

Effects of sprint training on extrarenal potassium regulation with intense exercise in Type 1 diabetes

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Harmer, Alison R., Patricia A. Ruell, Michael J. McKenna, Donald J. Chisholm, Sandra K. Hunter, Jeanette M. Thom, Norman R. Morris, and Jeff R. Flack. Effects of sprint training on extrarenal potassium regulation with intense exercise in Type 1 diabetes. *J Appl Physiol* 100: 26–34, 2006. First published September 22, 2005; doi:10.1152/jappphysiol.00240.2005.—Effects of sprint training on plasma K⁺ concentration ([K⁺]) regulation during intense exercise and on muscle Na⁺-K⁺-ATPase were investigated in subjects with Type 1 diabetes mellitus (T1D) under real-life conditions and in nondiabetic subjects (CON). Eight subjects with T1D and seven CON undertook 7 wk of sprint cycling training. Before training, subjects cycled to exhaustion at 130% peak O₂ uptake. After training, identical work was performed. Arterialized venous blood was drawn at rest, during exercise, and at recovery and analyzed for plasma glucose, [K⁺], Na⁺ concentration ([Na⁺]), catecholamines, insulin, and glucagon. A vastus lateralis biopsy was obtained before and after training and assayed for Na⁺-K⁺-ATPase content ([³H]ouabain binding). Pretraining, Na⁺-K⁺-ATPase content and the rise in plasma [K⁺] (Δ [K⁺]) during maximal exercise were similar in T1D and CON. However, after 60 min of recovery in T1D, plasma [K⁺], glucose, and glucagon/insulin were higher and plasma [Na⁺] was lower than in CON. Training increased Na⁺-K⁺-ATPase content and reduced Δ [K⁺] in both groups ($P < 0.05$). These variables were correlated in CON ($r = -0.65$, $P < 0.05$) but not in T1D. This study showed first that mildly hypoinsulinemic subjects with T1D can safely undertake intense exercise with respect to K⁺ regulation; however, elevated [K⁺] will ensue in recovery unless insulin is administered. Second, sprint training improved K⁺ regulation during intense exercise in both T1D and CON groups; however, the lack of correlation between plasma Δ [K⁺] and Na⁺-K⁺-ATPase content in T1D may indicate different relative contributions of K⁺-regulatory mechanisms.

glycemia; potassium regulation; Na⁺-K⁺-ATPase; high-intensity exercise; insulin

THE MAJOR SITES OF K⁺ CLEARANCE, which contribute to plasma K⁺ concentration ([K⁺]) regulation, include skeletal muscle, the liver, and excretion via the kidney (27). Acute K⁺ loads are cleared via extrarenal mechanisms, whereas renal K⁺ excretion contributes to longer term regulation (3). Insulin has long been known to acutely reduce plasma [K⁺] (4, 23) via an increase in muscle (1, 48, 49) and hepatic K⁺ uptake (9, 12). In nondiabetic subjects, insulin is secreted in response to increments in

plasma K⁺ (3); conversely, acute reduction of basal insulin secretion, induced by somatostatin, increases [K⁺] (8).

During exercise, muscle K⁺ efflux increases with increasing exercise intensity (18, 47), and therefore maximal exercise induces a marked elevation in arterial plasma [K⁺] (35, 47). The major mechanism by which acute K⁺ clearance is effected during intense exercise is via skeletal muscle Na⁺-K⁺-ATPase, which extrudes cellular Na⁺ in exchange for K⁺: this exchange is crucial in protecting muscle membrane excitability and contractility during intense muscle activity (43). The pump is subject to both acute and chronic regulation by a variety of stimuli, including hormones, contractile activity, exercise training, and electrolyte and nutritional status (7). Acute increases in Na⁺-K⁺-ATPase activation occur with increases in the catecholamines, insulin, intracellular Na⁺ concentration ([Na⁺]), and most dramatically via muscle excitation (6, 10, 36). Chronic stimulation by exercise training increases muscle Na⁺-K⁺-ATPase content (14, 33). We previously demonstrated in nondiabetic men that sprint cycle training increased muscle Na⁺-K⁺-ATPase content (33) and improved plasma K⁺ regulation during maximal exercise (20, 33, 35).

The only previous study to examine Na⁺-K⁺-ATPase content in human Type 1 diabetes mellitus (DM) reported 22% higher content in vastus lateralis muscle than in nondiabetic subjects (42). Therefore, K⁺ regulation during intense exercise, in which large plasma [K⁺] increments occur, may be enhanced in those with Type 1 DM. However, hyperkalemia may occur under resting conditions in subjects with Type 1 DM who are relatively hypoinsulinemic (46). Mild preexercise hypoinsulinemia is commonly recommended in Type 1 DM to prevent hypoglycemia during and after sustained exercise. However, the effect of intense exercise on plasma [K⁺] regulation in subjects with Type 1 DM who are mildly hypoinsulinemic is unknown.

The present study investigated the effects of high-intensity training on muscle Na⁺-K⁺-ATPase content and on plasma K⁺ regulation during intense exercise in subjects with Type 1 DM under real-life conditions. We hypothesized that 1) maximal exercise would increase plasma [K⁺] similarly in subjects with Type 1 DM who were mildly hypoinsulinemic compared with healthy controls and 2) sprint training would increase Na⁺-K⁺-ATPase content and improve plasma K⁺ regulation during maximal exercise in both groups.

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METHODS

Subjects. Eight subjects with Type 1 DM (T1D group; 3 women, 5 men) and seven healthy controls (CON group; 3 women, 4 men) were recruited concurrently over a period of 3 yr. In T1D subjects, duration of DM was 7.1 ± 4.0 yr (mean \pm SD), average daily insulin dose was 52 ± 11 U/day, and glycosylated hemoglobin (Hb) was $8.6 \pm 0.8\%$. Control subjects (glycosylated Hb, $5.3 \pm 0.3\%$) were chosen to closely match those with diabetes for age (T1D, 25 ± 4 yr; CON, 24 ± 5 yr), body mass index (T1D, 25.4 ± 3.2 kg/m²; CON, 23.8 ± 5.0 kg/m²), and peak O₂ uptake ($\dot{V}O_{2\text{ peak}}$; see RESULTS). Potential subjects with DM were excluded from the study if any of the following complications of diabetes were present: proteinuria or microalbuminuria; proliferative retinopathy, >10 microaneurysms in the previous year; or autonomic or peripheral neuropathies. Inclusion criteria for subjects with Type 1 DM were diabetes duration of ≥ 1.5 yr and glycosylated Hb of $<10\%$. Subjects without diabetes had no family history of metabolic disorders. No subject smoked, took regular medication (other than insulin in the T1D group), or had previously engaged in high-intensity cycle training. Each subject gave written, informed consent. This study was approved by the respective Human Research Ethics Committees of The University of Sydney and the South Western Sydney Area Health Service.

Experimental protocol. Testing was conducted in the postabsorptive state. Subjects in the T1D group reduced their usual nighttime dose of insulin by 1–2 U to prevent a hypoglycemic episode on the morning of the tests and delayed their morning insulin dose until after testing had been completed. Before training, all subjects recorded their dietary intake for the 2 days before blood testing. After exercise training, each subject replicated their pretraining diet for the 2 days before testing. Subjects abstained from alcohol consumption and vigorous exercise for 48 h before each test.

