Subject #	Fluid	Time	$[K^+]_p$	$[Na^+]_p$	Hct	Hb g/dl	Plasma Volume	OSM_p
6	No PJ	pre	4.1	141.5	44	16.46914	0	282.5
6	No PJ	during	5.2	145	47	17.85185	-12.6877	291.5
6	No PJ	immed post	4.6	145	45.66	17.67901	-9.605	289
6	No PJ	90 min post	4.4	144	44.66	17.18519	-5.29613	288
6	No PJ	120 min post	4.3	144	45	17.45679	-7.34239	286.5
6	1 dose	pre	4.4	140	44.5	14.66667	0	282
6	1 dose	during	5	144	47	17.60494	-20.4427	288.5
6	1 dose	immed post	4.5	143	45.33	17.11111	-15.5676	287.5
6	1 dose	90 min post	4.5	144	44.66	16.17284	-9.57442	288.5
6	1 dose	120 min post	4.5	143.5	45.16	16.66667	-13.0465	287.5
6	2 doses	pre	4.7	141.5	45.5	16.77129	0	283
6	2 doses	during	5	144.5	48	18.10949	-11.6377	286
6	2 doses	immed post	4.7	145	46	17.30657	-3.98198	289
6	2 doses	90 min post	4.2	145.5	42	16.6983	6.887217	287
6	2 doses	120 min post	4.8	145	45	17.30657	-2.20387	289
7	No PJ	pre	4.1	141	42	15.4321	0	280
7	No PJ	during	5.1	141.5	44.66	16.8642	-12.6887	282
7	No PJ	immed post	4.7	141	45	16.79012	-12.8423	282
7	No PJ	90 min post	4.6	141	44	16.54321	-9.93309	282.5
7	No PJ	120 min post	4.9	141.5	44	17.60494	-15.3649	280
7	1 dose	pre	4.2	141.5	40.33	14.59259	0	281
7	1 dose	during	5.05	142.5	43.33	16.02469	-13.5152	285.5
7	1 dose	immed post	4.85	143	42.33	16.07407	-12.2594	285
7	1 dose	90 min post	4.3	142	41	15.23457	-5.28946	283
7	1 dose	120 min post	4.3	143	41.16	16.07407	-10.4794	284.5
7	2 doses	pre	4.2	141.5	39.66	14.59259	0	278.5
7	2 doses	during	5	141	42.66	16.07407	-13.7302	284.5
7	2 doses	immed post	4.9	143	43	15.45679	-10.8169	283.5
7	2 doses	90 min post	4.5	142.5	41.33	15.62963	-9.21909	284
7	2 doses	120 min post	4.7	143	42	16.04938	-12.6029	285.5
8	No PJ	pre	4.7	142	44.66	16.84938	0	287
8	No PJ	during	5.05	145	46.75	17.78765	-8.85229	294
8	No PJ	immed post	4.5	143.5	45.91	17.04691	-3.39134	292
8	No PJ	90 min post	5.15	144	45.16	17.31852	-3.5879	291.5
8	No PJ	120 min post	5.3	144	46	18.2321	-9.82172	292.5
8	1 dose	pre	4.7	140	42	14.97284	0	287.5
8	1 dose	during	5.2	146	45.33	16.67654	-15.371	294.5
8	1 dose	immed post	4.8	145	44.33	16.03457	-10.3727	298
8	1 dose	90 min post	5.2	144.5	42.5	16.5284	-10.1923	292.5

(continued)

Table C1. Blood Data (continued)

Subject #	Fluid	Time	$[K^+]_p$	$[Na^+]_p$	Hct	Hb g/dl	Plasma Volume	OSM_p
8	1 dose	120 min post	4.85	144.5	42.85	16.65185	-11.4008	292
8	2 doses	pre	4.4	142.5	43.66	16.47901	0	285.5
8	2 doses	during	4.7	145	47.16	17.78765	-13.1123	291
8	2 doses	immed post	4.4	145	45.5	16.94815	-5.94355	291
8	2 doses	90 min post	4.9	145	45	17.39259	-7.50618	292.5
8	2 doses	120 min post	5	145	44.66	17.71358	-8.62084	292
9	No PJ	pre	4.25	142	42.67	15.44198	0	288
9	No PJ	during	4.7	142.5	42	16.10864	-3.01826	290
9	No PJ	immed post	4.3	143.5	42.08	15.76296	-1.02817	288.5
9	No PJ	90 min post	4.1	144	41.67	15.44198	1.744287	289
9	No PJ	120 min post	4.2	144.5	43	16.30617	-5.84493	290.5
9	1 dose	pre	4.2	143	42.41	16.05926	0	289
9	1 dose	during	4.75	145	43	16.60247	-4.26283	292.5
9	1 dose	immed post	4.5	144	42.25	15.61481	3.132034	291
9	1 dose	90 min post	4.4	144.5	42	15.39259	5.073851	289.5
9	1 dose	120 min post	4.5	145	43.41	17.14568	-7.96279	291
9	2 doses	pre	4.4	142	43.33	15.63951	0	286
9	2 doses	during	4.5	143	43.16	15.78765	-0.64121	288.5
9	2 doses	immed post	4.5	145	42.83	15.61481	1.041824	290.5
9	2 doses	90 min post	4.2	144.5	42.75	15.9358	-0.85487	291
9	2 doses	120 min post	4.2	145	42.91	16.42963	-4.10365	292.5

