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Effect of hydration state on resistance exercise-induced endocrine markers of anabolism, catabolism, and metabolism

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Judelson DA, Maresh CM, Yamamoto LM, Farrell MJ, Armstrong LE, Kraemer WJ, Volek JS, Spiering BA, Casa DJ, Anderson JM. Effect of hydration state on resistance exercise-induced endocrine markers of anabolism, catabolism, and metabolism. *J Appl Physiol* 105: 816–824, 2008. First published July 10, 2008; doi:10.1152/japplphysiol.01010.2007.—Hypohydration (decreased total body water) exacerbates the catabolic hormonal response to endurance exercise with unclear effects on anabolic hormones. Limited research exists that evaluates the effect of hypohydration on endocrine responses to resistance exercise; this work merits attention as the acute postexercise hormonal environment potently modulates resistance training adaptations. The purpose of this study was to examine the effect of hydration state on the endocrine and metabolic responses to resistance exercise. Seven healthy resistance-trained men (age = 23 ± 4 yr, body mass = 87.8 ± 6.8 kg, body fat = 11.5 ± 5.2%) completed three identical resistance exercise bouts in different hydration states: euhydrated (EU), hypohydrated by ~2.5% body mass (HY25), and hypohydrated by ~5.0% body mass (HY50). Investigators manipulated hydration status via controlled water deprivation and exercise-heat stress. Cortisol, epinephrine, norepinephrine, testosterone, growth hormone, insulin-like growth factor-I, insulin, glucose, lactate, glycerol, and free fatty acids were measured during euhydrated rest, immediately preceding resistance exercise, immediately postexercise, and during 60 min of recovery. Body mass decreased 0.2 ± 0.4, 2.4 ± 0.4, and 4.8 ± 0.4% during EU, HY25, and HY50, respectively, supported by humoral and urinary changes that clearly indicated subjects achieved three distinct hydration states. Hypohydration significantly *1*) increased circulating concentrations of cortisol and norepinephrine, *2*) attenuated the testosterone response to exercise, and *3*) altered carbohydrate and lipid metabolism. These results suggest that hypohydration can modify the hormonal and metabolic response to resistance exercise, influencing the postexercise circulatory milieu.

dehydration; hormone; muscle; strength; water

INDIVIDUAL EXERCISE BOUTS cause transient changes in physiological function that, when repeated over time, predispose the exercising organism to beneficial adaptations. In terms of resistance exercise training, the hormonal and metabolic milieu created by each acute exercise bout crucially modulates the magnitude and direction of adaptations. Exercise bouts that maximize the anabolic hormonal response (e.g., increased circulating concentrations of testosterone and growth hormone) and/or minimize the catabolic hormonal response (e.g., decreased circulating concentrations of cortisol) promote greater long-term adaptations to resistance exercise training than control exercise sessions (22, 54). Similarly, exercise bouts that limit the anabolic hormonal response and/or exacerbate the catabolic hormonal response suppress adaptations compared with control exercise sessions (27, 32). Thus any factor capable of influencing the balance of anabolic and catabolic hormones in response to an exercise session represents a potential modulator of resistance training adaptations.

Research employing endurance exercise models suggests hypohydration (reduced total body water) might be one such modulator. Independent of external thermal stress, hypohydration potently amplifies the exercise-induced responses of cortisol (7, 33, 35, 37), norepinephrine (45, 47, 56–58), and, in some cases, epinephrine (39, 45, 47, 56–58). The few investigations documenting the effects of hypohydration on the anabolic hormonal response to exercise produced inconsistent results (33, 35, 41, 46, 57). In total, available evidence suggests hypohydration *1*) enhances the catabolic hormonal response, and *2*) questionably alters the anabolic hormonal response to low-intensity endurance exercise.

The unique metabolic, mechanical, and homeostatic challenges of resistance exercise, however, cause significantly different physiological responses than endurance exercise (27). Unfortunately, no evidence describes the effect of hypohydration on the endocrine response to resistance exercise. If hydration state detrimentally affects this hormonal response (as suggested by associated endurance-based data), fluid balance might assume an important role to populations that often resistance train but might suffer chronic hypohydration (e.g., athletes, the elderly, and astronauts). Therefore, the purpose of this study was to determine the effect of hydration state on the hormonal and metabolic responses to resistance exercise.

METHODS

Subjects. Seven healthy, nonsmoking, resistance-trained men volunteered to complete this study [age = 23 ± 4 yr, height = 1.79 ± 0.58 m, body mass = 87.8 ± 6.8 kg, body fat = 11.5 ± 5.2%, back squat one repetition maximum (1 RM) = 152 ± 20 kg]. Inclusion criteria consisted of a minimum 6-mo experience in the parallel back squat exercise and a medical history free of musculoskeletal, cardiac, endocrine, and heat-related illnesses. Before commencing participation, our medical monitor reviewed all medical histories, and subjects signed an informed consent statement approved by the Institutional Review Board of the University of Connecticut.

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Experimental design. To assess the effects of hydration state on the endocrine and metabolic responses to resistance exercise, subjects completed three identical resistance exercise bouts in different hydration states: euhydrated (EU), hypohydrated by ~2.5% body mass (HY25), and hypohydrated by ~5.0% body mass (HY50). Although ~5.0% hypohydration represents a significant loss of total body water, we chose this research design to assess potential implications for individuals who experience very hypohydrated states (e.g., competitive wrestlers) and to maximize the magnitude of potential responses for this novel research question. Subjects completed each resistance exercise bout in a randomized order, at the same time of day (in the morning, ±1 h), and in temperate environmental conditions (~21°C). Investigators manipulated hydration status 1 day preceding each trial via controlled water deprivation and exercise-heat stress. Metabolic and hormonal variables were measured during euhydrated rest, immediately preceding resistance exercise (10–12 h after manipulation of hydration state), immediately postexercise, and during 60 min of recovery.