Exercise testing: incremental test. Before training (2 days after an identical familiarization trial), subjects cycled on an electronically braked ergometer (Ergoline 800s, Mijndhardt, The Netherlands) for 4 min at 60, 90, 120, and 150 W to obtain steady-state O₂ uptake ($\dot{V}O_{2\text{ peak}}$). After heart rate had returned to within 10 beats/min of resting values, a 10 W/30 s incremental test to volitional fatigue was commenced to obtain $\dot{V}O_{2\text{ peak}}$, defined as the highest $\dot{V}O_{2\text{ peak}}$ measured during a 30-s period. Expired volume was determined using a pneumotach (Hans Rudolph), and expired gas fractions were determined by O₂ and CO₂ analyzers (Ametek, Thermo Instruments, Pittsburgh, PA), which were calibrated immediately before and after each test. The power output required to elicit 130% $\dot{V}O_{2\text{ peak}}$ was calculated and used for the subsequent sprint tests as previously described (20). $\dot{V}O_{2\text{ peak}}$ and submaximal $\dot{V}O_{2\text{ peak}}$ were reassessed using an identical protocol 5–8 days after the final training session.

Exercise testing: constant-load sprint test. Before training, after an identical test on a separate day (used for familiarization and to collect respiratory data), a sprint test (Pre) was conducted to exhaustion on the electronically braked cycle ergometer. After a 3-min warm-up at 20 W, subjects pedaled at a power output equivalent to 130% $\dot{V}O_{2\text{ peak}}$ at 110 rpm until exhaustion, defined as the inability to maintain a cadence of ≥ 80 rpm despite strong verbal encouragement (20). Both muscle and blood were sampled in this sprint test. After training, a test was conducted at the same power output and for the same duration as the pretraining test, i.e., the work was matched (Post) with blood sampling times matched to Pre.

Muscle sampling and analyses. The skin and the fascia overlying the vastus lateralis muscle were anesthetized using 2% xylocaine (without epinephrine); then a percutaneous biopsy with suction was performed. Muscle samples ($n = 7$ T1D; $n = 6$ CON), obtained at rest before and after training, were immediately immersed in liquid nitrogen. Muscle Na⁺-K⁺-ATPase content was assayed by vanadate-facilitated [³H]ouabain binding using the standard method for small muscle samples (41). After incubation, washing, and blotting, the mean muscle wet weight (ww) was 5.99 ± 0.19 mg. The [³H] activity

was counted in a WinSpectral 1414 Liquid Scintillation Counter (Wallac, Turku, Finland). The mean intra-assay coefficient of variation was $5.6 \pm 0.7\%$. The [³H]ouabain binding site concentration is expressed as pmol·(g ww)^{−1}.

Blood sampling and analyses. A 22-gauge flexible catheter (Optiva 225, Johnson & Johnson, Australia) was inserted into a dorsal hand vein and secured with a waterproof dressing. Minimum volume extension tubing (25 cm; Tuta Laboratories) was connected to the catheter, and a one-way valve (Safsite, B. Braun) was attached to the tubing to enable rapid sampling. The hand was placed inside a plastic bag, then immersed in warm water for the duration of each test to arterialize the venous blood (20). The catheter was kept patent by periodic administration of sterile saline.

Blood sampling during exercise tests. At each time point, two to three blood samples were collected in the following order: catecholamines, blood gases, then insulin and glucagon. Plasma was analyzed for norepinephrine and epinephrine concentrations by high-performance liquid chromatography with electrochemical detection as previously described (20). Blood gases, pH, Hb, and plasma [Na⁺] and [K⁺] were analyzed in a Corning 865 analyzer (Chiron Diagnostics). Plasma glucose (PG) was analyzed using a commercial kit (Thermo Electron, Melbourne, Australia). Hematocrit (Hct) was measured in duplicate in microcapillary tubes. The percentage change in plasma volume (% Δ PV) relative to rest was calculated from measured Hct and Hb concentration, and the increase in plasma [K⁺] from rest to the end of exercise was calculated (Δ [K⁺]) (33). Osmolarity was estimated using the formula $2([Na^+] + [K^+]) + [PG]$, where brackets denote concentration. Urea was not included in the calculation because it is so diffusible as to be considered irrelevant (24). Blood was mixed with aprotinin (10,000 IU/ml), centrifuged, and the plasma stored at -85°C until analyzed for glucagon concentration by a double-antibody RIA method (Euro Diagnostica, Malmö, Sweden). Serum was analyzed for insulin by double-antibody RIA (NOVO Industri, Bagsvaerd, Denmark). In the T1D group, free insulin was measured by precipitating immunoglobulins from the sample with polyethylene glycol before RIA.

T1D resting study. On a separate day before training, each of the subjects in the T1D group attended the laboratory for collection of blood samples at rest. Plasma (or serum) was assayed for glucose, free insulin, [K⁺], and [Na⁺]. This test was designed to examine the effect of delaying insulin administration on metabolic and ionic control, thus providing a nonexercise comparison for the invasive exercise tests. Subject preparation for this test was identical to that for the other blood tests. The time of day, the total test time, the preparation and procedures for blood sampling, and the postures adopted in the 130% $\dot{V}O_{2\text{ peak}}$ tests in which blood and muscle were sampled were all replicated.

High-intensity exercise training program. Subjects undertook 7 wk of supervised, progressive high-intensity cycling training, conducted either at home or in the laboratory, three times per week, as previously described (20, 33). Each training session consisted of four to ten 30-s “all out” sprints on a mechanically braked cycle ergometer (Monark 668, Varberg, Sweden), with each sprint separated by a 3- to 4-min passive rest interval. Each subject completed 21 training sessions. The flywheel tension was kept constant for the duration of the training program at 0.075 kp/kg body mass. Training overload was imposed by progressively increasing the number of 30-s sprint bouts per session from 4 in week 1 to 6 in week 2, 8 in week 3, through to 10 in weeks 4–7, and by reducing the rest interval from 4 to 3 min in weeks 5–7 of the training period.

Statistics. Data were analyzed using repeated-measures ANOVA (within-subjects factors: training status, sample time; between-subjects factor: group; SPSS 10.0 for Windows). Significant *F* ratios were further examined using an ANOVA contrast technique (SPSS). Statistical significance was accepted at $P < 0.05$. Results are reported as means \pm SD.

