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J Appl Physiol 84:1889-1894, 1998. ;

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Hypohydration effects on skeletal muscle performance and metabolism: a ^{31}P -MRS study

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Montain, Scott J., Sinclair A. Smith, Ralph P. Mattot, Gary P. Zientara, Ferenc A. Jolesz, and Michael N. Sawka. Hypohydration effects on skeletal muscle performance and metabolism: a ^{31}P -MRS study. *J. Appl. Physiol.* 84(6): 1889–1894, 1998.—The purpose of this study was to determine whether hypohydration reduces skeletal muscle endurance and whether increased H^+ and P_i might contribute to performance degradation. Ten physically active volunteers (age 21–40 yr) performed supine single-leg, knee-extension exercise to exhaustion in a 1.5-T whole body magnetic resonance spectroscopy (MRS) system when euhydrated and when hypohydrated (4% body wt). ^{31}P spectra were collected at a rate of one per second at rest, exercise, and recovery, and were grouped and averaged to represent 10-s intervals. The desired hydration level was achieved by having the subjects perform 2–3 h of exercise in a warm room (40°C dry bulb, 20% relative humidity) with or without fluid replacement 3–8 h before the experiment. Time to fatigue was reduced ($P < 0.05$) by 15% when the subjects were hypohydrated [213 ± 12 vs. 251 ± 15 (SE) s]. Muscle strength was generally not affected by hypohydration. Muscle pH and $\text{P}_i/\beta\text{-ATP}$ ratio were similar during exercise and at exhaustion, regardless of hydration state. The time constants for phosphocreatine recovery were also similar between trials. In summary, moderate hypohydration reduces muscle endurance, and neither H^+ nor P_i concentration appears to be related to these reductions.

fatigue; acid-base balance

THE EFFECTS THAT HYPOHYDRATION (body water deficit) has on increasing heat strain (21, 22a, 26) and cardiovascular strain (21, 24) and on reducing and/or degrading aerobic exercise performance (2, 5, 23, 30) are well documented. Less understood are the effects of hypohydration on skeletal muscle performance and metabolism. Whereas results from two studies (3, 29) indicated that hypohydration reduced muscle endurance, other investigators found no difference in fatigability during handgrip exercise (27). Similarly, anaerobic exercise performance has been reported to be decreased (30) or not altered (13, 18, 19) by hypohydration. These previous studies are somewhat confounded, however, because they did not control for prior exercise and/or heat exposure and different caloric intake before the performance tests. Research is needed that controls for these confounding variables to determine whether hypohydration has direct effects on skeletal muscle that contribute to the well-documented reductions in aerobic performance.

Hypohydration might accelerate depletion of energy stores, accumulation of metabolites (e.g., lactate, H^+ , P_i), and changes in intracellular electrolyte concentra-

tions, and/or reduce buffering capacity (8, 13, 17, 22). Investigators examining the effects of hypohydration on muscle glycogen use have found either no effect (8) or a small increase in muscle glycogen utilization (16). Similarly, hypohydration has been reported to not alter (8) or increase muscle lactate concentration (16). The effects of hypohydration on intracellular H^+ or P_i concentrations in skeletal muscle have not been studied. Elevated H^+ and P_i concentrations reduce muscle force production during repeated contractions (12), and intracellular concentrations would be increased by simply reducing intracellular water. These two metabolites can be measured noninvasively and repeatedly during exhaustive exercise with the use of ^{31}P -magnetic resonance spectroscopy (MRS).

The purpose of this study was to determine whether hypohydration reduces skeletal muscle performance and whether increased H^+ and P_i concentrations might contribute to performance degradation. We hypothesized that hypohydration would reduce skeletal muscle endurance and act via increased H^+ and P_i concentrations. To test these hypotheses, we used ^{31}P -MRS to measure high-energy phosphates and pH during exhaustive single-leg, knee-extension exercise when subjects were euhydrated and hypohydrated.

METHODS

Subjects. Ten healthy, physically active persons (5 men and 5 women), 21–40 yr of age, participated in this study. The study was approved by the appropriate institutional review boards, and all volunteers gave their voluntary and informed consent before participation.