Study controls. Approximately 1 wk separated the experimental trials, during which time subjects completed self-directed workouts. To maintain similar training status throughout the study, subjects were asked to record and replicate individual exercise sessions between experimental testing sessions. Similar controls existed for dietary intake during the 2 days preceding experimental sessions. To minimize the potential effect of reduced caloric intake on exercise performance (34, 36), investigators encouraged subjects to consume a normal diet throughout the study. To limit physiological fluctuations, subjects did not exercise, consume alcohol, or ingest stimulants for 36 h before each testing session. Finally, subjects arrived for the three baseline testing sessions and the three 28-h experimental trials in a euhydrated condition [urine specific gravity ≤ 1.020 (6)] after a 12-h overnight fast. To promote euhydration on these specific days, subjects drank approximately 1 liter of water the night before and 1 liter of water the morning of these testing sessions.

Baseline testing and familiarization. During three preliminary visits, investigators obtained baseline subject characteristics. On arrival to the laboratory for each preliminary testing session, subjects immediately emptied their bladder; urinary measures of specific gravity and osmolality quantitatively documented hydration state (6). Body mass was then measured via platform scale (DS44L, Ohaus, Florham Park, NJ). Cheuvront et al. (11) defined baseline body mass as the average euhydrated body mass measured on 3 separate days. This study required identification of a stable, accurate, baseline body mass because changes in body mass are the gold standard measure of altered hydration state (5).

Additional measures collected during the first preliminary visit included subjects' height, body composition (via skinfold analysis), and back squat 1 RM. Briefly, subjects cycled for 5–10 min at a modest intensity and then completed several submaximal sets of back squat. After warming up, subjects attempted to lift a mass representing ~90% of their estimated 1 RM. If successful, trials continued with gradually increasing resistance until the subject failed to lift a mass with correct form. Subjects performed all resistance exercise on a modified Smith Machine (LifeFitness, Rosemont, IL) (limiting movement to one plane of motion) and rested a minimum of 3 min between attempts. 1 RM was defined as the greatest mass a subject lifted with correct form through a full range of motion, i.e., top of the thigh parallel to the floor (29).

During the second visit, subjects also completed a high-intensity resistance exercise challenge (REC) following measures of body mass. The REC consisted of six sets of the parallel back squat at 80% of subjects' predetermined 1 RM; subjects attempted to complete 10 repetitions per set. If unable to complete 10 repetitions, subjects stopped at exhaustion, but still attempted to complete all remaining sets with the original load. Subjects rested 2 min between each of the six sets. The total number of repetitions completed during the six sets served as the standard for subsequent experimental REC testing.

Experimental trials. Each subject completed three experimental trials, differing only in hydration status during exercise testing. Figure 1 displays an approximate timeline of these study procedures and ideal corresponding changes in subjects' body mass throughout each 28-h trial.

To begin, euhydrated subjects reported to the laboratory in the morning, ~24 h before exercise (see *Study controls*). After 10 min of seated rest, subjects provided a baseline blood sample from an antecubital vein. Following blood collection, subjects abstained from the intake of fluids or fluid-rich foods for the remainder of the day. Subjects left the laboratory after the blood draw, returning later that afternoon to enhance water loss by walking on a motor-driven treadmill (initial speed = 1.5 m/s, initial incline = 3% grade) in a heated

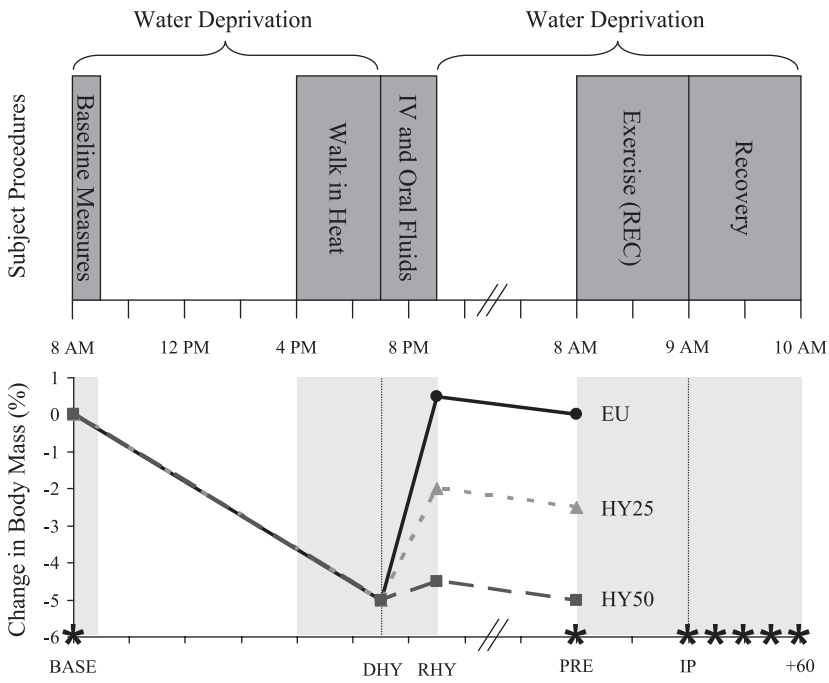

Fig. 1. Subject procedures (*top*) and idealized corresponding changes in body mass (*bottom*) occurring during each experimental trial. Asterisks indicate blood draws. See text for complete description of procedures. Base, baseline; DHY, postdehydration; EU, euhydrated trial; HY25, hypohydrated by ~2.5% trial; HY50, hypohydrated by ~5.0% trial; IP, immediately postexercise; Pre, immediately preexercise; REC, resistance exercise challenge; RHY, postrehydration; +60, 60 min postexercise; IV, intravenous.