Table 1. Hematocrit and hemoglobin at rest, during, and after maximal exercise tests conducted before and after sprint training

			Recovery Time, min					
	Rest	End of Exercise	1	2	5	20	45	60
<i>Hct, %*†</i>								
T1D								
Pre	43±3	47±3	48±3	49±2	48±3	44±4	43±2	43±3
Post	44±2	47±2	48±3	49±2	48±2	44±2	43±2	42±3
CON								
Pre	40±4	44±4	46±3	46±2	46±2	41±3	40±4	41±3
Post	41±4	44±3	45±3	45±2	44±4	40±2	38±4	40±3
<i>[Hb], g/dl*‡</i>								
T1D								
Pre	14.1±0.7	15.3±0.7	15.6±1.0	16.0±0.6	15.4±0.9	13.9±0.9	13.9±0.6	13.9±0.7
Post	14.2±0.9	15.3±1.0	15.6±1.0	15.6±1.1	15.3±1.0	14.1±0.6	14.0±0.9	14.2±0.6
CON								
Pre	14.0±1.1	14.9±1.2	15.4±1.0	15.2±0.8	15.0±0.9	13.7±0.6	13.8±1.1	13.7±1.1
Post	13.9±1.1	14.6±1.0	14.7±1.0	14.3±1.1	14.2±1.1	13.1±1.0	13.0±0.8	13.0±0.9

Values are means ± SD. End of exercise at 130% of pretraining peak O₂ uptake. T1D, group with Type 1 diabetes mellitus; CON, control group; Pre, before training; Post, after training. *Main effect of time ($P < 0.001$), with Hct and hemoglobin concentration ([Hb]) at end of exercise through 5 min of recovery greater than at rest, and 45 and 60 of recovery less than at rest. †Main effect of group ($P < 0.05$), with T1D > CON for Hct. ‡Training status-by-time interaction for [Hb] ($P < 0.05$), with [Hb] lower after training at 1, 2, and 5 min of recovery. For Hct, $n = 8$ T1D, except at 1 and 2 min of recovery where $n = 7$; $n = 7$ CON, except at 2 and 20 of recovery where $n = 6$. For [Hb], $n = 6$ T1D, except at 1, 2, 20, and 60 min of recovery where $n = 5$; $n = 6$ CON, except at 2, 5, and 20 min of recovery where $n = 5$.

RESULTS

Increased incremental exercise performance. After training, $\dot{V}O_{2\text{ peak}}$ was increased $6 \pm 10\%$ (T1D, pretraining, 3.30 ± 0.97 l/min, posttraining, 3.38 ± 1.00 l/min; CON, pretraining, 3.17 ± 0.79 l/min, posttraining, 3.43 ± 0.98 l/min; $P < 0.05$) and peak incremental power was increased $11 \pm 10\%$ (T1D, pretraining, 269 ± 80 W, posttraining, 298 ± 95 W; CON, pretraining, 270 ± 64 W, posttraining, 297 ± 69 W; $P < 0.01$), with no differences between the groups.

Constant-load tests. Before training in the 130% $\dot{V}O_{2\text{ peak}}$ test (Pre), time to exhaustion was 78 ± 21 s for T1D (cumulative work 25 ± 7 kJ) and 62 ± 17 s for CON (work 21 ± 7 kJ), with no group differences. As designed, both power output and exercise time were identical in Pre and Post tests. Consequently, there was a small reduction in relative exercise intensity after training in Post ($P = 0.05$), with the power output being calculated to elicit 127 ± 10 (T1D) and $122 \pm 12\%$ $\dot{V}O_{2\text{ peak}}$ (CON), with no difference between groups.

Vanadate-facilitated [³H]ouabain binding. Muscle [³H]ouabain binding site ($\text{Na}^+/\text{K}^+/\text{ATPase}$) content before training was 328 ± 62 and 313 ± 72 pmol/g wet wt in T1D and CON, respectively, with no difference between groups. After training, $\text{Na}^+/\text{K}^+/\text{ATPase}$ content was $8.2 \pm 8.1\%$ higher ($P < 0.05$), with no difference between groups [T1D, 354 ± 77 pmol/g wet wt; CON, 337 ± 60 pmol/g wet wt; $P = 0.90$].

Hematology and plasma volume shifts during constant load exercise. Hct and Hb both increased with exercise, peaked at 1–2 min of recovery, then fell below resting values at 45 and 60 min of recovery (Table 1). There was no effect of training on Hct; however, overall Hct was 3.1% higher in T1D than CON ($P < 0.05$). After training, [Hb] did not differ at rest; however, an interaction effect between time and training status was evident whereby [Hb] was lower after training at 1, 2, and 5 min of recovery ($P < 0.05$; Table 1), with no difference between groups.

The nadir for $\%\Delta\text{PV}$ from rest occurred 1–2 min after exercise ($P < 0.001$), followed by a return to rest by 20 min, then a relative expansion at 45 and 60 min of recovery ($P < 0.01$; Fig. 1). After training, $\%\Delta\text{PV}$ was similar immediately after exercise, but there was less hemoconcentration at 1, 2, and 5 min of recovery ($P < 0.01$; training status-by-time interaction; Fig. 1). In addition, after training, there was greater relative plasma volume expansion at 5 and 20 min of recovery

Percent change in plasma volume with exercise and training

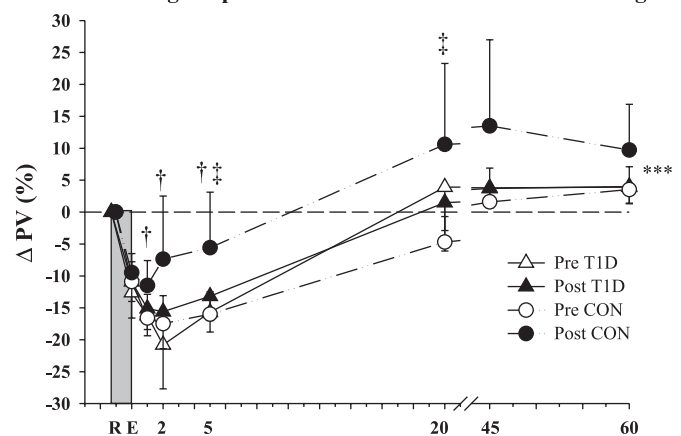


Fig. 1. Percent change in plasma volume (PV; means ± SD) from rest (R) for end of exercise (E) at 130% pretraining peak O₂ uptake ($\dot{V}O_{2\text{ peak}}$), and at 1, 2, 5, 20, 45, and 60 min of recovery before (Pre) and after training (Post) in the group with Type 1 diabetes mellitus (T1D; $n = 8$, except at 1–60 min of recovery where $n = 6$) and the nondiabetic group (CON; $n = 7$, except at 1–60 min of recovery where $n = 6$). The shaded bar represents the period of exercise. A main effect for time ($***P < 0.001$) was evident with E through 5 min of recovery <R, and 45 and 60 min of recovery >R. Interaction effects were evident for training status-by-time ($†P < 0.05$) with less contraction of PV at 1, 2, and 5 min of recovery after training and training status-by-time-by-group ($‡P < 0.05$) with less contraction of PV after training in the CON than T1D group at 5 and 20 min of recovery.

in the CON than T1D group ($P < 0.01$; training status-by-time-by-group interaction).