Experimental procedure. After several practice sessions to familiarize the volunteers with the experimental procedures and to determine the appropriate exercise intensity for the experimental trials, the volunteers reported to the laboratory on two occasions separated by a minimum of 1 wk. On arrival, at 1100–1300, an initial nude body weight was obtained to establish baseline body weight. The volunteers then entered a hot room (40°C, 20% relative humidity) to perform 2–3 h of moderate-intensity treadmill and cycling exercise. For the euhydrated trial, water was available ad libitum during the exercise. For the hypohydration trial, drinking was restricted to produce a 4% body weight loss (BWL). The exercise mode, duration, and intensity were held constant for each trial. In the event that the exercise protocol did not elicit the desired BWL, supplemental sauna exposure was added. Trial order was randomly assigned and balanced across subjects. After exercise, the volunteers were given a small standardized meal (~400 kcal; 70% carbohydrate) and 200 ml of fruit juice.

Three to eight hours of recovery separated the dehydration sessions and experimental testing in the magnetic resonance (MR) system. During the recovery period, for the euhydration trial, the volunteers were provided ad libitum access to water and other beverages that did not contain sugar or caffeine, but, for the hypohydration trial, fluids were restricted to maintain the desired water deficit. This recovery period was spent resting in a temperate climate.

For the experimental trials, volunteers performed single-leg, knee-extension exercise to exhaustion while lying supine inside a whole body 1.5-T MR system (GE SIGNA, GE Medical Systems, Milwaukee, WI). The experimental setup is illustrated in Fig. 1. The ergometer was made of nonferrous materials and isolated a relatively large muscle mass to enhance the signal-to-noise ratio (20). Single-leg, knee-extension exercise was performed at 37 contractions/min through an $\sim 110\text{--}140^\circ$ range of motion of knee extension. The resistance was determined from practice sessions and set to elicit exhaustion in $\sim 4\text{--}5$ min. The same resistance was used for both trials and was achieved by adding frictional resistance with known quantities of lead weight suspended over a flywheel (set in motion by the knee-extension exercise). An elastic cord returned the lever arm to the starting position after each knee-extension motion. Force, range of motion, and kick duration were measured by a computer-based, data-acquisition system (Strawberry Tree, Sunnyvale, CA) interfaced to a force transducer and 360° potentiometer located in-line between the knee-extension lever arm and the flywheel. The average power output was 19 ± 1 W. The volunteers were instructed to perform the knee-extension task as long as possible. Endurance time was defined as the time when the power that generated each kick declined 20% below the average value during the initial minute of exercise. The subjects were given verbal encouragement to produce maximal effort. Both legs were tested in each hydration condition, and the results were treated as independent observations.

Before exhaustive exercise and at select times during recovery, measurements of muscle efficiency (left leg) or muscle strength (right leg) were obtained. For the muscle efficiency tests, subjects performed six knee extensions, within the 10-s ^{31}P -MRS sampling periods, 100 and 50 s before exhaustive exercise, at 30 s of recovery, and every minute thereafter through 5 min of recovery. Muscle strength was measured by having the subjects perform a maximal voluntary isometric contraction for 5 s with the knee at $\sim 110^\circ$ extension. The procedure was performed 100 and 50 s before exhaustive exercise, at every 30 s of recovery for 2 min, and every minute thereafter through 5 min of recovery.

^{31}P spectra were collected at rest and during exercise through an 11-cm $^1\text{H}/^{31}\text{P}$ dual radio-frequency (RF) transmit-receive coil (USAsia, Columbus, OH) placed over the quadri-