Table C2. Demographics and Ingestion Data

Subject	Age	Height (in)	Height (cm)	Trial	PJ Volume Ingested (mL)	Total Na Ingested (g)	Total K Ingested (g)
1	19	71	180.34	0 bolus	0	0	0
1				1 bolus	69	0.84111	0.077694
1				2 bolus	136	1.65784	0.153136
2	22	76	193.04	0 bolus	0	0	0
2				1 bolus	106	1.29214	0.119356
2				2 bolus	212	2.58428	0.238712
3	25	69	175.26	0 bolus	0	0	0
3				1 bolus	67	0.81673	0.075442
3				2 bolus	134	1.63346	0.150884
4	19	72	182.88	0 bolus	0	0	0
4				1 bolus	86	1.04834	0.096836
4				2 bolus	172	2.09668	0.193672
5	19	71	180.34	0 bolus	0	0	0
5				1 bolus	81	0.98739	0.091206
5				2 bolus	160	1.9504	0.18016
6	20	72	182.88	0 bolus	0	0	0
6				1 bolus	72	0.87768	0.081072
6				2 bolus	146	1.77974	0.164396
7	28	68	172.72	0 bolus	0	0	0
7				1 bolus	68	0.82892	0.076568
7				2 bolus	136	1.65784	0.153136
8	25	70	177.8	0 bolus	0	0	0
8				1 bolus	84	1.02396	0.094584
8				2 bolus	168	2.04792	0.189168
9	28	72	182.88	0 bolus	0	0	0
9				1 bolus	95	1.15805	0.10697
9				2 bolus	192	2.34048	0.216192

Table C3. Body Weight, Sweat Rate, Percent Hypohydration, Urine Volume

Subject	BW 1 (pre-ingest)	BW 2 (immed pre-ex)	BW 3 (post-ex)	BW 4 (post-urine)	Sweat Rate (L/h)	% Hypohydration	Urine Vol (mL)
1	68.9	68.85	67.24	66.76	1.61	3.035585	465
1	69	68.97	67.77	67.38	1.2	2.30535	390
1	67.75	67.73	66.32	66.18	1.41	2.288498	150
2	107.23	107.2	106.22	105.57	0.98	1.520522	640
2	105.6	105.57	104.7	104.1	0.87	1.392441	600
2	105.94	105.91	105.07	104.31	0.84	1.510717	700
3	65.95	65.92	64.71	64.51	1.21	2.138956	190
3	66.6	66.56	65.36	65.04	1.2	2.283654	310
3	66.59	66.56	65.58	65.46	0.98	1.652644	110
4	84.59	84.55	83.14	82.86	1.41	1.998817	250
4	85.64	85.61	84.31	83.88	1.3	2.020792	420
4	85.66	85.63	84.19	83.77	1.44	2.172136	425
5	81.19	81.16	80.03	79.08	1.13	2.562839	940
5	80.71	80.66	79.37	78.5	1.29	2.677907	850
5	79.83	79.8	78.77	77.98	1.03	2.280702	800
6	72.04	72.02	70.97	70.61	1.05	1.95779	350
6	71.9	71.87	70.91	70.61	0.96	1.753165	300
6	72.79	72.75	71.86	71.35	0.89	1.924399	520
7	68.44	68.41	66.87	66.67	1.54	2.543488	180
7	68.3	68.26	66.63	66.41	1.63	2.710226	210
7	68.11	68.08	66.3	66.04	1.78	2.996475	250
8	82.88	82.85	81.68	81.57	1.17	1.544961	95
8	83.7	83.66	82.59	82.16	1.07	1.792972	435
8	83.96	83.92	83.04	82.66	0.88	1.50143	380
9	95.73	95.7	94.67	94.26	1.03	1.504702	405
9	94.69	94.66	93.84	93.62	0.82	1.098669	207
9	95.47	95.45	94.61	94.38	0.84	1.121006	245