Plasma $[K^+]$. Plasma $[K^+]$ at rest did not differ between groups. Plasma $[K^+]$ peaked in the final seconds of exercise ($P < 0.001$), then briefly fell below rest at 5 min of recovery (Fig. 2). Interestingly, in T1D, plasma $[K^+]$ then increased after the postexercise nadir to be $10 \pm 5\%$ above the resting value at 60 min of recovery ($P < 0.001$). This response differed from CON (time-by-group interaction, $P < 0.05$, observed power 0.86), in whom the 60-min recovery plasma $[K^+]$ was similar to rest. After training, plasma $[K^+]$ was lower across all times by a mean difference of 0.17 ± 0.34 mM (Fig. 2; $P < 0.05$; main effect) and was $7 \pm 9\%$ lower during exercise (by 0.39 ± 0.50 mM) and at 1 min of recovery (by 0.36 ± 0.42 mM) ($P < 0.01$; training status-by-time interaction effect; observed power 0.98), with no difference in plasma $[K^+]$ between groups ($P = 0.58$; training status-by-time-by-group interaction effect; observed power 0.40).

The mean rise from rest in plasma $[K^+]$ with exercise ($\Delta[K^+]$) was 1.43 ± 0.51 mM in Pre with no significant difference between groups. After training, $\Delta[K^+]$ was $21 \pm 27\%$ lower (T1D, $-20 \pm 22\%$; CON, $-22 \pm 34\%$; $P < 0.05$).

The ratio of $\Delta[K^+]$ to work ($\Delta[K^+]/\text{work}$) did not differ significantly between groups and was reduced $21 \pm 28\%$ in Post (T1D, Pre 55 ± 21 , Post 43 ± 16 ; CON, Pre 77 ± 32 , Post 59 ± 32 $\text{nmol} \cdot \text{l}^{-1} \cdot \text{J}^{-1}$; $P < 0.01$).

Relationships between $\text{Na}^+ \cdot \text{K}^+ \cdot \text{ATPase}$ content, $\Delta[K^+]$, and work. $\Delta[K^+]$ was significantly inversely correlated with $\text{Na}^+ \cdot \text{K}^+ \cdot \text{ATPase}$ content for the CON group alone ($r = -0.65$, $n = 12$ (6 Pre, 6 Post values), $P < 0.05$; Fig. 3A) and when data were pooled for both groups ($r = -0.41$, $n = 26$, $P < 0.05$). However, when the T1D group was considered alone, there was no correlation between $\Delta[K^+]$ and $\text{Na}^+ \cdot \text{K}^+ \cdot \text{ATPase}$ content ($r = -0.05$, $n = 14$, $P = 0.86$; Fig. 3A). Similarly, significant relationships were found between $\text{Na}^+ \cdot \text{K}^+ \cdot \text{ATPase}$ content and $\Delta[K^+]/\text{work}$ for the CON group ($r = -0.59$, $n = 12$, $P < 0.05$) but not for the T1D group ($r = -0.46$, $n = 14$, $P = 0.10$; Fig. 3B).

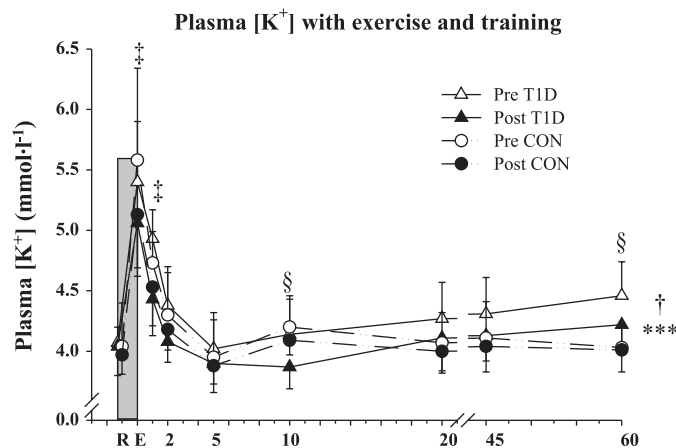


Fig. 2. Plasma K^+ concentration ($[K^+]$) at R, at E at 130% pretraining $\dot{V}O_2$ peak, and in recovery (1–60 min) before and after training in T1D ($n = 8$, except at 1, 2, 20, and 60 min of recovery where $n = 7$) and CON ($n = 7$, except at 2, 5, and 20 min of recovery where $n = 6$). Symbols, the shaded bar, and x-axis markings are the same as in Fig. 1. Main effects were evident for time ($***P < 0.001$) with $[K^+]$ different from R at E through 5 and 20 through 60 min of recovery and training status (lower posttraining, $†P < 0.05$), and interaction effects were evident for training status by time ($‡P < 0.01$) with E and 1 min lower after training, and time by group ($§P < 0.05$) with differences at 10 and 60 min of recovery in the T1D group.

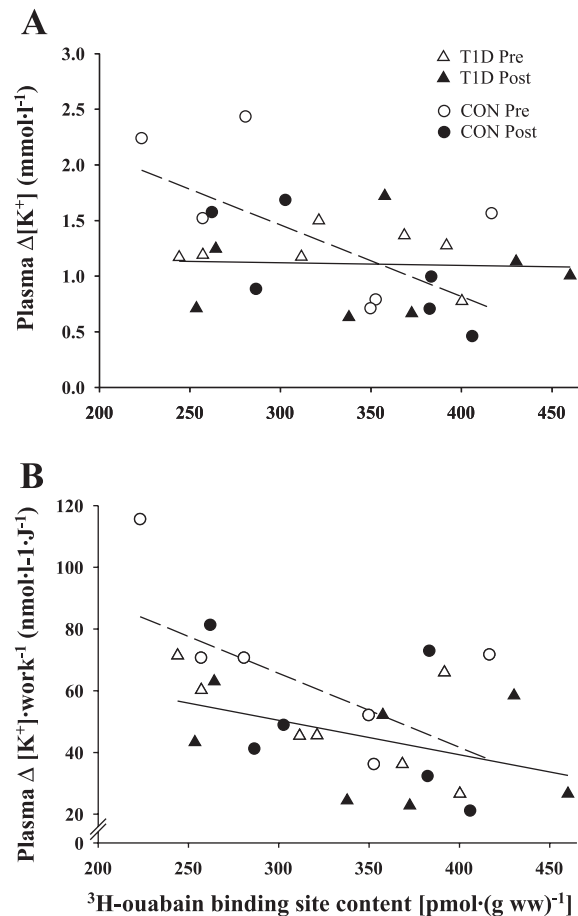


Fig. 3. A: relationship between $\Delta[K^+]$ (exercise – rest $[K^+]$) and muscle $[^3\text{H}]\text{ouabain}$ binding site content ($\text{Na}^+ \cdot \text{K}^+ \cdot \text{ATPase}$ content) before and after training for T1D ($n = 14$; solid line, $y = -0.0002x + 1.19$; $r = -0.05$; $P = 0.86$) and CON ($n = 12$; broken line, $y = -0.006x + 3.39$; $r = -0.65$; $P < 0.05$). B: relationship between the ratio of $\Delta[K^+]$ to work ($\Delta[K^+]/\text{work}$) and muscle $[^3\text{H}]\text{ouabain}$ binding site content before and after training for T1D ($n = 14$; solid line, $y = -0.112x + 84.0$; $r = -0.46$; $P = 0.10$) and CON ($n = 12$; broken line, $y = -0.24x + 137.5$; $r = -0.59$; $P < 0.05$).