ceps muscles. Data were acquired by using hard-pulse 25.85-MHz excitation (pulse width 600 μs , transmission rate 1,000 ms, spectral width 2,000 Hz, and 1,024 sampled free induction decay) signals. Before exercise, a proton MR image was acquired axially by using the $^1\text{H}/^{31}\text{P}$ RF coil to verify coil placement and muscle group participation. A special linear gradient shim procedure was performed to reduce magnetic field inhomogeneity within the sensitive volume. RF coil transmitter and receiver gains for ^{31}P -MRS were set once to maximize the phosphocreatine (PCr) signal acquired from the muscle and were kept constant throughout the study. Ten free induction decay signals were averaged, producing one average spectrum every 10 s. Postprocessing consisted of apodization of 10-Hz line broadening, zero-filling to 4,096 points, and Fourier transformation, followed by zero- and first-order phasing. Peak areas from the PCr, P_i , and $\beta\text{-ATP}$ peaks were used to determine phosphorus ratios. Muscle pH was calculated from the frequency shift between P_i and PCr by using the following equation: $\text{pH} = 6.73 + \log_{10}[(a - 3.275)/(5.685 - a)]$, where a is the chemical shift from P_i to PCr (14). Monovalent P_i (H_2PO_4^-) was calculated by using the following equation: $[\text{H}_2\text{PO}_4^-] = ([\text{H}^+][\text{P}_i])/(K_p + [\text{H}^+])$, where $[\text{H}_2\text{PO}_4^-]$, $[\text{H}^+]$, and $[\text{P}_i]$ are concentrations of H_2PO_4^- , H^+ , and P_i , respectively, and $[\text{P}_i]$ was estimated from ratio of P_i to $\beta\text{-ATP}$ ($\text{P}_i/\beta\text{-ATP}$) and assumed muscle ATP concentration of 5.5 mM. The equilibrium constant K_p was taken to be 1.86×10^{-7} M. Recovery kinetics for PCr resynthesis were determined by calculating the time constant for the left-leg ratio of PCr to $\beta\text{-ATP}$ (PCr/ $\beta\text{-ATP}$) data. Recovery data were fitted to a monoexponential curve, and the time constant was calculated from the derived rate constant. MRS system calibration was periodically verified by using known standards.

Statistical analysis. The data were analyzed by using one- and two-way repeated-measures analysis of variance where appropriate. For all analyses, the data obtained from each leg were treated as an independent set of measurements. During one trial, time to fatigue was not reached due to technical difficulties. Therefore, data from that leg were not included in statistical analysis. For three other trials, collected spectra were uninterpretable, and all MR data for those legs were excluded from statistical analysis. Tukey's highly significant difference procedure was used to identify differences between means when statistical significance was achieved. Statistical significance was tested at the $P < 0.05$ level. Data in the text are reported as means \pm SE.

RESULTS

BWL. Before exercise was performed in the hot room, the subjects' body weights were 65.9 ± 4.1 and 66.1 ± 4.1 kg for euhydration and hypohydration, respectively. The dehydration-rehydration procedures resulted in

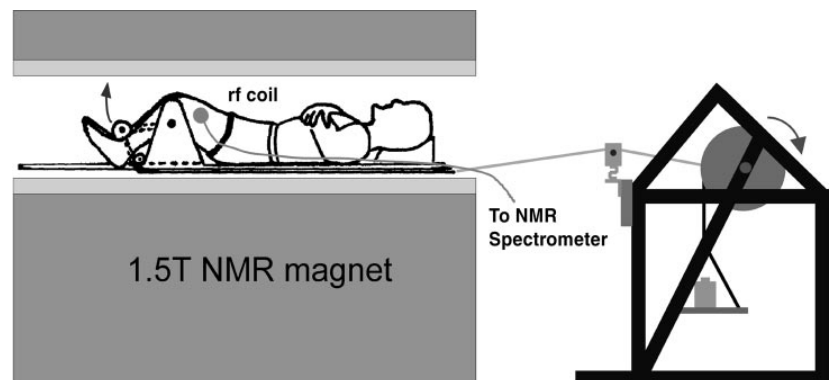


Fig. 1. Experimental setup for ^{31}P -magnetic resonance spectroscopy studies. NMR, nuclear magnetic resonance; RF, radio frequency.

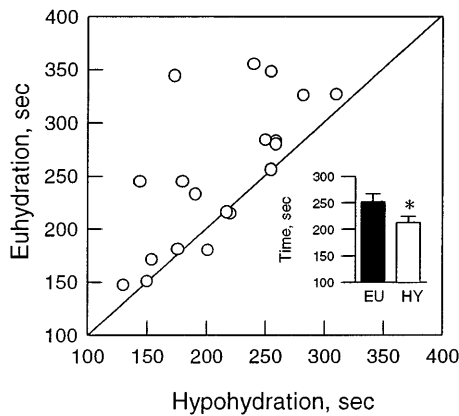


Fig. 2. Individual (○) and group mean (solid line) results for time to fatigue for subjects euhydrated (EU) and hypohydrated (HY) by 4% of initial body weight. Inset: hypohydration less than euhydration, **P* < 0.05.

0.6 ± 0.2 and 4.0 ± 0.2% BWL, respectively, before the MRS tests.