Table C4. Plasma Sodium Content Changes

Subject #	Fluid	Blood Sample	$[Na^+]_p$	Hct	Hb g/dl			Na^+ Content (%)
1	0 bolus	1	142.5	43.33	15.72506			0
1	0 bolus	2	146	44.33	17.16058	148.4075	148.4075	-1.622233631
1	0 bolus	3	146	44	16.23601	146.4347	146.4347	-0.296864046
1	0 bolus	4	145.5	44.66	16.23601	150.4038	150.4038	-3.260454772
1	0 bolus	5	145	45	16.86861	152.4857	152.4857	-4.909136752
1	1 bolus	1	142	42.33	14.80049			0
1	1 bolus	2	145	42.66	15.65207	143.9306	143.9306	0.742984447
1	1 bolus	3	146	44	16.33333	152.0039	152.0039	-3.949820905
1	1 bolus	4	145.5	41.33	14.67883	136.2823	136.2823	6.763708029
1	1 bolus	5	145.5	43.33	16.382	147.9195	147.9195	-1.635703641
1	2 bolus	1	142	40.66	15.95062			0
1	2 bolus	2	145	43.33	16.91358	158.4543	158.4543	-8.490956947
1	2 bolus	3	146	42.33	16.88889	152.1132	152.1132	-4.018832867
1	2 bolus	4	145.5	41.25	16.88889	145.5072	145.5072	-0.004973617
1	2 bolus	5	146	42.33	16.91358	152.1132	152.1132	-4.018832867
2	0 bolus	1	142	42.33	15.48148			0
2	0 bolus	2	144	44.66	17.1358	156.124	156.124	-7.765605545
2	0 bolus	3	143.5	44	16.19753	152.0039	152.0039	-5.594515752
2	0 bolus	4	143	43	15.62963	145.9431	145.9431	-2.016621446
2	0 bolus	5	143	43.33	16.71605	147.9195	147.9195	-3.325811826
2	1 bolus	1	142	41.83	15.21411			0
2	1 bolus	2	144	43	15.94404	148.9681	148.9681	-3.334979231
2	1 bolus	3	143	42.33	15.6764	144.9432	144.9432	-1.340666597
2	1 bolus	4	143	41.33	15.18978	139.107	139.107	2.798594484
2	1 bolus	5	143.5	41.83	15.84672	142	142	1.056338028
2	2 bolus	1	141	41	14.54321			0
2	2 bolus	2	144.5	42.8	16.98765	151.8221	151.8221	-4.822818612
2	2 bolus	3	143.5	41.33	15.40741	142.9343	142.9343	0.395747223
2	2 bolus	4	143	41.33	15.7037	142.9343	142.9343	0.045936257
2	2 bolus	5	144	40.66	16.37037	139.0295	139.0295	3.575107375
3	0 bolus	1	139.5	39	15.98519			0
3	0 bolus	2	142	43.5	18.03457	167.9888	167.9888	-15.47053901
3	0 bolus	3	142	43.66	17.24444	169.0855	169.0855	-16.0188108
3	0 bolus	4	142	42	15.96049	158.0013	158.0013	-10.12733667
3	0 bolus	5	143	42.5	17.61481	161.2726	161.2726	-11.33024336
3	1 bolus	1	142	38.66	15.0963			0
3	1 bolus	2	145	41.16	16.77531	157.6061	157.6061	-7.998473374
3	1 bolus	3	144.5	40.25	15.56543	151.7743	151.7743	-4.792841054

(continued)

Table C4. Plasma Sodium Content Changes (continued)