environmental chamber (36–37°C, 40–50% relative humidity) (model 200, Minus Eleven, Malden, MA). Every 20 min, subjects stopped exercising, dried all sweat off their bodies, and were weighed. Investigators measured rectal temperature (model 401, Yellow Springs Instruments, Yellow Springs, OH), heart rate (Vantage XL, Polar Electro, Woodbury, NY), and ratings of perceived exertion immediately before body mass measurements. Subjects repeated this routine (exercise, safety measures, and body mass determination) until *1*) they lost 5% of baseline (prewater deprivation) body mass; *2*) heart rate exceeded 180 beats/min for 5 consecutive min; *3*) rectal temperature exceeded 39.5°C; *4*) they displayed signs or symptoms of an exercise-induced heat illness; or *5*) they requested to stop exercising. Investigators gradually decreased exercise intensity on an individual basis to prolong the dehydration stress and maximize the opportunity for subjects to lose body mass without exceeding safety criteria. To minimize the influence of the dehydration protocol on subsequent performance, subjects completed identical walking bouts during all three trials (i.e., characteristics of the first exercise-heat stress, regardless of the hydration state achieved, were repeated during *trials 2* and *3*).

After dehydration, subjects exited the environmental chamber and sat in temperate conditions while investigators rehydrated them such that the following morning subjects were ~0%, ~2.5%, or ~5% hypohydrated. To account for urination and overnight fluid losses (53), subjects rehydrated with sufficient fluid to achieve a hydration state that was 0.5% of body mass greater than desired for the following morning (i.e., subjects rehydrated to +0.5% body mass for EU, −2.0% body mass for HY25, and −4.5% body mass for HY50). Rehydration consisted of equal volumes of intravenous infusion of normal saline (rate = 1 l/h) and oral ingestion of an electrolyte-fortified, noncaloric, flavored solution (rate = 1 l/h in 15-min increments). Subjects rehydrated using both techniques to speed the rate of fluid delivery, decreasing time demands on the subjects and allowing them to return home and get a full night of sleep before the following day's data collection. Following rehydration, subjects consumed a high-calorie (13 kcal/kg), carbohydrate-rich (2.25 g carbohydrate/kg) meal (Classic Hand-Tossed Cheese Pizza, Domino's Pizza, Ann Arbor, MI). Table 1 displays the full nutritional composition of the recovery meal. Finally, subjects left the laboratory with instructions not to exercise or ingest anything, including water.

The following morning (10–12 h after rehydration), subjects returned to the laboratory and emptied their bladders; investigators documented subject body mass. Subjects then sat while a trained phlebotomist inserted a Teflon catheter into an antecubital vein and obtained a resting, preexercise (Pre) blood sample from the subject. Subjects then briefly inserted a rectal thermistor (model 401, Yellow Springs Instruments, Yellow Springs, OH) 10 cm beyond the anal sphincter to determine core temperature. After removing the thermistor, subjects cycled for 5–10 min at a modest intensity and completed 5–10 min of self-directed stretching. Immediately after warming up, subjects completed several nonfatiguing tests of muscular strength and power. These data reflecting the effect of hypohydration on exercise performance are reported elsewhere (24).

Table 1. *Nutritional composition of the recovery meal*

Serving Size, g	121 (1 slice)
Calories, kcal	290
Protein, g	12
Carbohydrates, g	42
Dietary fiber, g	3
Total sugars, g	4
Fat, g	9.0
Saturated fat, g	3.5
Calories from fat, kcal	80
Transfatty acids, g	0
Cholesterol, mg	5.0
Sodium, mg	470

Data were obtained from Domino's Pizza Nutrition Guide, Domino's Pizza LLC, 2005.

Subjects then repeated the REC (6 sets of the squat exercise at 80% 1 RM; see above). If a subject failed to complete the same total number of repetitions during the six sets as during baseline testing, he completed additional "make-up" sets until total repetitions during the experimental REC equaled total repetitions during the baseline REC. Although this practice decreased the external validity of the exercise protocol, it equated exercise volume among trials and ensured that hydration-induced differences in total work between trials would not uncontrollably influence the resulting hormonal or metabolic environments (28, 30). Investigators obtained blood samples from the catheter immediately following the REC [immediately postexercise (IP)] and every 15 min during 1 h of seated recovery (+15, +30, +45, and +60).

Biochemical analysis. At each of the seven blood collections (Base, Pre, IP, +15, +30, +45, +60), blood was drawn into plain plastic tubes or tubes pretreated with EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Aliquots of whole blood were analyzed in triplicate for hematocrit via microcentrifugation and in duplicate for hemoglobin via photometric analysis (Hb 201+, HemoCue, Lake Forest, CA). Plasma volume shifts were calculated using hematocrit and hemoglobin values (15). The remaining whole blood was then centrifuged at 1,500 *g* for 15 min at 4°C. An aliquot of plasma was immediately assessed in duplicate for osmolality via freezing point depression (3DII, Advanced Digimatic, Needham Heights, MA). The remaining serum and plasma were aliquoted and frozen at −80°C until analysis.

