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EFFECT OF ACETAZOLAMIDE ON ISOLATED QUADRICEPS MUSCLE ENDURANCE PERFORMANCE AT SEA LEVEL AND DURING ACUTE ALTITUDE EXPOSURE

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13. ABSTRACT *(Maximum 200 words)*

Acetazolamide (AZ) can be taken at sea level (SL) to prevent acute mountain sickness during subsequent altitude (ALT) exposure. AZ causes metabolic acidosis at SL and ALT, and increases arterial oxygen saturation (SaO2) at ALT. HYPOTHESIS: AZ will impair muscle endurance at SL but not ALT (4300 m, <3 h). METHODS: Six subjects (20±2 yr; X±SD) performed exhaustive constant work rate 1-leg knee extension exercise (25±2 watts) once per wk for 4 wks (2 wks at SL and 2 wks at ALT). Each week, subjects took either AZ (250 mg) or placebo orally (double blind) every 8 h starting one-day prior to exercise. All exercise bouts began 3 h after the last (i.e., 4th) dose and 2 bouts began 2 h after ALT exposure. RESULTS: AZ caused similar acidosis in the same subjects at SL and ALT, but muscle endurance was impaired only at SL. CONCLUSION: Lack of endurance impairment at ALT during AZ was likely due to offsetting secondary effects resulting from the acidosis (e.g., increased SaO2) that improved muscle oxygen delivery.

(*P<0.05 from placebo; #P<0.05 from SL):

	Sea Level		Altitude	
	Placebo	AZ	Placebo	AZ
Arterialized Blood pH	7.43 ± 0.02	7.34 ± 0.02*	7.48 ± 0.07#	7.37 ± 0.03*
SaO2 (%)	97 ± 1	98 ± 1	86 ± 2#	89 ± 3*,#
Endurance Time (min)	48 ± 9	36 ± 12*	17 ± 6#	20 ± 8#

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BACKGROUND

The physical demands upon the soldier are always intense but are particularly so during acute altitude exposure. Whether troops climb to altitude or are transported there, they must immediately prepare defensive positions, establish logistical resupply and evacuation routes, set up outposts, and move weapons, ammunition and supplies about. These tasks must be performed over rugged terrain and often over snow and ice. If military mountaineering and possible offensive or defensive combat tasks are also required, the physical endurance capabilities of the soldier will be tasked nearly all of the time. At altitude, the maximal capacity for physical activity is reduced and, as a consequence, the ability to exercise or work at a given rate for prolonged periods of time is also reduced. Therefore, anything that has the potential to further reduce physical performance at altitude can significantly interfere with mission goals.

Acute mountain sickness (AMS) can have a significant negative impact on attainment of mission objectives in military units rapidly deployed to altitude. The Department of the Army Technical Bulletin 288 (TB MED 288) defines AMS as "a self-limited syndrome of unacclimatized persons typically manifest several hours after arrival at high altitude, and are characterized by headache, nausea, dizziness, tiredness, weakness, and insomnia". Acetazolamide is currently the only drug approved by the Federal Drug Administration to prevent AMS and has been demonstrated convincingly to prevent or reduce AMS symptoms in most individuals. However, there is a paucity of available information on how the drug affects physical performance at sea level and altitude. Reports to date regarding the effects of acetazolamide on physical performance indicate that acetazolamide generally reduces endurance exercise performance at sea level (e.g., during pre-deployment administration), but reduces, improves, or has no effect on physical performance at altitude. The reason for the inconsistent results at altitude is unclear.

The current investigation was performed to understand how acetazolamide affects physical performance at sea level and altitude. This was accomplished by systematically assessing responses to identical work performed by the same subjects under all experimental conditions (i.e., at sea level and altitude, with and without acetazolamide treatment).