Subject #	Fluid	Blood Sample	$[Na^+]_p$	Hct	Hb g/dl			Na^+ Content (%)
3	1 bolus	4	144	39.33	15.88642	146.0563	146.0563	-1.407864322
3	1 bolus	5	145	40.08	15.88642	150.7045	150.7045	-3.785211898
3	2 bolus	1	141.5	39.33	13.12099			0
3	2 bolus	2	145	42.33	16.18272	160.2156	160.2156	-9.496953881
3	2 bolus	3	144	38.9	15.46667	138.968	138.968	3.620960265
3	2 bolus	4	144	38.33	14.70123	135.6661	135.6661	6.142944914
3	2 bolus	5	144	40.08	15.6642	146.0032	146.0032	-1.372029741
4	0 bolus	1	141	47.33	17.06326			0
4	0 bolus	2	143	49.66	18.15815	154.7888	154.7888	-7.616033033
4	0 bolus	3	142.5	50	18.20681	156.9083	156.9083	-9.18262647
4	0 bolus	4	143	48.66	17.3309	148.7175	148.7175	-3.84455753
4	0 bolus	5	143	49.33	18.20681	152.7588	152.7588	-6.388350095
4	1 bolus	1	142	46.16	16.60097			0
4	1 bolus	2	145	49.33	17.3309	161.2456	161.2456	-10.07505195
4	1 bolus	3	145	49.33	17.98783	161.2456	161.2456	-10.07505195
4	1 bolus	4	145	47.33	16.96594	148.8335	148.8335	-2.575716459
4	1 bolus	5	144.5	47.83	17.13625	151.8473	151.8473	-4.838625241
4	2 bolus	1	143	46.16	16.84428			0
4	2 bolus	2	146	48.66	17.72019	158.0853	158.0853	-7.64480556
4	2 bolus	3	146	47.66	17.18491	151.8783	151.8783	-3.870375132
4	2 bolus	4	146	46	17.23358	142.0821	142.0821	2.757491878
4	2 bolus	5	146	46.33	17.6472	143.9813	143.9813	1.402079725
5	0 bolus	1	140.5	40.66	15.58025			0
5	0 bolus	2	144	43.16	16.17284	155.6983	155.6983	-7.513438492
5	0 bolus	3	143	41.66	15.45679	146.423	146.423	-2.337754694
5	0 bolus	4	143	40.33	15.1358	138.589	138.589	3.182812482
5	0 bolus	5	143	41	15.55556	142.4913	142.4913	0.35700696
5	1 bolus	1	140	41.33	14.83951			0
5	1 bolus	2	143	44	15.77778	156.1505	156.1505	-8.421680586
5	1 bolus	3	143	44.33	15.80247	158.2542	158.2542	-9.639045697
5	1 bolus	4	142	41.66	14.81481	141.9161	141.9161	0.059144402
5	1 bolus	5	142	42.33	14.83951	145.8737	145.8737	-2.655524666
5	2 bolus	1	141	41.33	14.79012			0
5	2 bolus	2	144	43.16	16.07407	151.9838	151.9838	-5.25303009
5	2 bolus	3	144	41.66	15.03704	142.9298	142.9298	0.748793884
5	2 bolus	4	143	39.66	15.25926	131.558	131.558	8.697320357
5	2 bolus	5	144	40.16	15.35802	134.3297	134.3297	7.198961282
6	0 bolus	1	141.5	44	16.46914			0

(continued)

Table C4. Plasma Sodium Content Changes (continued)

Subject #	Fluid	Blood Sample	$[Na^+]_p$	Hct	Hb g/dl			Na^+ Content (%)
6	0 bolus	2	145	47	17.85185	159.7033	159.7033	-9.206611748
6	0 bolus	3	145	45.66	17.67901	151.3241	151.3241	-4.179167709
6	0 bolus	4	144	44.66	17.18519	145.3354	145.3354	-0.918827382
6	0 bolus	5	144	45	17.45679	147.3471	147.3471	-2.27158001
6	1 bolus	1	140	44.5	14.66667			0
6	1 bolus	2	144	47	17.60494	154.8399	154.8399	-7.000739341
6	1 bolus	3	143	45.33	17.11111	144.7764	144.7764	-1.226968199
6	1 bolus	4	144	44.66	16.17284	140.9096	140.9096	2.193181602
6	1 bolus	5	143.5	45.16	16.66667	143.7863	143.7863	-0.199111867
6	2 bolus	1	141.5	45.5	16.77129			0
6	2 bolus	2	144.5	48	18.10949	156.4514	156.4514	-7.639046477
6	2 bolus	3	145	46	17.30657	144.3795	144.3795	0.429750761
6	2 bolus	4	145.5	42	16.6983	122.7334	122.7334	18.54961585
6	2 bolus	5	145	45	17.30657	138.6728	138.6728	4.562662315
7	0 bolus	1	141	42	15.4321			0
7	0 bolus	2	141.5	44.66	16.8642	157.1366	157.1366	-9.950965623
7	0 bolus	3	141	45	16.79012	159.3117	159.3117	-11.49425287
7	0 bolus	4	141	44	16.54321	152.9898	152.9898	-7.836990596
7	0 bolus	5	141.5	44	17.60494	152.9898	152.9898	-7.510171413
7	1 bolus	1	141.5	40.33	14.59259			0
7	1 bolus	2	142.5	43.33	16.02469	160.0736	160.0736	-10.97845399
7	1 bolus	3	143	42.33	16.07407	153.6677	153.6677	-6.942053514
7	1 bolus	4	142	41	15.23457	145.4843	145.4843	-2.394960036
7	1 bolus	5	143	41.16	16.07407	146.4492	146.4492	-2.355209102
7	2 bolus	1	141.5	39.66	14.59259			0
7	2 bolus	2	141	42.66	16.07407	160.1667	160.1667	-11.96671373
7	2 bolus	3	143	43	15.45679	162.4062	162.4062	-11.94918063
7	2 bolus	4	142.5	41.33	15.62963	151.6556	151.6556	-6.037078494
7	2 bolus	5	143	42	16.04938	155.8943	155.8943	-8.271201539
8	0 bolus	1	142	44.66	16.84938			0
8	0 bolus	2	145	46.75	17.78765	154.4795	154.4795	-6.136397457
8	0 bolus	3	143.5	45.91	17.04691	149.3479	149.3479	-3.915616203
8	0 bolus	4	144	45.16	17.31852	144.899	144.899	-0.62040455
8	0 bolus	5	144	46	18.2321	149.8901	149.8901	-3.929590965
8	1 bolus	1	140	42	14.97284			0
8	1 bolus	2	146	45.33	16.67654	160.3036	160.3036	-8.92284169
8	1 bolus	3	145	44.33	16.03457	153.9513	153.9513	-5.814346943
8	1 bolus	4	144.5	42.5	16.5284	142.8986	142.8986	1.120689655