Individual samples were thawed only once, and all samples from a given subject were evaluated in the same analytic run. Serum electrolyte concentrations (Na^+, K^+, and Cl^-) were assessed in duplicate via ion-sensitive electrodes (EasyElectrolyte, Medica, Bedford, MA). Intra-assay coefficients of variation (CV) for Na^+, K^+, and Cl^- were 0.2, 0.2, and 0.1%, respectively. Lactate and glucose were assessed in duplicate via enzymatic techniques (model 2300 Glucose/Lactate Analyzer, Yellow Springs Instruments). Intra-assay CVs for glucose and lactate were 0.5 and 0.6%, respectively. Free fatty acids (Wako Chemicals, Richmond, VA) and glycerol (Sigma-Aldrich, St. Louis, MO) were assessed in duplicate via enzymatic, colorimetric assays. Intra-assay CVs for free fatty acids and glycerol were 7.9 and 7.8%, respectively. Testosterone, growth hormone, insulin-like growth factor-I (IGF-I), cortisol (Diagnostic Systems Laboratories, Webster, TX), insulin (LINCO Research, St. Charles, MO), epinephrine, and norepinephrine (Rocky Mountain Diagnostics, Colorado Springs, CO) were assessed via enzyme-linked immunosorbent assay. Intra-assay CVs for testosterone, growth hormone, IGF-I, cortisol, insulin, epinephrine, and norepinephrine were 4.7, 9.4, 2.7, 5.2, 2.9, 16.1, and 17.4%, respectively. To accurately reflect the actual exposure of the target tissues to the hormones and metabolites, concentrations are reported as measured values not corrected for plasma volume shifts.

Statistical analysis. Descriptive data (means, SDs, and SEs of the mean) were calculated for all test variables. Selected data violating the assumption of normality (norepinephrine, insulin, glycerol, and testosterone) were $\log_{10}$ transformed to reduce variability. Area under the curve (AUC) was calculated using the trapezoidal method. Differences among trials were analyzed with a 3 (hydration state) × 7 (time) repeated-measures ANOVA. In the event of a significant *F*-ratio, specific pairwise differences were examined with Fisher's least significant difference post hoc. Effect sizes [partial η^2 (η_p^2)] were calculated for specific variables that approached statistical significance. Significance was set at $P < 0.05$. Data are presented as means ± SD, unless otherwise noted.

RESULTS

Dehydration procedures. No significant differences existed among trials for room temperature, relative humidity, or duration of the exercise-heat stress (all trial averages = 36 ± 1°C, 44 ± 6%, and 184 ± 14 min, respectively). Heart rate, rectal temperature, and rating of perceived exertion data measured at the conclusion of the exercise-heat stress were also similar among trials (all trial averages = 150 ± 14 beats/min, 38.53 ± 0.28°C, and 14 ± 2, respectively). Following the exercise-heat stress, subjects replaced significantly different volumes of fluid (EU = 4.669 ± 0.308 liters, HY25 = 2.531 ± 0.336 liters, HY50 = 0.594 ± 0.328 liters), but consumed similar energy (EU = 1,122 ± 92 kcal, HY25 = 1,076 ± 113 kcal, HY50 = 1,098 ± 128 kcal). Core temperature decreased to normal resting values by the next morning, but hypohydration significantly increased Pre core temperature (HY25 = 36.84 ± 0.32°C, HY50 = 36.98 ± 0.42°C) compared with EU (36.56 ± 0.37°C).

Hydration measures. No significant differences existed in body mass between the euhydrated baseline determined during familiarization trials and the three Base experimental measures (familiarization average = 87.78 ± 6.82 kg, EU Base = 87.81 ± 7.48 kg, HY25 Base = 87.71 ± 6.96 kg, HY50 Base = 87.99 ± 7.38). Immediately pre-resistance exercise, percent change in body mass significantly differed among all trials and averaged −0.2 ± 0.4, −2.4 ± 0.4, and −4.8 ± 0.4% for EU, HY25, and HY50, respectively.

Table 2 shows the humoral hydration measures. Base electrolyte concentrations were similar among trials. Sodium concentrations significantly differed among all trials at each time point postdehydration, but no significant differences existed among trials in potassium concentrations. Chloride concentrations significantly differed among all trials at +30, +45, and +60. No consistent, physiologically significant differences occurred among trials in plasma volume shift from Pre. Plasma osmolality (data not shown) corresponded to changes in sodium, significantly differing among all trials at each time point postdehydration.

REC performance. All subjects successfully completed the REC. Of the combined 21 trials, one subject required additional "make-up" sets during one trial (HY50) to successfully match the number of total repetitions to baseline REC testing. He completed 78.1% of his repetitions (25 repetitions) during the initial six sets of exercise and finished the remaining 21.9% of his repetitions (7 repetitions) during three additional sets. Data regarding the effect of the hydration state on performance of the REC are reported elsewhere (24).

Hormonal and metabolic responses. Figure 2 describes the stress hormonal responses to hypohydration and resistance exercise. HY50 cortisol significantly exceeded EU cortisol at Pre. HY50 cortisol was significantly greater than other trials throughout recovery; all three conditions significantly differed at +45. Hypohydration incrementally increased cortisol AUC above EU by 16.2% [HY25, not significant (NS)] and 46.2% (HY50, $P < 0.05$). Epinephrine concentrations were similar among trials at all points, but hypohydration tended to increase epinephrine AUC ($P = 0.075$, $\eta_p^2 = 0.350$). HY50 norepinephrine significantly exceeded EU and HY25 norepinephrine at Pre. Norepinephrine was significantly greater during HY25 and HY50 than EU at IP; all trials significantly differed throughout recovery. Hypohydration incrementally increased norepinephrine AUC above EU by 40.3% (HY25, NS) and 81.5% (HY50, $P < 0.05$).