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EXECUTIVE SUMMARY

Acetazolamide can be taken at sea level (SL) for the prevention of acute mountain sickness during subsequent altitude (ALT) exposure. While acetazolamide reduces sickness symptomatology in most individuals, there is a paucity of available information on how the drug affects exercise performance at ALT. During treatment at SL, endurance exercise performance is impaired; but at ALT, it is reported to be improved, not affected, or impaired. One reason often postulated for the conflicting findings at ALT relates to a difference in the relative balance of physiological responses associated with acetazolamide-induced metabolic acidosis. On the one hand, metabolic acidosis *per se* tends to hinder exercise performance at SL and ALT. On the other hand, opposing secondary responses resulting from the acidosis (e.g., increased ventilation that could enhance oxygen delivery to active muscle) tend to improve exercise performance but only at ALT where arterial oxygen saturation (SaO_2) is reduced.

We hypothesized that acetazolamide would impair muscle endurance at SL but not ALT (4300 m, <3 h). Six subjects (20±1 yr; X±SD) performed exhaustive constant work rate 1-leg knee extension exercise (25±2 watts) once per wk for 4 wks (2 wks at SL and 2 wks at ALT). Each week, subjects took either acetazolamide (250 mg) or placebo orally (double blind) every 8 h starting one-day prior to exercise. All exercise bouts began 3 h after the last (i.e., 4th) dose and 2 bouts began 2 h after ALT exposure. The table shows that acetazolamide caused similar acidosis at SL and ALT, but muscle endurance was impaired only at SL. Ventilation also was higher ($P<0.05$) during treatment with acetazolamide than with placebo at SL and ALT. *(*P<0.05 from placebo; #P<0.05 from SL)*

	SL		***ALT***	
	Placebo	***Acetazolamide***	***Placebo***	***Acetazolamide***
Arterialized Blood pH	7.43 ± 0.02	7.34 ± 0.02*	7.48 ± 0.07#	7.37 ± 0.03*
SaO2 (%)	97 ± 1	98 ± 1	86 ± 2#	89 ± 3*,#
Endurance Time (min)	48 ± 9	36 ± 12*	17 ± 6#	20 ± 8#

We conclude that lack of endurance impairment at ALT during treatment was likely due to offsetting secondary effects resulting from the acidosis (e.g., ventilatory-induced increase in SaO_2) that improved muscle oxygen delivery.

INTRODUCTION

Acute mountain sickness (AMS) is a symptom complex that includes headache, nausea, dizziness, tiredness, weakness, and insomnia, and is most common when low-altitude residents ascend rapidly to altitudes exceeding 3,000 m (10;27). Acetazolamide has been used for over 30 years to prevent AMS (1). Acetazolamide inhibits carbonic anhydrase and causes increased loss of water, bicarbonate, sodium, and potassium in the urine, a reduced concentration of bicarbonate in extracellular fluid, a metabolic acidosis, and increased ventilation (1;25;26;29). When taken prophylactically as indicated (i.e., 24 to 48 hours prior to ascent), it prevents AMS in 30% to 50% of individuals and reduces symptoms in most others (6;12). It is currently the only drug approved by the Federal Drug Administration for this purpose (1).

While acetazolamide has been demonstrated convincingly to prevent or reduce AMS symptoms in most individuals, there is a paucity of available information on how the drug treatment affects work and exercise performances at sea level and altitude. Moreover, the data available are incomplete, inconsistent, and difficult to interpret. Collective findings from previous studies indicate that acetazolamide causes similar physiological changes at sea level and altitude. Yet, endurance exercise performance during drug treatment compared to performance without drug treatment has been reported as impaired at sea level but improved, not affected, or impaired at altitude (3;11;17;22;23;26).

One reason often postulated for the conflicting findings at altitude versus sea level relates to a difference in the relative balance of opposing physiological responses associated with the acetazolamide-induced metabolic acidosis. On the one hand, metabolic acidosis *per se* likely impairs ability to buffer metabolic acids in active muscle and thereby tends to *hinder* exercise performance at sea level and altitude (13;20;24). On the other hand, the opposing secondary responses resulting from the acidosis --- an elevation in minute ventilation that may enhance oxygen delivery to active muscle --- tend to *improve* exercise performance; but only at altitude where PO_2 and arterial oxygen saturation are reduced (3;22). Whether this postulate is true has been difficult to determine because of previous problems associated with: 1. the use of exercise modes

(e.g., conventional whole-body treadmill or bicycle ergometry) having marked inter- and intra-subject variability that may have obscured changes in exercise performance, 2. performance during conventional ergometry that may have been restricted by a central circulatory or pulmonary diffusion limitation (19), and 3. the use of different individuals or exercise intensities and power outputs at sea level compared to altitude that made performance comparisons difficult to interpret (23).