(continued)

Table C4. Plasma Sodium Content Changes (continued)

Subject #	Fluid	Blood Sample	$[Na^+]_p$	Hct	Hb g/dl			Na^+ Content (%)
8	1 bolus	5	144.5	42.85	16.65185	144.9577	144.9577	-0.315756649
8	2 bolus	1	142.5	43.66	16.47901			0
8	2 bolus	2	145	47.16	17.78765	164.119	164.119	-11.64949441
8	2 bolus	3	145	45.5	16.94815	153.5193	153.5193	-5.549310594
8	2 bolus	4	145	45	17.39259	150.4519	150.4519	-3.623710402
8	2 bolus	5	145	44.66	17.71358	148.3978	148.3978	-2.289673906
9	0 bolus	1	142	42.67	15.44198			0
9	0 bolus	2	142.5	42	16.10864	138.1557	138.1557	3.144464204
9	0 bolus	3	143.5	42.08	15.76296	138.6101	138.6101	3.527822553
9	0 bolus	4	144	41.67	15.44198	136.2948	136.2948	5.653362841
9	0 bolus	5	144.5	43	16.30617	143.9267	143.9267	0. 398356576
9	1 bolus	1	143	42.41	16.05926			0
9	1 bolus	2	145	43	16.60247	146.4902	146.4902	-1.017240129
9	1 bolus	3	144	42.25	15.61481	142.0658	142.0658	1.361475494
9	1 bolus	4	144.5	42	15.39259	140.6165	140.6165	2.761800913
9	1 bolus	5	145	43.41	17.14568	148.9584	148.9584	-2.657372023
9	2 bolus	1	142	43.33	15.63951			0
9	2 bolus	2	143	43.16	15.78765	141.0198	141.0198	1.40416721
9	2 bolus	3	145	42.83	15.61481	139.1338	139.1338	4.216204434
9	2 bolus	4	144.5	42.75	15.9358	138.6799	138.6799	4.196792694
9	2 bolus	5	145	42.91	16.42963	139.589	139.589	3.876345624