$K^+ \cdot \text{ATPase}$ content and $\Delta[K^+]/\text{work}$ for the CON group ($r = -0.59$, $n = 12$, $P < 0.05$) but not for the T1D group ($r = -0.46$, $n = 14$, $P = 0.10$; Fig. 3B).

PG concentration. PG was 8.6 mM higher across both days and all times in the T1D group than in the CON group ($P < 0.001$); however, resting concentrations did not differ within each group before and after sprint training. Before training in the CON group, PG increased during early recovery to peak after 5 min at 6.42 ± 0.53 mM ($P < 0.001$), then fell to resting values by 20 min of recovery. In the T1D group, PG rose during exercise, and continued to rise unabated ($P < 0.001$), peaking at 16.8 ± 4.1 mM after 60 min of recovery. The peak change in PG from rest (ΔPG) was 0.9 ± 0.3 mM in CON and 3.8 ± 1.7 mM in T1D (Fig. 4). Sprint training had no significant effect on either PG or ΔPG in either group (Fig. 4).

Plasma catecholamine concentrations. Plasma norepinephrine concentration ($[\text{NEpi}]$) rose sharply with exercise, peaked after 1 min of recovery ($P < 0.001$), then declined to resting levels after 60 min of recovery in both groups (Fig. 5A). $[\text{NEpi}]$ was 65% higher in the T1D group in the final seconds of exercise ($P < 0.05$; time-by-group interaction). After training,

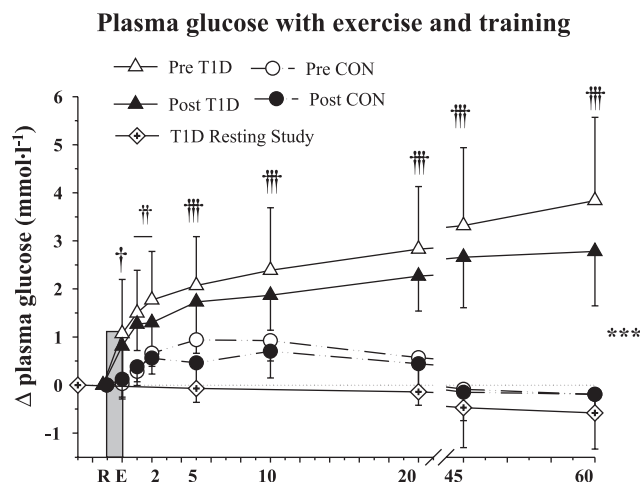


Fig. 4. Change in plasma glucose (Δ PG = difference from PG concentration at rest) before and after training for the T1D ($n = 7$) and CON groups ($n = 7$). Symbols, the shaded bar, and x-axis markings are similar to Fig. 1. A main effect was evident for time ($***P < 0.001$) and an interaction effect was evident for time by group ($\dagger\dagger\dagger P < 0.001$), whereby T1D > CON from E through 60 min of recovery (different from CON: $\dagger P < 0.05$; $\dagger\dagger P < 0.01$; $\dagger\dagger\dagger P < 0.001$). Also included is Δ PG for the T1D resting study that was conducted on a separate day. Δ PG in the T1D resting study ($n = 8$) fell slightly over time ($P < 0.05$).

[NEpi] tended to be lower (14%, mean difference, 0.84 ± 2.4 nM; $P = 0.07$) in both groups (Fig. 5A).

Plasma epinephrine concentration rose with exercise, peaked at 1 min of recovery ($P < 0.001$), then returned to resting levels by 10 min of recovery (Fig. 5B). No statistical difference was found between groups. Sprint training did not significantly alter epinephrine concentration, although a tendency for a training status-by-time interaction was found ($P = 0.09$).

Serum immunoreactive insulin and glucagon concentrations. Immunoreactive insulin (IRI; T1D, free insulin; CON, total insulin) did not differ between groups at rest or during exercise. Before training in the CON group, IRI rose briskly after exercise to be $100 \pm 60\%$ above resting values at 5 min of recovery ($P < 0.01$), then declined to resting values by 45 min (Fig. 6A). In contrast, in T1D (time-by-group interaction, $P < 0.001$), free IRI did not differ from resting values at 5 min of recovery, and then fell slightly below resting values by 20 min of recovery ($P < 0.05$; Fig. 6A). Hence, the mean IRI, across all times and both days was 6.8 mU/l (40.9 pM) lower in the T1D group ($P < 0.01$). IRI was not changed significantly after sprint training in either group.

Immunoreactive glucagon (IRG) did not differ with time, between groups or with training (Fig. 6B).

Molar ratio of IRG to IRI. The IRG-to-IRI ratio (IRG/IRI; free insulin in the T1D group) increased with exercise in both groups ($P < 0.05$; data not shown). In CON, IRG/IRI declined in early recovery then slowly rose toward resting values. However, in T1D, the ratio remained elevated in early recovery and continued to rise in later recovery ($P < 0.01$; group-by-time interaction). IRG/IRI did not differ after training. Across both days and all times, IRG/IRI was 0.71 higher in the T1D group ($P < 0.05$).

Plasma $[Na^+]$. Plasma $[Na^+]$ at rest did not differ between groups or with training. Exercise induced a mild increase in plasma $[Na^+]$, which peaked at 1 min of recovery at $5 \pm 3\%$

above resting values ($P < 0.001$), then slowly declined to resting values by 20 min of recovery (Fig. 7A). After training, plasma $[Na^+]$ was lower at 2 and 5 min of recovery ($P < 0.01$; training status-by-time interaction). In the T1D group, both before and after training, plasma $[Na^+]$ was lower than in the CON group at 20, 45, and 60 min of recovery ($P < 0.05$; time-by-group interaction).

Osmolarity. As expected, due to the progressively higher PG, calculated plasma osmolarity was higher in the T1D group across all times and both days (7.8 mM; $P < 0.001$); however, resting values did not differ within groups before and after training. Osmolarity rose with exercise ($P < 0.001$), peaked after 1 min of recovery, then declined to resting levels by 20 min of recovery (Fig. 7B). After training, osmolarity was lower (2.3 ± 4.7 mM mean difference; main effect of training status, $P < 0.05$), with no difference between groups.

Arterialization. Mean PO_2 did not differ between groups or with training and was 74 ± 20 Torr across both tests (Pre, Post), indicating an adequate level of arterialization (data not shown).