The current investigation was designed to minimize these confounding factors and determine the effect of acetazolamide on muscle endurance exercise performance at sea level and altitude. This was accomplished by comparing endurance times of the same subjects performing isolated muscle exercise at identical power outputs under all experimental conditions using an exercise model having low test-retest variability (8).

OBJECTIVES

The main objective of this study was to determine how acetazolamide --- a drug primarily administered prophylactically at sea level to prevent AMS during subsequent altitude exposure --- affected muscle exercise performance at sea level and during initial altitude exposure (4,300 m, < 3 hours). The overall study hypothesis was that acetazolamide would impair muscle endurance at sea level but not at altitude.

METHODS

SUBJECTS

Five men and one woman (mean ± SD; 20 ± 3 yrs, 78 ± 4 kgs, 173 ± 5 cm) volunteered to participate after being fully informed of the nature of the study. All provided verbal and written consent. All were born at altitudes less than 1500 m and resided near sea level (<100 m) for at least six months before the study started.

STUDY DESIGN

The study used a two factor (placebo\acetazolamide treatment and sea level\altitude exposure) experimental design with crossover on both factors. Both the

subjects and the investigators directly involved were blinded to the specific treatment (placebo or acetazolamide) intervention status until the entire study was completed.

OVERALL EXPERIMENTAL TIMETABLE AND LOCATION

A three-week long preliminary testing phase was followed by a four-week long definitive testing phase. The presentation of the definitive exercise testing bouts (i.e., sea level\placebo; sea level\acetazolamide; altitude\placebo; and altitude\acetazolamide) was randomly assigned among subjects. Each subject performed only one exercise bout each week during the definitive testing phase. At least one day prior to all exercise bouts in the preliminary and definitive testing phases, the subjects were not allowed to run or perform leg weight-training exercise. Preliminary and definitive phase testing took place in the Altitude Chamber Facility, USARIEM, Natick, MA. Temperature and relative humidity were maintained at 21± 2° C and 45 ± 5%, respectively.

1. Preliminary Testing Phase:

The preliminary testing phase included one-hour of hypobaric chamber familiarization and safety training at 4300 m, knee extensions on a conventional weightlifting machine, a bicycle ergometer $\dot{V}O_2$peak test, a dynamic knee extension (DKE) $\dot{V}O_2$peak test, and four to six daily sessions to practice DKE exercise (both static and dynamic contractions) to ensure habituation and proper training (e.g., DKE exercise and leg pacing coordination) prior to the definitive testing phase. Another important goal during the preliminary testing phase was to determine a work rate that would elicit exhaustion in approximately 45 minutes during DKE exercise at sea level without acetazolamide treatment. (This work rate would then be used for each of the four exercise tests in the definitive phase).

Other measures such as body weight and height, Environmental Symptoms Questionnaire (ESQ), bioelectrical impedance, arterialized finger tip blood sample, ratings of perceived exertion, respiratory gas exchange, heart rate, and arterial oxygen saturation were obtained at rest and/or during exercise during at least one of the preliminary test sessions for the purpose of either establishing baseline values, characterizing subjects in terms of conditioning, or to familiarize the subject to measurements that would be obtained during the definitive test phase. All subjects

participated in one to four, < 2 hour daily sessions in each of the three weeks of the preliminary testing phase. The exact days, times, and composition of the sessions were individually scheduled to ensure appropriate exercise recovery times.