Table 2. *Humoral hydration measures*

Time Trial	Na^+, mmol/l	K^+, mmol/l	Cl^-, mmol/l	PV Shift, %
Base				
EU	135.0±2.3	4.16±0.49	102.3±1.3	
HY25	135.4±2.1	4.18±0.43	102.0±1.8	
HY50	134.9±3.0	4.36±0.44	101.3±2.5	
Pre				
EU	137.6±1.0*	3.82±0.29	106.3±0.4*	
HY25	139.8±3.0†	3.98±0.35	108.3±3.3	
HY50	142.8±2.8‡	3.86±0.22	109.3±2.6	
IP				
EU	142.4±2.2*	4.26±0.37	106.9±1.6*	−22.2±2.8
HY25	145.1±1.2†	4.34±0.39	109.1±2.2	−21.2±3.8
HY50	146.9±1.7‡	4.36±0.43	110.2±1.4	−18.3±5.3‡
+15				
EU	138.6±0.8*	3.82±0.28	106.5±0.9*	−9.3±3.5
HY25	140.9±1.9†	3.85±0.32	107.9±2.3	−9.5±3.6
HY50	142.9±2.2‡	3.88±0.32	108.7±2.1	−7.5±4.9
+30				
EU	138.1±0.9*	4.13±0.31	106.8±0.7*	−3.3±3.4
HY25	140.3±1.6†	4.12±0.36	108.3±2.3†	−1.4±3.1
HY50	142.6±2.1‡	4.20±0.24	109.6±1.9‡	−3.0±3.7
+45				
EU	137.6±0.9*	4.17±0.30	107.1±0.8*	0.1±4.0
HY25	140.0±1.4†	4.31±0.36	108.5±2.3†	0.6±3.4§
HY50	142.6±2.2‡	4.28±0.23	110.5±2.0‡	−2.2±1.9
+60				
EU	137.9±1.0*	4.32±0.28	107.5±1.2*	−0.1±3.3
HY25	139.9±1.3†	4.33±0.36	109.1±2.2†	0.4±4.6
HY50	142.4±2.0‡	4.42±0.12	110.4±2.1‡	−1.2±5.3

Values are means ± SD. Na^+, sodium; K^+, potassium; Cl^-, chloride, PV shift, plasma volume shift from preexercise (Pre); Base, baseline; IP, immediately postexercise; EU, euhydrated; HY25 and HY50: hypohydrated by 2.5 and 5.0% body mass, respectively; +15, +30, +45, +60: minutes postexercise. *EU significantly differs from HY25 and HY50 at a given time point; †HY25 significantly differs from EU and HY50 at a given time point; §HY50 significantly differs from EU and HY25 at a given time point; ‡HY25 significantly differs from HY50 at a given time point: $P < 0.05$.

Figure 3 displays insulin and glucose responses to hypohydration and resistance exercise. HY50 insulin significantly exceeded EU and HY25 insulin at Pre. Insulin was significantly greater during HY50 than *1*) EU throughout recovery and *2*) EU and HY25 at +45; all three trials significantly differed at +30. Hypohydration incrementally increased insulin AUC above EU by 17.2% (HY25, NS) and 47.4% (HY50, $P < 0.05$). HY50 glucose significantly exceeded EU glucose at Pre. HY50 glucose was significantly greater than EU and HY25 throughout recovery; all trials significantly differed at IP, +15, and +30. Glucose AUC significantly differed among all trials (HY25 = 6.4% > EU, HY50 = 19.0% > EU). Table 3 presents lactate responses to hypohydration and resistance exercise. Isolated statistically significant pairwise differences occurred (HY50 > EU at Base, EU > HY25 at Pre, and EU > HY50 at +45), but no consistent, physiologically meaningful patterns emerged. No differences existed in lactate AUC (HY25 = 1.8% > EU, HY50 = 1.9% < EU).

Figure 4 presents glycerol and free fatty acid responses to hypohydration and resistance exercise. HY50 glycerol exceeded EU and HY25 glycerol at IP and +60. Hypohydration tended to increase glycerol AUC ($P = 0.060$, $\eta_p^2 = 0.375$). Free fatty acid concentrations were similar among trials at all

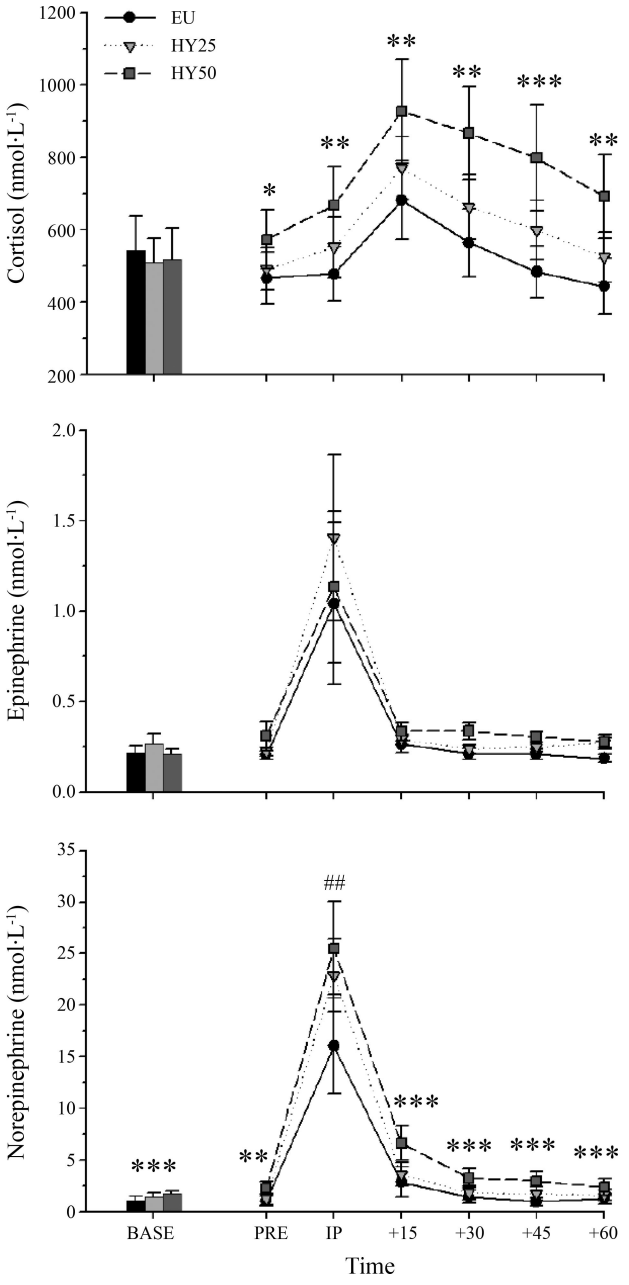

Fig. 2. Cortisol (*top*), epinephrine (*middle*), and norepinephrine (*bottom*) responses (means ± SE) to hypohydration and resistance exercise. Significant difference between *HY50 and EU; **HY50 and both EU and HY25; ##EU and both hypohydrated trials; and ***all trials at a given time: $P < 0.05$.

time points; however, hypohydration incrementally increased free fatty acid AUC above EU by 21.2% (HY25, NS) and 43.1% (HY50, $P < 0.05$).