T1D resting study. PG concentration fell slightly ($P < 0.05$; mean fall in PG, -0.31 ± 0.50 mM; Fig. 4), whereas plasma $[K^+]$ (mean, 4.23 ± 0.47 mM), $[Na^+]$ (mean, 133.1 ± 2.5 mM), and free IRI (mean, 6.7 ± 1.9 mU/l) were unchanged for

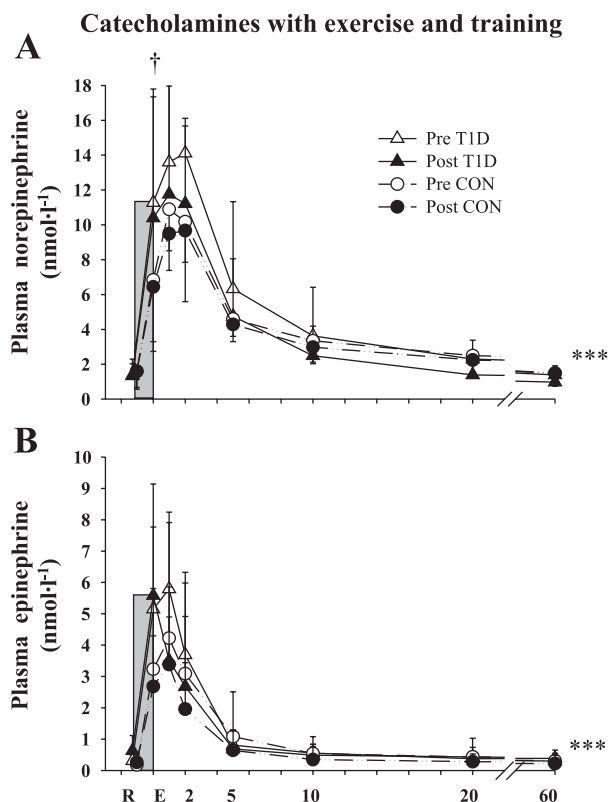


Fig. 5. Plasma concentrations of norepinephrine (A; T1D, $n = 8$, except E, 1 and 2 min of recovery where $n = 7$; CON, $n = 7$) and epinephrine (B; T1D, $n = 7$, except E, 1 and 2 min of recovery where $n = 6$; CON, $n = 7$) in Pre and Post. Symbols, the shaded bar, and x-axis markings are similar to Fig. 1. For norepinephrine, a main effect for time ($***P < 0.001$) with all times > R except 60 min of recovery and a time-by-group interaction ($\dagger P < 0.05$) in which E was higher for the T1D group were evident. For epinephrine, a main effect for time ($***P < 0.001$) was evident with E through 5 min of recovery > R.

Insulin and glucagon with exercise and training

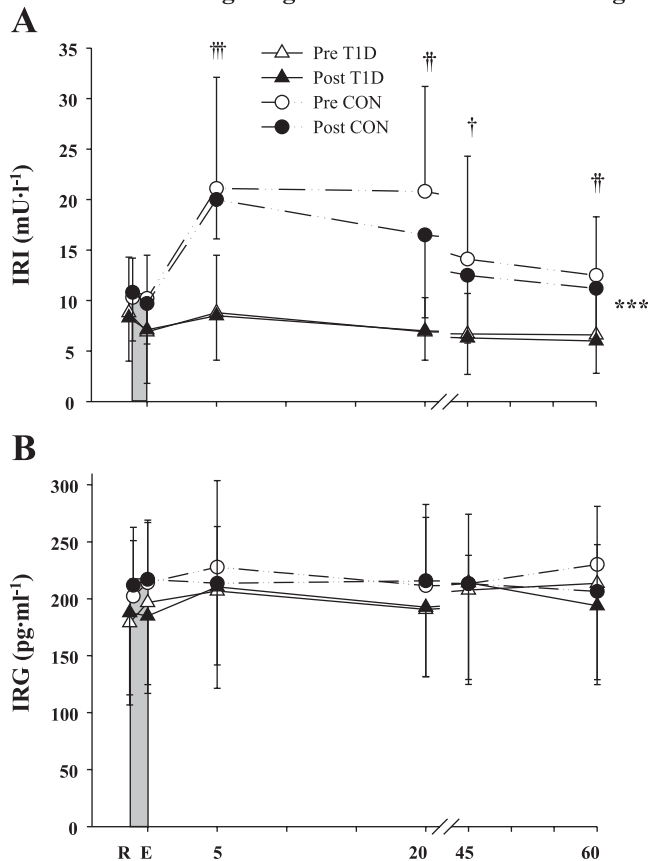


Fig. 6. Immunoreactive insulin (IRI; T1D, $n = 8$, except E and 20 min of recovery where $n = 7$; CON, $n = 7$; A), and glucagon concentrations [immunoreactive glucagon (IRG); T1D, $n = 8$; CON, $n = 7$; B] before and after training. Symbols, the shaded bar, and x-axis markings are similar to Fig. 1. For IRI, a main effect of time ($***P < 0.001$) and a time-by-group interaction ($†††P < 0.001$) with IRI in CON $>$ T1D from 5 through 60 min of recovery ($†P < 0.05$; $‡P < 0.01$; $††P < 0.001$) were evident.

the duration of the resting study. PG, $[K^+]$, $[Na^+]$, and IRI in the resting study did not differ from resting samples in the exercise studies (Pre, Post).

DISCUSSION

This is the first study to investigate, in subjects with Type 1 DM, both the acute effects of intense exercise on plasma K^+ regulation and the effects of high-intensity exercise training on muscle $Na^+-K^+-ATPase$ content and K^+ regulation during intense exercise. This is also the first study to examine the relationship between the rise in plasma $[K^+]$ during exercise and muscle $Na^+-K^+-ATPase$ content in Type 1 DM. We demonstrated first that muscle $Na^+-K^+-ATPase$ content and the acute rise in plasma $[K^+]$ during intense exercise did not differ between subjects with Type 1 DM who were mildly hypoinsulinemic and nondiabetic subjects. Second, sprint training improved plasma K^+ regulation during intense exercise in both groups; however, the relative contribution of K^+ -regulatory mechanisms may differ in the subjects with Type 1 diabetes. Additionally, since the T1D resting study demonstrated that there was no deterioration in metabolic or ionic control over the duration of the period of testing, changes

observed with the exercise tests were attributable to the effects of exercise and training alone.

Intense exercise, plasma $[K^+]$, and Type 1 DM. Acute intense exercise induced a comparable increase in plasma $[K^+]$ in the T1D and CON groups in the present study, confirming our first hypothesis. This result both extends and is consistent with the findings in the only other study to have reported plasma $[K^+]$ during exercise in Type 1 DM (5). However, in that study, only a small increment in plasma $[K^+]$ (~ 0.3 mM) was evident due to the mild exercise intensity (40% maximal $\dot{V}O_2$), and subjects were maintained on a basal insulin infusion. In the present study, the rise in plasma $[K^+]$ ($\Delta[K^+]$) during intense exercise at 130% $\dot{V}O_{2\text{ peak}}$ was ~ 4.5 -fold higher, with a peak plasma $[K^+]$ of 5.48 ± 0.62 mM being reached, presenting a major acute challenge to K^+ -regulatory mechanisms. Similar peak plasma $[K^+]$ during intense exercise in the T1D and CON groups is interesting, given that the T1D