2. Definitive Testing Phase:

For each subject during the definitive testing phase, there was only one DKE exercise bout each week for four consecutive weeks. Placebo or acetazolamide treatment and several experimental procedures took place over two consecutive days in each of the four weeks. Any medications, caffeine, and carbonated beverages were strictly prohibited from the beginning of day 1 until after the DKE exercise bout on Day 2. (Carbonated beverages taste awful during acetazolamide treatment and thus were prohibited to assure maintenance of the blinded experimental design). For each subject, the week-to-week experimental schedule and data collection procedures were identical except for presentation of sea level\altitude days and placebo\ acetazolamide treatments.

The following briefly outlines the sequence of events that a subject adhered to during each of the four definitive testing weeks:

Day 1. Twenty-seven hours (t = -27 h, 0700 h, while fasting) and 18.5 hours (t = -18.5 h, 1530h) prior to the scheduled exercise bout at 0930h on day 2, the subject reported to the Altitude Chamber Facility. On each occasion, the subject was weighed; filled out an Environmental Symptoms Questionnaire (ESQ), had total body water determined using a bioelectric impedance procedure, and swallowed a capsule in the presence of an investigator. Prior to leaving, the subject was provided with a capsule and instructed to ingest it either just before going to sleep or no later than 2200 h (t = -12 h).

Day 2. The subject again reported to the Altitude Chamber Facility on day 2 while fasting (t = -3 h, 0700h). After body weight and bioelectrical impedance measurement were obtained, and an ESQ was filled out, the subject was provided a light meal (i.e., 400 to 500 kcal and > 50% carbohydrate) consisting of a commercially available Power© bar, and peanut butter crackers. For each subject over the four-week definitive testing period, this meal was identical. An arterialized capillary blood sample was then taken from a fingertip (0730 h). At 0745 h, the subject took the 4th and last capsule of the week and was required to remain at or around the Altitude Chamber Facility. At 0820 h (t ~ -1.5 h), body weight and bioelectrical impedance measurements

were again obtained. At 0900 h, the 2nd arterialized capillary blood sample was taken. At approximately 0920 h, the subject was prepared (i.e., electrodes placed, secured to knee extension device, etc), for DKE testing to start at 1000 h.

During the altitude testing bouts, the timing of the above events remained identical except that the chamber (with the volunteer and investigative staff) was decompressed starting at 0800 h at a rate of 45 mmHg/min to a pressure of 446 mmHg, which is equivalent to a terrestrial altitude of 4300 m (14,110 feet). Decompression to 4300 m (and subsequent recompression) therefore took approximately 15 min.

PLACEBO\ACETAZOLAMIDE TREATMENT

On four occasions prior to each of the definitive DKE exercise bouts (t = -27 h, t = -18.5 h, t = -12 h, and t = -2 h) the subjects received either acetazolamide (250 mg) or an identical appearing placebo (lactose) in capsule form (prepared by The Massachusetts College of Pharmacy, Boston). The initiation of treatment (i.e., day before ascent), administration frequency, and the amount (i.e., 250 mg) are consistent with current recommendations for acetazolamide treatment prior to altitude exposure (1;12).

TEST PROCEDURES AND MEASUREMENTS

1. $\dot{V}O_2$ peak During Conventional Bicycle Ergometer Exercise

$\dot{V}O_2$peak was determined during continuous, graded bicycle exercise on an electrically-braked ergometer once at sea level during the preliminary testing phase. A pedal rate of 65 to 70 rpm was used. Subjects warmed-up for 3 min at 100 watts with the workload increased by 30 watts every two min thereafter. $\dot{V}O_2$peak was defined as the point at which oxygen consumption began to plateau with increased work rate or at the point where the volunteer could no longer maintain the work rate despite strong encouragement.

2. $\dot{V}O_2$ peak During Dynamic Knee Extension Exercise

$\dot{V}O_2$peak was determined during graded dynamic knee extension to peak exercise once at sea level during the preliminary testing phase. Data collection procedures were identical to those in Section 6 below with the exceptions of four-minute

stages of one-leg dynamic knee extension exercise of graded intensity separated by four minutes of rest and no recovery measurements. Increments of work rate applied to each stage to exhaustion were individually determined for each subject. $\dot{V}O_2$peak was defined as the highest value just prior to a deviation from a linear relationship between a change in oxygen consumption and a change in work rate (8). Four to seven exercise stages were used for each subject.