Table C5. Plasma Potassium Content Changes

Subject #	Fluid	Blood Sample	[K]p	Hct	Hb g/dl			K Content (%)
1	0 bolus	1	4.8	43.33	15.72506			0
1	0 bolus	2	5	44.33	17.16058	4.99899	4.99899	0.020203394
1	0 bolus	3	4.45	44	16.23601	4.932538	4.932538	-9.782746737
1	0 bolus	4	4.6	44.66	16.23601	5.066235	5.066235	-9.20278938
1	0 bolus	5	4.6	45	16.86861	5.136362	5.136362	-10.44244129
1	1 bolus	1	4.65	42.33	14.80049			0
1	1 bolus	2	5	42.66	15.65207	4.713221	4.713221	6.084566493
1	1 bolus	3	4.7	44	16.33333	4.977592	4.977592	-5.576830861
1	1 bolus	4	4.9	41.33	14.67883	4.462764	4.462764	9.797417946
1	1 bolus	5	4.9	43.33	16.382	4.843844	4.843844	1.159335486
1	2 bolus	1	4.5	40.66	15.95062			0
1	2 bolus	2	5.05	43.33	16.91358	5.021439	5.021439	0.568788924
1	2 bolus	3	4.6	42.33	16.88889	4.820488	4.820488	-4.573975082
1	2 bolus	4	4.5	41.25	16.88889	4.611145	4.611145	-2.410352259
1	2 bolus	5	4.6	42.33	16.91358	4.820488	4.820488	-4.573975082
2	0 bolus	1	4.1	42.33	15.48148			0
2	0 bolus	2	5	44.66	17.1358	4.507805	4.507805	10.91873317
2	0 bolus	3	4.5	44	16.19753	4.388844	4.388844	2.532683665
2	0 bolus	4	4.35	43	15.62963	4.213851	4.213851	3.230995962
2	0 bolus	5	4.5	43.33	16.71605	4.270916	4.270916	5.363817573
2	1 bolus	1	4.2	41.83	15.21411			0
2	1 bolus	2	5.2	43	15.94404	4.406097	4.406097	18.01827271
2	1 bolus	3	4.6	42.33	15.6764	4.287053	4.287053	7.299827803
2	1 bolus	4	4.4	41.33	15.18978	4.114431	4.114431	6.940662394
2	1 bolus	5	4.3	41.83	15.84672	4.2	4.2	2.380952381
2	2 bolus	1	4.2	41	14.54321			0
2	2 bolus	2	5.2	42.8	16.98765	4.522361	4.522361	14.98419739
2	2 bolus	3	4.6	41.33	15.40741	4.257619	4.257619	8.041615478
2	2 bolus	4	4.5	41.33	15.7037	4.257619	4.257619	5.692884707
2	2 bolus	5	4.6	40.66	16.37037	4.141306	4.141306	11.07608241
3	0 bolus	1	4.15	39	15.98519			0
3	0 bolus	2	4.6	43.5	18.03457	4.997515	4.997515	-7.954259094
3	0 bolus	3	4.4	43.66	17.24444	5.030142	5.030142	-12.52731388
3	0 bolus	4	4.3	42	15.96049	4.700398	4.700398	-8.518382664
3	0 bolus	5	4.6	42.5	17.61481	4.797715	4.797715	-4.12101637
3	1 bolus	1	4.4	38.66	15.0963			0
3	1 bolus	2	4.9	41.16	16.77531	4.883569	4.883569	0.33646117
3	1 bolus	3	4.6	40.25	15.56543	4.702866	4.702866	-2.187297541

(continued)

Table C5. Plasma Potassium Content Changes (continued)

Subject #	Fluid	Blood Sample	[K]p	Hct	Hb g/dl			K Content (%)
3	1 bolus	4	4.6	39.33	15.88642	4.525687	4.525687	1.642018663
3	1 bolus	5	4.75	40.08	15.88642	4.669716	4.669716	1.719239146
3	2 bolus	1	4.1	39.33	13.12099			0
3	2 bolus	2	5	42.33	16.18272	4.64229	4.64229	7.705475406
3	2 bolus	3	4.65	38.9	15.46667	4.026635	4.026635	15.48103206
3	2 bolus	4	4.5	38.33	14.70123	3.930961	3.930961	14.4758133
3	2 bolus	5	4.7	40.08	15.6642	4.230482	4.230482	11.09846142
4	0 bolus	1	4	47.33	17.06326			0
4	0 bolus	2	4.7	49.66	18.15815	4.391171	4.391171	7.032963128
4	0 bolus	3	4.7	50	18.20681	4.451299	4.451299	5.587146383
4	0 bolus	4	4.4	48.66	17.3309	4.218937	4.218937	4.291672217
4	0 bolus	5	4.8	49.33	18.20681	4.333582	4.333582	10.76287527
4	1 bolus	1	4.6	46.16	16.60097			0
4	1 bolus	2	5.1	49.33	17.3309	5.223448	5.223448	-2.363347256
4	1 bolus	3	4.7	49.33	17.98783	5.223448	5.223448	-10.02112394
4	1 bolus	4	4.6	47.33	16.96594	4.821368	4.821368	-4.59139129
4	1 bolus	5	4.7	47.83	17.13625	4.918998	4.918998	-4.452081369
4	2 bolus	1	4.1	46.16	16.84428			0
4	2 bolus	2	4.5	48.66	17.72019	4.532516	4.532516	-0.71739455
4	2 bolus	3	4.45	47.66	17.18491	4.354551	4.354551	2.191925802
4	2 bolus	4	4.5	46	17.23358	4.073683	4.073683	10.46516208
4	2 bolus	5	4.7	46.33	17.6472	4.128134	4.128134	13.85288637
5	0 bolus	1	4.25	40.66	15.58025			0
5	0 bolus	2	4.8	43.16	16.17284	4.709735	4.709735	1.916563858
5	0 bolus	3	4.2	41.66	15.45679	4.429166	4.429166	-5.174017351
5	0 bolus	4	4.1	40.33	15.1358	4.192193	4.192193	-2.19916227
5	0 bolus	5	4.2	41	15.55556	4.310235	4.310235	-2.557515743
5	1 bolus	1	4	41.33	14.83951			0
5	1 bolus	2	4.7	44	15.77778	4.461443	4.461443	5.347087717
5	1 bolus	3	4.1	44.33	15.80247	4.521548	4.521548	-9.323098305
5	1 bolus	4	4.1	41.66	14.81481	4.054745	4.054745	1.116107195
5	1 bolus	5	4.4	42.33	14.83951	4.16782	4.16782	5.570769024
5	2 bolus	1	3.9	41.33	14.79012			0
5	2 bolus	2	4.7	43.16	16.07407	4.203806	4.203806	11.803449
5	2 bolus	3	4.1	41.66	15.03704	3.953376	3.953376	3.708827892
5	2 bolus	4	4	39.66	15.25926	3.638838	3.638838	9.925208322
5	2 bolus	5	4.2	40.16	15.35802	3.715501	3.715501	13.03993033
6	0 bolus	1	4.1	44	16.46914			0