Plasma $[Na^+]$ and osmolarity with exercise and training

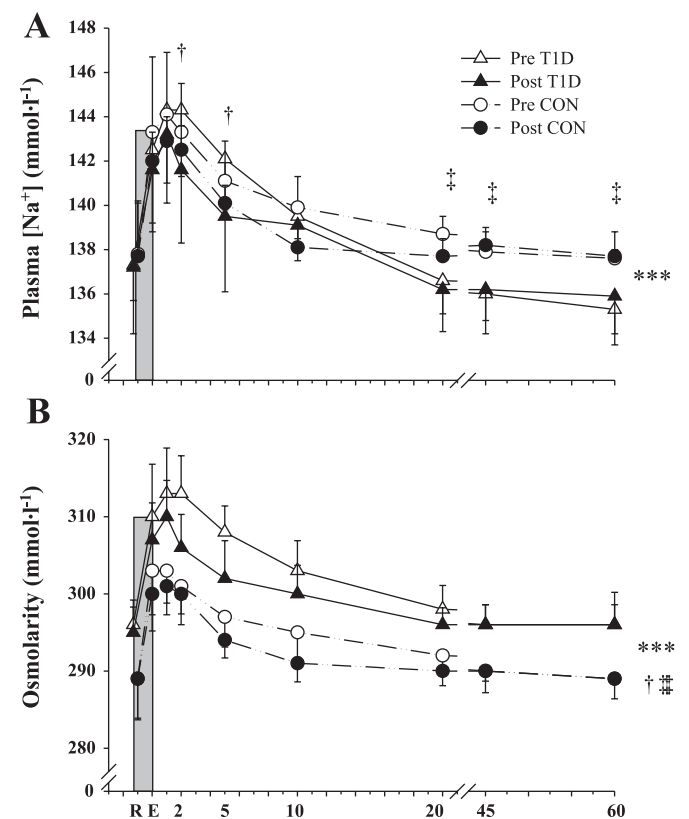


Fig. 7. A: plasma sodium concentration ($[Na^+]$; T1D, $n = 8$, except 1, 2, and 60 min of recovery where $n = 7$; CON, $n = 7$, except 2, 45, and 60 min of recovery where $n = 6$, and 5 and 20 min of recovery where $n = 5$). B: estimated osmolarity (T1D, $n = 7$, except 1, 2, 20, and 60 min of recovery where $n = 6$; CON, $n = 7$, except 2, 45, and 60 min of recovery where $n = 6$, and 5 and 20 min of recovery where $n = 5$) before and after training. Symbols, the shaded bar, and x-axis markings are similar to Fig. 1. For plasma $[Na^+]$, a main effect for time ($***P < 0.001$; E through 10 $>$ R) and interaction effects for training status by time with $[Na^+]$ lower after training at 2 and 5 min of recovery and time by group ($‡P < 0.05$) with CON $>$ T1D from 20 through 60 min of recovery were evident. For osmolarity, main effects were evident for time ($***P < 0.001$; E through 10 $>$ R) and training status ($†P < 0.05$) with $[Na^+]$ lower after training and T1D $>$ CON ($†††P < 0.001$) at all times. Note that pre- and posttraining values are overlaid for each group at 45 and 60 min of recovery.

subjects had mild relative hypoinsulinemia and slightly higher Hct (consistent with the mild dehydration that is anticipated with hyperglycemia), both of which would increase plasma $[K^+]$. These imply either that insulin is of relatively minor importance to plasma K^+ regulation during a single brief bout of intense exercise or that other mechanisms compensate for the K^+ -raising effect of mild relative hypoinsulinemia.

Potassium regulation during intense exercise is achieved by increased activation of the skeletal muscle $Na^+-K^+-ATPase$, which, in isolated muscles in rats, occurs within a few seconds of muscle excitation (22). Catecholamines are important in accelerating $Na^+-K^+-ATPase$ activity at the start of exercise and in maintaining activation at the cessation of exercise when muscle contraction is absent (17, 18). During mild exercise, subjects with Type 1 DM are more sensitive to the effects of adrenergic stimulation (on glucose metabolism) than nondiabetic subjects (45). The significance of the prevailing insulin concentration or of insulin resistance with regard to K^+ regulation during intense exercise is unknown. However, if increased sensitivity to adrenergic stimulation also applies to K^+ metabolism, then it is conceivable that the higher norepinephrine concentrations during exercise in the T1D subjects in the present study may have compensated for any effect of the relative hypoinsulinemia to increase plasma $[K^+]$.

Potassium clearance during and after intense exercise is also affected by inactive tissues remote from the exercising muscles (28, 30). This contributes to arterial K^+ being lower than femoral venous K^+ during intense leg exercise (29, 35, 47). Antecubital venous $[K^+]$ is also expected to be lower than femoral venous $[K^+]$ (providing that the arms are relatively inactive) as a consequence of forearm K^+ uptake (28, 29). In the present study, we sampled arterialized blood from a dorsal hand vein to provide a close estimate of arterial $[K^+]$. Although simultaneous arterial and femoral venous sampling would have been ideal to assess effects of exercise and training on K^+ regulation, we were mindful that, even though our subjects in the T1D group were young and had no evidence of cardiovascular disease, people with diabetes are at higher risk of cardiovascular disease and may have vascular changes that are not clinically evident. Arterialized venous samples may be used to obtain accurate estimates of arterial K^+ during non-steady-state exercise, albeit with a slight underestimation (37); and extraction of K^+ by inactive muscles is minimized if superficial venous blood is well arterialized (33).

Given that we did not assess blood flow or the arteriovenous difference for K^+ , it is difficult to determine the extent of the redistribution of K^+ during exercise in the present study since a considerable muscle mass may be anticipated to have been relatively inactive. However, during brief, intense exercise, K^+ lost from the exercising muscle is mixed with a redistribution volume that is probably not much larger than the plasma volume due to low perfusion of other tissues (43). Hence, the exercising muscle bed would be of primary importance. However, during recovery from exercise, the high catecholamine concentrations in the first 5–10 min would also promote $Na^+-K^+-ATPase$ activity in resting muscle and other remote tissues, facilitating clearance of plasma $[K^+]$.

Increased plasma $[K^+]$ and reduced $[Na^+]$ in late recovery in Type 1 DM. An interesting finding in the T1D group in the present study was the presence of increased plasma $[K^+]$ and reduced $[Na^+]$ compared with the CON group, coincident with

hyperglycemia, that occurred after 60 min of recovery from the intense single bout of exercise. A phenomenon called hyperglycemia-induced hyperkalemia has been described under resting conditions in hyperglycemic insulin-dependent subjects (38, 39, 44, 46). The findings in the present study may be consistent with this; however, the relationship was only evident in late recovery and not when mildly hyperglycemic before exercise commenced. Rather than an effect of hyperglycemia per se, it has been suggested that increases in plasma $[K^+]$ may be due to hyperosmolality (46). However, osmolality in the T1D group did not differ significantly between rest and 40–60 min of recovery, suggesting that the effects of relative hypoinsulinemia and the associated hyperglycemia on plasma $[K^+]$ were more important than hyperosmolality in the present study. It is also possible that higher plasma $[K^+]$ at 60 min of recovery in the T1D group may reflect altered renal handling of $[K^+]$ compared with the CON group. However, although aldosterone concentration was increased four- to fivefold during 40 min of mild exercise and remained elevated during 30 min of recovery, urinary K^+ excretion was unchanged in subjects with Type 1 DM or controls (5).