Muscle endurance. Figure 2 presents the individual leg and mean endurance times to exhaustion. Hypohydration reduced endurance time ≥8% (coefficient of variation for time to fatigue) in 12 of 19 of the trials performed, and mean endurance was reduced (*P* < 0.05) from 251 ± 15 to 213 ± 12 s (15%). Four of ten subjects had reduced endurance time in both legs when hypohydrated, whereas in three others only one leg was affected. For these three subjects, the reduced exercise performance occurred in the second leg tested. For one subject, endurance time was reduced in one leg but was not tested in the other leg due to technical problems. These results were similar to our pilot work (*n* = 5 subjects) in which 4–5% BWL reduced endurance in 8 of 10 trials and reduced (*P* < 0.05) mean endurance time from 230 ± 34 to 192 ± 32 s (17%). These combined results demonstrate that hypohydration decreased mean endurance time (20 out of 29 tests) by 15–17% by using this exercise paradigm.

Muscle strength. Figure 3 presents maximal voluntary contraction (MVC) data. Hypohydration did not alter preendurance exercise maximal isometric force. Hypohydrated persons produced a 16% higher (*P* < 0.05) maximal isometric force 30 s after exhaustive exercise. No other difference between trials existed

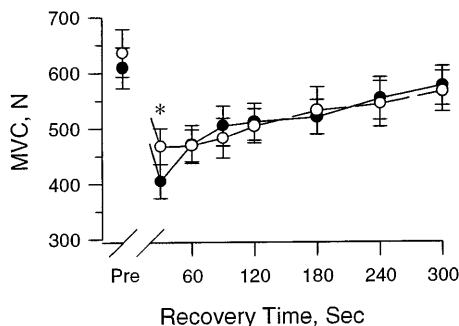


Fig. 3. Maximal isometric force before (Pre) and after exhaustive exercise when subjects were euhydrated (●) and hypohydrated (○). Data are means ± SE for 9 subjects. MVC, maximal voluntary contraction. Hypohydration greater than euhydration, **P* < 0.05.

during recovery from exhaustive exercise. Furthermore, there was no statistical correlation [not significant (NS)] between the increased MVC at 30 s postexercise and the reduction in endurance time when the subjects were hypohydrated, whether expressed as absolute change in MVC (*r* = 0.58) or percent change (*r* = 0.28).

³¹P-MRS. Figure 4 presents P_i/β-ATP, pH, and ratio of P_i to PCr (P_i/PCr) data collected during all experimental trials, and these variables were not altered by hydration, and these variables were not altered by hydration. P_i/β-ATP values were similar at rest, averaging 1.20 ± 0.08 and 1.14 ± 0.06 during euhydration and hypohydration trials, respectively. During exhaustive exercise, the P_i/β-ATP rose progressively to peak values of 5.66 ± 0.35 and 5.55 ± 0.33 during euhydration and hypohydration, respectively. Similarly, P_i/PCr rose from resting values, averaging 0.17 ± 0.01 to 3.79 ± 0.47 and 3.46 ± 0.40 at exhaustion during euhydration and hypohydration, respectively. The pH fell progressively from 7.04 ± 0.02 at rest to 6.49 ± 0.09 at exhaustion during euhydration and hypohydration, respectively. H₂PO₄⁻ levels rose from 2.2 ± 0.2 mM at rest to similar levels (NS) at exhaustion (19.1 ± 1.9 and 20.5 ± 1.8 mM for euhydration and hypohydration trials, respectively). Similar to exercise data, hypohydration did not alter (*P* < 0.07) the time constant of PCr synthesis after exhaustive

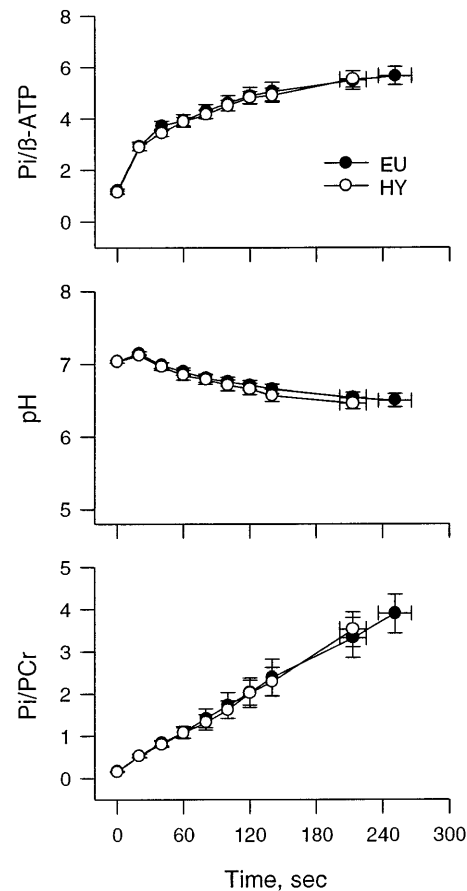


Fig. 4. Muscle P_i/β-ATP ratio, pH, and P_i/phosphocreatine (PCr) ratio data for all tests when subjects were euhydrated (●) and hypohydrated (○). Data are means ± SE for 15 paired comparisons.