(continued)

Table C5. Plasma Potassium Content Changes (continued)

Subject #	Fluid	Blood Sample	[K]p	Hct	Hb g/dl			K Content (%)
6	0 bolus	2	5.2	47	17.85185	4.627444	4.627444	12.3730447
6	0 bolus	3	4.6	45.66	17.67901	4.384656	4.384656	4.911319997
6	0 bolus	4	4.4	44.66	17.18519	4.211131	4.211131	4.484989959
6	0 bolus	5	4.3	45	17.45679	4.269421	4.269421	0.716221448
6	1 bolus	1	4.4	44.5	14.66667			0
6	1 bolus	2	5	47	17.60494	4.866398	4.866398	2.745395299
6	1 bolus	3	4.5	45.33	17.11111	4.550114	4.550114	-1.101382653
6	1 bolus	4	4.5	44.66	16.17284	4.428587	4.428587	1.612538525
6	1 bolus	5	4.5	45.16	16.66667	4.518998	4.518998	-0.420399867
6	2 bolus	1	4.7	45.5	16.77129			0
6	2 bolus	2	5	48	18.10949	5.196619	5.196619	-3.783590344
6	2 bolus	3	4.7	46	17.30657	4.795645	4.795645	-1.994415636
6	2 bolus	4	4.2	42	16.6983	4.076658	4.076658	3.025570955
6	2 bolus	5	4.8	45	17.30657	4.606094	4.606094	4.20977292
7	0 bolus	1	4.1	42	15.4321			0
7	0 bolus	2	5.1	44.66	16.8642	4.569221	4.569221	11.61641062
7	0 bolus	3	4.7	45	16.79012	4.632468	4.632468	1.457807682
7	0 bolus	4	4.6	44	16.54321	4.448639	4.448639	3.402400795
7	0 bolus	5	4.9	44	17.60494	4.448639	4.448639	10.14603563
7	1 bolus	1	4.2	40.33	14.59259			0
7	1 bolus	2	5.05	43.33	16.02469	4.751301	4.751301	6.286668735
7	1 bolus	3	4.85	42.33	16.07407	4.561161	4.561161	6.332571136
7	1 bolus	4	4.3	41	15.23457	4.318262	4.318262	-0.422893082
7	1 bolus	5	4.3	41.16	16.07407	4.346902	4.346902	-1.078965997
7	2 bolus	1	4.2	39.66	14.59259			0
7	2 bolus	2	5	42.66	16.07407	4.754064	4.754064	5.173167913
7	2 bolus	3	4.9	43	15.45679	4.820538	4.820538	1.648410943
7	2 bolus	4	4.5	41.33	15.62963	4.501437	4.501437	-0.031929375
7	2 bolus	5	4.7	42	16.04938	4.627252	4.627252	1.572156871
8	0 bolus	1	4.7	44.66	16.84938			0
8	0 bolus	2	5.05	46.75	17.78765	5.113053	5.113053	-1.233177721
8	0 bolus	3	4.5	45.91	17.04691	4.943205	4.943205	-8.965940772
8	0 bolus	4	5.15	45.16	17.31852	4.795951	4.795951	7.382237223
8	0 bolus	5	5.3	46	18.2321	4.96115	4.96115	6.83006773
8	1 bolus	1	4.7	42	14.97284			0
8	1 bolus	2	5.2	45.33	16.67654	5.381622	5.381622	-3.374859735
8	1 bolus	3	4.8	44.33	16.03457	5.168364	5.168364	-7.127279745
8	1 bolus	4	5.2	42.5	16.5284	4.797308	4.797308	8.394113331
8	1 bolus	5	4.85	42.85	16.65185	4.866438	4.866438	-0.337773341

(continued)

Table C5. Plasma Potassium Content Changes (continued)