Sprint training improved plasma $[K^+]$ regulation during intense exercise in T1D. We demonstrated that sprint training enhanced plasma K^+ regulation in Type 1 DM, with attenuated plasma $\Delta[K^+]$ during maximal exercise and a reduced $\Delta[K^+]/work$, in agreement with our hypothesis. Similar adaptations were evident in the CON group, supporting our previous findings (20). The 21% reduction in plasma $\Delta[K^+]$ during maximal matched-work exercise after training could not be explained by any difference in ΔPV during exercise and was only partially explained by the small reduction ($\sim 5\%$) in relative exercise intensity after training. It may be argued that the magnitude of the reduction in peak plasma $[K^+]$ during exercise is of little clinical significance. Certainly this would be the case if plasma $[K^+]$ was reduced to a similar degree under resting conditions in patients with Type 1 DM. However, with regard to intense exercise and performance, high interstitial K^+ accumulation is a strong contributor to muscle fatigue (40). Thus the small reduction in plasma $[K^+]$, probably reflecting to some degree the change in interstitial $[K^+]$, during intense exercise in the present study may have contributed to improving performance in both the T1D and CON groups by delaying fatigue.

Factors that may reduce hyperkalemia during intense exercise after exercise training include increased content and/or activity of skeletal muscle $Na^+-K^+-ATPase$, altered blood flow, or altered muscle recruitment (for review, see Ref. 34). The present study examined the effects of sprint training on muscle $Na^+-K^+-ATPase$ content.

$Na^+-K^+-ATPase$ content did not differ between T1D subjects and controls. We found no difference in $Na^+-K^+-ATPase$ content between the T1D and CON groups, both being within the range previously reported for healthy, untrained nondiabetic subjects (15, 16, 19, 26, 33). In contrast, the only other study to examine $Na^+-K^+-ATPase$ content in human Type 1 diabetes reported 22% higher $Na^+-K^+-ATPase$ content compared with nondiabetic subjects (42). However, this might in part reflect that $Na^+-K^+-ATPase$ content in nondiabetic subjects was on the lower end of normal values (42). Insulin, a proposed acute translocator of $Na^+-K^+-ATPase$ in rat muscle (32), was ~ 3.2 -fold higher in the subjects with diabetes com-

pared with the nondiabetic subjects in that study (42). Hence, it is possible that elevated insulin may have increased the number of pumps at the muscle surface in the subjects with diabetes. However, another recent study demonstrated no increase in ouabain binding with insulin stimulation, arguing against translocation (36). Insulin is also a chronic regulator of $\text{Na}^+\text{-K}^+\text{-ATPase}$ content (7), with streptozotocin-induced diabetes reducing $\text{Na}^+\text{-K}^+\text{-ATPase}$ content in rats (25, 42). Thus it is possible that an acute effect of mild hypoinsulinemia was observed in the present study in which subjects delayed their morning insulin dose, whereas both acute and chronic effects of higher insulin levels were evident in the insulin-replete subjects of Schmidt et al. (42). In the present study, it is also possible that we failed to detect a difference between groups due to a type II error. However, although our observed power to detect differences between groups was 0.07, the P value was 0.69 and the nonsignificant difference in $\text{Na}^+\text{-K}^+\text{-ATPase}$ content between groups was only 4.7%. Additionally, due to our repeated-measures design, 14 values for the T1D group were compared with 12 values in the CON group.

Increased muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ content after sprint training in T1D. The present study demonstrated for the first time that intense exercise training increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ content in T1D. Our findings are supported by an animal study in which endurance training maintained [^3H]ouabain binding site content at nondiabetic control levels in untreated diabetic rats, whereas their inactive diabetic counterparts displayed a 19% reduction after 10 wk (42). Both studies demonstrate that intense exercise training increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ content in diabetes.

The increase in $\text{Na}^+\text{-K}^+\text{-ATPase}$ content with sprint training in the CON group supports a previous study in nondiabetic men that used the same training protocol (33). The finding is consistent with other studies that induced increases after endurance training (14, 15) or intensified exercise training (11, 31).

Muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and its relationship to plasma $[\text{K}^+]$. An inverse correlation was found between $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and plasma $\Delta[\text{K}^+]$ during maximal exercise in the CON group. This correlation links improved K^+ regulation during maximal exercise after sprint training with greater muscle content of $\text{Na}^+\text{-K}^+\text{-ATPase}$. Similarly, $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and $\Delta[\text{K}^+]/\text{work}$ during exercise were inversely correlated in the CON group, a finding supported by Fraser et al. (13) who used an incremental cycling protocol.

A number of previous studies have demonstrated no relationship between $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and plasma $\Delta[\text{K}^+]$. $\Delta[\text{K}^+]$ during exercise was lower after moderate-intensity training; however, $\text{Na}^+\text{-K}^+\text{-ATPase}$ content was unchanged in one study (26). It is possible that a significant relationship between $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and plasma K^+ accumulation may only be demonstrable with maximal exercise in which high K^+ fluxes occur. However, another study, which used repeated maximal exercise bouts, found increased muscle $\text{Na}^+\text{-K}^+\text{-ATPase}$ content but no significant reduction in $\Delta[\text{K}^+]$ during exercise after sprint training (33). The reason for the difference between studies is not clear. However, recent work indicates that maximum $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity is depressed with fatiguing maximal exercise (2, 13). Hence it is conceivable that, during repeated bouts of maximal exercise, the extent of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activation may be more important than small differences in content in healthy subjects.

In contrast to the CON group, in the T1D group, there was no correlation between attenuated $\Delta[\text{K}^+]$ and $\Delta[\text{K}^+]/\text{work}$ and increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ content. These findings imply that other factors may be more important than absolute content during intense exercise in Type 1 DM. This may include the extent of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activation in relation to the current hormonal milieu. This contention may be supported by the finding of higher [NEpi] during exercise, both before and after training, in the T1D group. Alternatively, the lack of correlation between $\Delta[\text{K}^+]$ and $\text{Na}^+\text{-K}^+\text{-ATPase}$ content in the T1D group may reflect a type II error. However, this seems unlikely given our repeated-measures design, which strengthens our conclusions by doubling the subject numbers ($n = 12$, CON; $n = 14$, T1D) and the markedly different P values for the correlations for the CON ($P = 0.022$) and T1D groups ($P = 0.86$).

In conclusion, first, resting $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and K^+ regulation during a single fatiguing bout of maximal exercise were not different in subjects with Type 1 DM and nondiabetic subjects. With respect to plasma $[\text{K}^+]$ regulation, subjects with Type 1 DM may safely undertake maximal exercise when mildly hypoinsulinemic; however, elevation in plasma $[\text{K}^+]$ and reduction in plasma $[\text{Na}^+]$ will ensue in late recovery, coincident with hyperglycemia, if insulin administration is delayed. Second, sprint training increased $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and improved K^+ regulation during intense exercise in both groups, but whereas $\text{Na}^+\text{-K}^+\text{-ATPase}$ content and plasma $\Delta[\text{K}^+]$ were inversely correlated in the nondiabetic group, there was no correlation in the group with Type 1 DM. This may imply that the relative contribution of mechanisms by which K^+ regulation is improved after training differs in subjects with Type 1 DM compared with nondiabetic subjects.

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