exercise (63 ± 6 and 72 ± 7 s for euhydration and hypohydration trials, respectively).

To further investigate whether elevated levels of P_i or H^+ could contribute to reduced endurance time, we subdivided the data to compare only spectra from trials when endurance times were reduced. Figure 5 presents this subset. Note that P_i/β -ATP and pH were similar (NS) between euhydration and hypohydration during rest and exercise. In contrast, P_i/PCr increased more rapidly and to a higher ($P < 0.05$) level at the time of exhaustion during hypohydration compared with during euhydration. Examination of the individual data revealed that the higher P_i/PCr during hypohydration was largely attributable to 3 of 10 trials, and there was no significant correlation between the higher P_i/PCr values and reduced endurance times. $H_2PO_4^-$ levels were also similar (NS) between trials at the time of hypohydration exhaustion.

DISCUSSION

To our knowledge, this study is the first to examine simultaneously the impact of hypohydration on skeletal muscle performance and muscle metabolism. The level of hypohydration studied is commonly achieved by athletes during competition and training (1). To mini-

mize the likelihood of hypoglycemia and to replace some of the carbohydrate metabolized during the dehydration procedures, the subjects were given a small meal during the recovery period before the experimental trials. To isolate the effects of hypohydration on muscle from the potentially confounding effects of elevated body temperature (22), a minimum of 3 h of rest separated the heat exposures from experimental testing, and the MRS experiments were conducted in a cool room ($\sim 18^\circ C$).

We found that hypohydration reduced muscular endurance by 15% but had no effect on muscle strength. These findings agree with data from earlier studies, which indicated that hypohydration can impair muscle endurance (3, 29) but had no effect on muscle strength (3, 15, 23, 27, 28). They also agree with studies demonstrating that hypohydration can reduce aerobic endurance (see Ref. 25 for review). Our results extend the findings of these earlier studies by separating the effects of hypohydration from the confounding effects of elevated body temperature, cardiovascular strain, heat exposure, and differing quantities of exercise before experimental testing. The data from the present study also demonstrate that hypohydration has no effect on recovery of muscle strength after exhausting exercise.

In subjects during exercise, we employed ^{31}P -MRS to assess whether hypohydration would accelerate the accumulation of H^+ or P_i during exhaustive exercise. These two variables were chosen as both have been shown to reduce cross-bridge formation and force production and are the two variables within muscle often considered to be responsible for fatigue during high-intensity exercise (11, 12). We hypothesized that, if hypohydration had direct effects on muscle metabolism, then the hypohydration trials would likely be associated with elevated exercise H^+ and/or P_i concentrations. The results of this study did not support this hypothesis, however, as P_i/β -ATP, pH, and $H_2PO_4^-$ responses to exercise were not affected by 4% BWL. The only observation which suggested that hypohydration had an effect on muscle metabolism was the accelerated increase in P_i/PCr in the subgroup of trials with shortened time to fatigue. This would suggest that hypohydration required greater reliance on creatine kinase to sustain muscle ATP in these trials. The fact that P_i/PCr was not consistently elevated even in this subgroup or correlated with maintenance of endurance time, however, further supports the contention that moderate levels of hypohydration had little or no effect on muscle metabolism.