Subject #	Fluid	Blood Sample	[K]p	Hct	Hb g/dl			K Content (%)
8	2 bolus	1	4.4	43.66	16.47901			0
8	2 bolus	2	4.7	47.16	17.78765	5.067535	5.067535	-7.25274119
8	2 bolus	3	4.4	45.5	16.94815	4.740244	4.740244	-7.177770756
8	2 bolus	4	4.9	45	17.39259	4.645534	4.645534	5.477655504
8	2 bolus	5	5	44.66	17.71358	4.582108	4.582108	9.120074204
9	0 bolus	1	4.25	42.67	15.44198			0
9	0 bolus	2	4.7	42	16.10864	4.134943	4.134943	13.66541244
9	0 bolus	3	4.3	42.08	15.76296	4.148541	.148541	3.650893135
9	0 bolus	4	4.1	41.67	15.44198	4.079245	4.079245	0.50880367
9	0 bolus	5	4.2	43	16.30617	4.307664	4.307664	-2.499361104
9	1 bolus	1	4.2	42.41	16.05926			0
9	1 bolus	2	4.75	43	16.60247	4.302508	4.302508	10.40072191
9	1 bolus	3	4.5	42.25	15.61481	4.172562	4.172562	7.847403241
9	1 bolus	4	4.4	42	15.39259	4.129994	4.129994	6.537691768
9	1 bolus	5	4.5	43.41	17.14568	4.375001	4.375001	2.857111828
9	2 bolus	1	4.4	43.33	15.63951			0
9	2 bolus	2	4.5	43.16	15.78765	4.369629	4.369629	2.983570958
9	2 bolus	3	4.5	42.83	15.61481	4.311189	4.311189	4.379552716
9	2 bolus	4	4.2	42.75	15.9358	4.297123	4.297123	-2.260196346
9	2 bolus	5	4.2	42.91	16.42963	4.325294	4.325294	-2.896782868

Table C6. Heat Chamber and Laboratory Temperature

Subject	Trial	Temp Start	Temp End	Avg Temp	Humidity Start	Humidity End	Avg Humidity	Treatment Drink	Room Temp
1	0 bolus	37	37	37	16	25	20.5	PJ	22
1	1 bolus	38	37	37.5	16	16	16	PJ	20
1	2 bolus	36	38	37	16	22	19	PJ	21
2	0 bolus	36	36	36	16	16	16	PJ	23
2	1 bolus	38	38	38	16	23	19.5	PJ	22
2	2 bolus	37	37	37	16	19	17.5	PJ	21
3	0 bolus	37	36	36.5	16	16	16	PJ	20
3	1 bolus	38	38	38	16	16	16	PJ	21
3	2 bolus	35	36	35.5	16	16	16	PJ	20
4	0 bolus	35	36	35.5	16	26	21	PJ	23
4	1 bolus	35	36	35.5	16	26	21	PJ	22
4	2 bolus	37	37	37	16	25	20.5	PJ	22
5	0 bolus	39	38	38.5	16	16	16	PJ	21
5	1 bolus	36	26	31	16	25	20.5	PJ	23
5	2 bolus	39	37	38	16	16	16	PJ	22
6	0 bolus	36	36	36	16	22	19	PJ	20
6	1 bolus	36	36	36	16	20	18	PJ	20
6	2 bolus	38	38	38	16	24	20	PJ	23
7	0 bolus	38	38	38	16	19	17.5	PJ	23
7	1 bolus	38	38	38	16	16	16	PJ	21
7	2 bolus	38	38	38	16	16	16	PJ	21
8	0 bolus	36	38	37	16	16	16	PJ	20
8	1 bolus	38	38	38	16	16	16	PJ	22
8	2 bolus	36	36	36	16	16	16	PJ	21
9	0 bolus	36	35	35.5	16	16	16	PJ	23
9	1 bolus	36	36	36	16	16	16	PJ	22
9	2 bolus	36	35	35.5	16	16	16	PJ	21

Table C7. Urine Specific Gravity

Subject	0 bolus	1 bolus	2 bolus
1	1.003	1.01	1.005
2	1.011	1.015	1.017
3	1.015	1.015	1.016
4	1.011	1.005	1.005
5	1.014	1.005	1.004
6	1.009	1.018	1.017
7	1.005	1.004	1.004
8	1.015	1.007	1.004
9	1.011	1.008	1.006

APPENDIX D. RECOMMENDATIONS FOR FUTURE RESEARCH

1. Determine the gastric emptying rate of pickle juice during and after exercise when multiple boluses of pickle juice are ingested.
2. Conduct this study over consecutive days to determine if hyperkalemia will occur with repeated pickle juice supplementation.
3. Collect sweat during exercise to determine exact electrolyte losses with this exercise protocol.
4. Exercise subjects until they achieve 3% hypohydration to determine if percent hypohydration will affect changes in plasma sodium content.