Figure 5 shows the anabolic hormonal response to hypohydration and resistance exercise. Testosterone concentrations were similar among trials at all time points, but hypohydration incrementally decreased testosterone AUC below EU by 10.8% (HY25, NS) and 16.8% (HY50, $P < 0.05$). No statistically significant differences in growth hormone existed among trials at any point or growth hormone AUC (HY25 = 9.3% < EU, HY50 = 9.4% < EU). IGF-I concentrations were similar among trials at all time points, but hypohydration significantly increased IGF-I AUC above EU by 10.1% (HY25, $P < 0.05$) and 11.0% (HY50, $P < 0.05$).

DISCUSSION

The primary findings of this study were that hypohydration *1*) strongly enhanced the catabolic hormonal response to resistance exercise; *2*) altered the anabolic hormonal response to resistance exercise; and *3*) increased circulating concentrations of metabolic substrates. Overall, these results indicate hydration state can significantly modify the endocrine and metabolic responses to resistance exercise and importantly influences the postexercise internal environment.

We recognize that equating REC exercise volume among experimental trials decreased the investigation's external validity for training athletes who rarely "make-up" failed repetitions. Despite this limitation, two facts justify the current research design. First, mandating an identical number of repetitions isolated the independent effect of hypohydration from the potentially confounding effects of differing total work (28, 30). Second, several nonathlete populations, such as military personnel and laborers, generally complete exercise bouts based on total work (e.g., loading all the boxes onto a truck) rather than a number of attempted sets and repetitions (e.g., loading as many boxes as possible in six attempts).

Catabolic hormones and metabolic markers. Hypohydration strongly increased cortisol, epinephrine, and norepinephrine

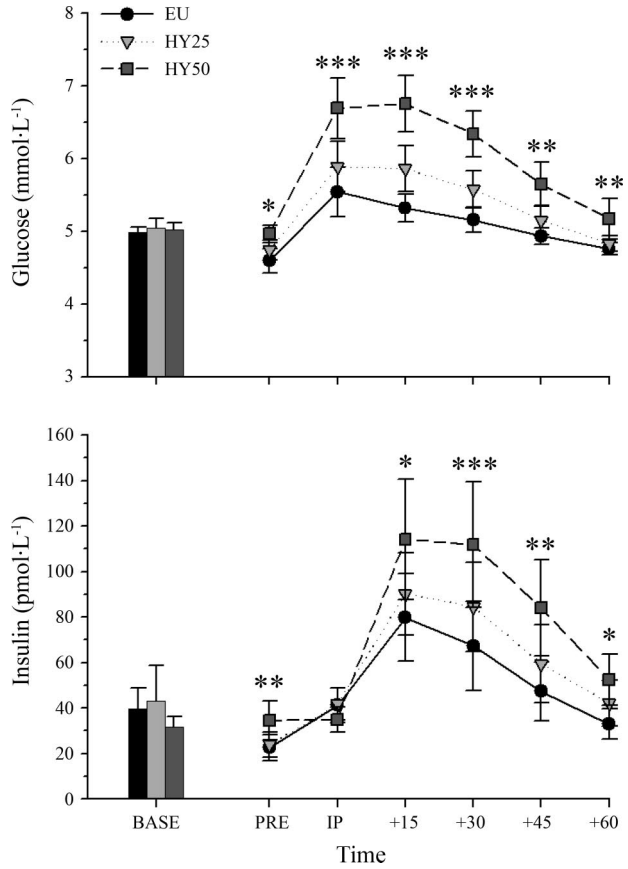

Fig. 3. Glucose (*top*) and insulin (*bottom*) responses (means ± SE) to hypohydration and resistance exercise. Significant difference between *HY50 and EU; **HY50 and both EU and HY25; and ***all trials at a given time: $P < 0.05$.

Table 3. *Lactate responses to hypohydration and resistance exercise*

	Lactate, mmol/l						
Trial	Base	Pre	IP	+15	+30	+45	+60
EU	1.8±1.2	1.7±0.6	13.4±3.7	10.8±2.0	7.1±1.7	5.0±1.3	3.6±0.9
HY25	2.0±1.4	1.4±0.6†	14.6±3.0	11.1±2.8	6.8±2.0	4.7±1.1	3.5±0.8
HY50	2.2±1.4*	1.6±0.5	15.3±2.6	10.5±3.0	6.1±1.8	4.1±1.0*	3.1±0.7

Values are means ± SD. Significant difference between *HY50 and EU, and †HY25 and EU: $P < 0.05$.

(Fig. 2), the primary stress hormones. These hydration-induced hormonal responses mirror those produced by hypohydrated endurance exercise (7, 33, 35, 37, 39, 45, 47, 56–58) and suggest that hypohydration significantly enhances the stress of resistance exercise. Hypohydration likely stimulated the catabolic hormones by increasing core temperature (supported by the significantly different resting core temperatures noted immediately Pre between the hypohydrated and EU conditions) (37, 43) and cardiovascular demand resulting from decreased plasma volume (45, 47, 56).