How hypohydration reduces muscle endurance remains an intriguing question. Approximately 50% of the water lost would be expected to come from the intracellular water compartments, and 4% BWL would be expected to reduce intramuscular water by 4–5% (7). It is unlikely that insufficient oxygen delivery was responsible for the shortened time to fatigue. The muscle mass activated during exhaustive exercise was not large enough to limit leg blood flow, and the exercise device was designed to reduce any isometric and eccen-

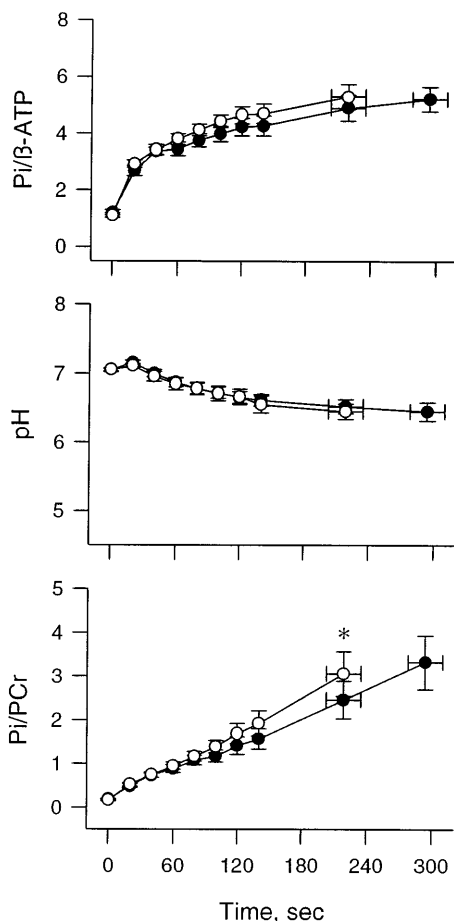


Fig. 5. Muscle P_i/β -ATP ratio, pH, and P_i/PCr ratio data for subgroup of tests when hypohydration shortened time to fatigue. Data are means \pm SE for 10 paired comparisons. Hypohydration (\circ) greater than euhydration (\bullet), * $P < 0.05$.

tric muscle loading during the recovery phase of the contraction, thus minimizing disruptions in muscle blood flow when the muscle was not performing the knee-extension movement. Furthermore, any impairment in oxygen delivery would be expected to increase muscle glycolytic flux and formation of lactate and H^+ . We found no difference in muscle pH during exhaustive exercise regardless of whether the exercise was performed when the subjects were euhydrated or hypohydrated.

Alternative mechanisms within muscle include altered cell depolarization and changes in Ca^{2+} release and/or uptake by the sarcoplasmic reticulum. Dehydration-induced changes in the ionic status of the T-tubular lumen and intracellular compartments could contribute to the development of fatigue by negatively affecting the T-tubular charge movement (12). Similarly, longer Ca^{2+} transients might reduce Ca^{2+} flux on depolarization and reduce force production (12). The possibility that elevated intracellular Mg^{2+} plays a role appears unlikely, as Costill and Saltin (8) found no difference in intracellular Mg^{2+} concentration during exercise when the subjects were euhydrated vs. hypohydrated by 4% of initial body weight.

An alternative explanation for the detrimental effects of hypohydration on muscle endurance is that hypohydration alters central nervous system function. In the subgroup of trials in which muscle strength was measured by performance of MVC before and after exercise, muscle endurance time was reduced ($P < 0.05$) by 14%, yet volunteers were able to generate greater absolute force during the initial period of recovery, suggesting that the subjects were either less willing or unable to sustain voluntary concentric exercise when hypohydrated, despite having adequate muscle strength. An unwillingness or inability to generate or maintain adequate central nervous system drive to the working muscle is thought to be responsible for the debilitating fatigue that accompanies many infections and illnesses, recovery from injury, and chronic fatigue syndrome (9). Hypohydration may impair performance in a similar manner. These conditions are characterized by an increased perception of effort during physical activity, yet afflicted patients are capable of generating maximal force (9). Body water loss also increases perception of effort during physical activities (10, 21) yet has no apparent effects on maximal strength (3, 23, 25). In addition, hypohydration is known to alter neuronal firing of osmoreceptive cells located in the organum vasculosum laminae terminalis and cells near the preoptic/anterior hypothalamic areas of the brain (4). Neuronal activation mediated by hypohydration might also alter the magnitude of corollary discharge from the motor cortex.

In summary, we found that moderate hypohydration 1) decreases skeletal muscle performance by reducing endurance by 15%, 2) does not alter muscle strength or recovery of muscle strength after exhaustive exercise, and 3) does not alter pH and P_i/β -ATP response to exhaustive exercise. These findings clearly identify

another physiological system by which hypohydration adversely affects human exercise performance; however, the mechanisms for this action remain unclear.

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision.

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Received 18 July 1997; accepted in final form 11 February 1998.

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