The experimental trials also significantly modified circulating concentrations of insulin and metabolic markers (Figs. 3 and 4). Rather than a direct effect of hydration on these variables, these changes likely occurred secondary to hydration-induced increases of the catabolic hormones, which potently stimulate glycogenolysis, gluconeogenesis, and lipolysis (8, 42). The importance of their relative metabolic influences, however, likely differed over time. Before exercise and during recovery (Pre, +15, +30, +45, and +60), hydration-induced differences in cortisol increased blood glucose. As expected, insulin increased to match the rise in glucose, simultaneously blunting lipolysis and producing similar glycerol concentrations during all trials. During exercise and IP, exercise-induced increases in the catecholamines (differing by hydration state in the case of norepinephrine) stimulated hepatic glucose production but suppressed insulin release; removal of insulin inhibition and addition of the catecholamines promoted lipolysis, significantly increasing glycerol (again, differing by hydration state). Overall, hypohydration increased the stress hormone response to resistance exercise, stimulating a massive substrate release. Teleologically, these actions might reflect the hypohydrated body's attempt to cope with the increased physiological demands of completing and recovering from intense resistance exercise.

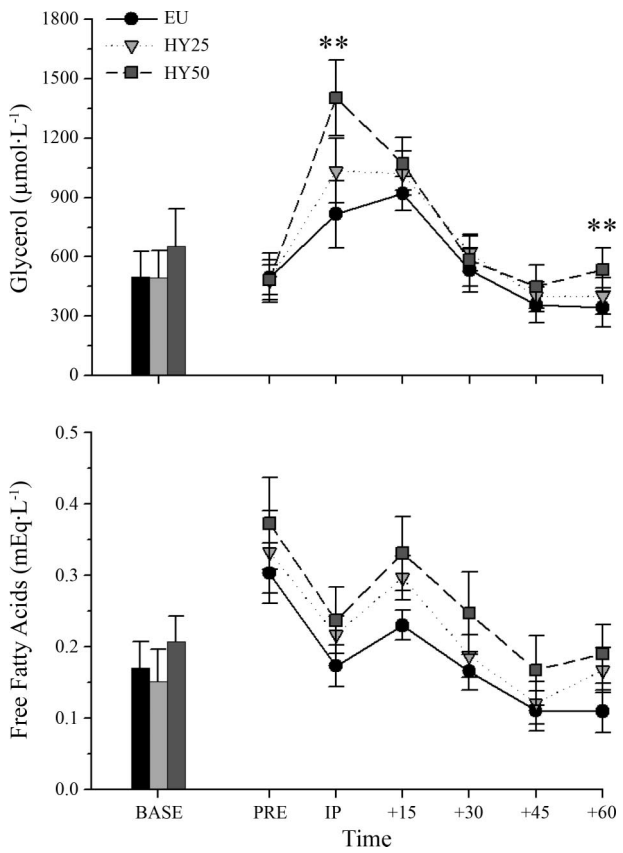

Fig. 4. Glycerol (*top*) and free fatty acid (*bottom*) responses (means ± SE) to hypohydration and resistance exercise. **Significant difference between HY50 and both EU and HY25, $P < 0.05$.

Alternately, the increased glucose noted during recovery (+15 to +60 min) and the greater free fatty acid AUC might result from hypohydration-induced insulin resistance. We know of no studies that directly examine this hypothesis in exercising humans; however, a decrease in cell volume caused by dehydration promotes insulin resistance (50–52), and two previous studies examining the effect of hypohydration on endurance exercise indirectly support this hypothesis (16, 20). Resistance exercise might exacerbate the effects of hypohydration on insulin resistance, considering the strong relationships between *1*) intense resistance exercise and muscle damage (9, 55), and *2*) muscle damage and insulin resistance (14, 25, 26).

Anabolic hormones. Although no time points significantly differed between trials, testosterone AUC was significantly decreased during HY50 compared with EU (Fig. 5), conflicting previous research (23, 33) utilizing very different exercise modalities. This finding, combined with the consistent stepwise arrangement of postexercise data points (a pattern occurring at random in only 1 of 7,776 cases) suggests that hypohydration attenuates the resistance exercise-induced increases in circulating concentrations of testosterone. Interestingly, this decrease occurred in the face of increased catecholamine concentrations, contradicting significant animal (17, 38) and in vitro (1–4) work that suggests catecholamines stimulate testosterone synthesis and release. Hypohydration might overcome this effect and blunt the testosterone responses to resistance exercise by stimulating increases in insulin and/or cortisol, analytes associated with decreases in testosterone synthesis or secretion (10, 18, 31, 40).

Hypohydration also failed to alter growth hormone responses to exercise (Fig. 5), unlike previous literature demonstrating increased (19, 48, 57) or decreased (41) growth hormone during endurance exercise with hypohydration. The very different exercise stimulus we employed might explain these conflicting results, but specific methodological choices likely

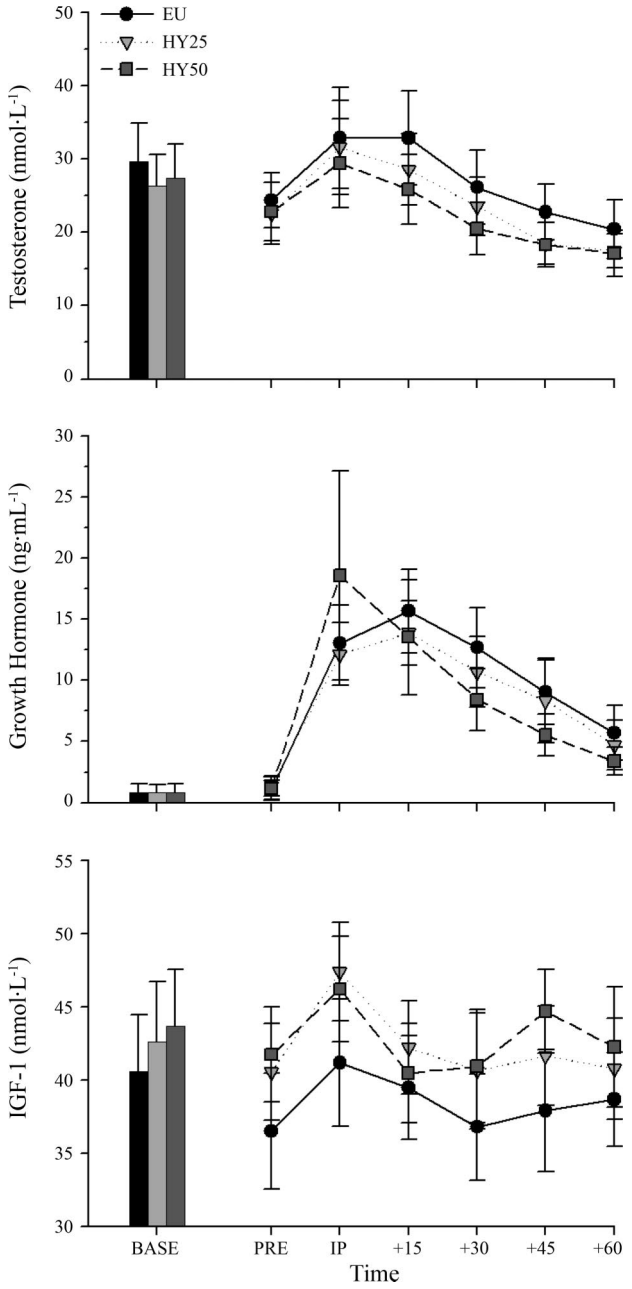

Fig. 5. Testosterone (*top*), growth hormone (*middle*), and insulin-like growth factor-I (IGF-I; *bottom*) responses (means ± SE) to hypohydration and resistance exercise. For testosterone, $n = 6$. No significant differences existed between trials at any point.

better clarify the apparently diverse responses. In the present investigation, hypohydration failed to directly influence growth hormone but clearly altered several growth hormone stimulators (the catecholamines) and inhibitors (glucose and free fatty acids) (8, 44). Presumably, these opposing influences balanced each other, and no change occurred in growth hormone. Research documenting hypohydration-induced increases in growth hormone employed lengthy protocols (140–180 min) in hot conditions (35–49°C) (19, 48) or maximal-intensity aerobic exercise (57); the duration, ambient temperature, and/or intensity of these exercise bouts promote increased catecholamines and/or hypoglycemia, stimulating growth hormone. The lone study displaying a hypohydration-induced decrease in growth hormone (41) examined short-duration (40 min) exercise in temperate conditions (25°C), likely limiting sympathetic nervous response. Furthermore, subjects supplemented ~500 kcal 2 h before exercise, likely increasing substrate concentrations and attenuating hypoglycemia. The apparent susceptibility of potent growth hormone influences to hypohydration suggests that "real-life" exercise situations (i.e., less intense exercise not preceded by an overnight fast) might show a susceptibility of growth hormone to hydration state. Alternately, the lack of differences among trials in growth hormone might reflect the similarity among hydration states in blood lactate, a proposed mediator of growth hormone release (12, 21).

Unexpectedly, IGF-I AUC during HY25 and HY50 exceeded EU; the enhanced IGF-I noted during hypohydration trials appeared to result from higher resting values, not an altered exercise response (Fig. 5). These results imply hypohydration directly affects IGF-I; unfortunately, no previous research confirms or refutes this hypothesis. Alternately, the dehydration stimulus might have caused the differences between trials. As previously mentioned, long-duration, low-intensity endurance exercises in hot conditions stimulate a growth hormone response (19, 48), suggesting the walking bout completed the day before the REC stimulated an increase in circulating concentrations of growth hormone. As *1*) subjects replaced less fluid during HY25 and HY50 than EU, and *2*) hypohydration diminishes thermoregulatory capabilities (49), core temperatures during the hypohydration trials were likely maintained at a greater magnitude for a longer duration than during the EU trial (a hypothesis supported by the significant differences between trials in rectal temperature immediately before the REC). Growth hormone directly stimulates IGF-I after a significant delay (13), so core temperature-induced increases in growth hormone the evening before the REC also might explain the increased IGF-I noted during HY25 and HY50.

Implications of chronic hypohydration. The results of this novel research indicate that hypohydration produces a less beneficial postexercise hormonal milieu, increasing catabolism and potentially decreasing anabolism. Combined with evidence documenting the deleterious effect of hypohydration on performance of multiset, multirepetition resistance exercise tasks (24), these findings suggest individuals who routinely complete resistance exercises in a hypohydrated state might attenuate overall training adaptations (22, 27, 54). Resistance exercise is recommended to improve quality of life (e.g., elderly), maintain occupational safety (e.g., soldiers and astronauts), and/or maximize exercise performance (e.g., athletes); the propensity of these groups to experience some degree of hypohydration suggests the current results might represent an important step in maximizing the physiological adaptations to resistance exercise. Confirmation of this hypothesis clearly awaits documented evidence, but these results and the significant populations potentially affected indicate the importance of future research examining this topic.

Conclusions. In conclusion, hypohydration up to 4.8% body mass loss significantly altered the endocrine and metabolic internal environments before and after intense resistance exercise. The stress of hypohydration significantly enhanced the exercise-induced increase in catabolic hormones and modified

the anabolic hormonal response. Likely secondary to these hormonal shifts, hypohydration stimulated a large influx of metabolic substrates. These data demonstrate that body water status is an important consideration in modulating the hormonal and metabolic responses to resistance exercise.

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GRANTS

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