3. Bioelectrical Impedance

A bioelectrical impedance measurement procedure was used to qualitatively estimate potential changes in total body water of the subjects. Subjects were assessed while in the supine resting position once during the preliminary testing phase, and at t = -27 h and –18.5 h on Day 1, and at t –3 h and t –1.5 h on day 2 of each week of the definitive testing phase. The measurements were made after 5 minutes of supine rest. A small imperceptible electrical current (800 μA maximum at 50 KHZ) was passed through their body from electrodes on the hand to similar electrodes on the ankle. Single frequency impedance data was collected using a bioelectrical analyzer (Model BIA-101, RJL Systems, Detroit, MI).

4. Arterialized Capillary Blood Sample

An arterialized blood sample of 100 to 200 μL from the fingertip was obtained twice during Day 2 of each week during the four definitive exercise testing days (t = - 2.5 h and t = - 45 min). During the two altitude testing days, the blood samples were obtained after the subjects had been at altitude for 1.25 h. Arterialization was achieved by warming the fingertip to enhance regional blood flow. The sample was analyzed on a blood gas analyzer (ABL555, Radiometer, Sweden) for pH; bicarbonate concentration [HCO_3^-] was calculated using the Henderson-Hasselbach equation. All staff involved with exercise testing was blinded to the blood analyses results until the end of the entire study.

5. Symptoms Assessment

The Environmental Symptoms Questionnaire (ESQ) is a self-reported 68-item inventory typically used to document symptoms induced by altitude and other stressful

environments. However, in the proposed study it was unlikely that any of the volunteers would experience AMS since each altitude exposure was scheduled to last no longer than about 3 hours --- at least a full hour before symptoms become conspicuous at 4300 m altitude. The purpose of not testing volunteers prior to the time AMS would likely be present was to eliminate the possibility that exercise performance would be confounded by illness. Therefore, the main intention of administering the ESQ was to assess potential symptoms associated with acetazolamide treatment and early altitude exposure, and not illness. The ESQ was administered once during the preliminary testing phase. For each of the four days during the definitive testing phase, the ESQ was administered just prior to the first, second, and fourth placebo\acetazolamide pill ingestion (i.e., t = -27 h and t = -18.5 h on Day 1, and t = - 2 h, respectively) and 15 min (t = -15 min) on Day 2 prior to initiating the exercise bout. The questionnaire required approximately 5 minutes to complete on each occasion. A total of the items, and a weighted average of scores from "cerebral" symptom items (AMS-c) and from "respiratory" symptom items (AMS-r) were calculated as previously described (21).

6. Knee Extension Exercise

The specially designed device for performing 1-leg (right leg) dynamic knee extension exercise interspersed with maximal static 1-leg knee extension contractions has been described in detail (8). Briefly, it consisted of a platform on which the subject sat, an attached minimal-friction weight-pulley system with an ankle harness, transducers for measurement of force (model SSM-250, Interface, Scottsdale, AZ; sensitivity 1.5 mV/kg) and ankle displacement (Celesco Transducer Products Inc., Canoga Park, CA, Model PT101-0100-111-1110) during dynamic knee extension and separate force transducers for measurement of force of static knee extension MVCs. In order to precisely control work rate, two vertical columns of 14 light emitting diodes (LEDs) were placed in front of the subject. The right LED column was wired in series to the position transducer such that the number of LEDs lighted was proportional to ankle displacement during knee extension. The left LED column was connected to a synthesizer/function generator that automatically and sequentially lit from one (at the 90° knee angle starting position) to 14 (corresponding to ankle displacement on reaching 160° of knee extension) to one (return to 90° starting position) at a pre-determined knee extension rate of 1 Hz.

To maintain correct distance and rate of dynamic knee extension, the subject continuously matched the column of LEDs controlled by leg movement with that controlled by the synthesizer/function generator. The LED units simplified subject and investigator monitoring of adherence to the required work rate. Because the knee extension movement encompassed 70° and there were 13 intervals between LEDs, the maximum allowable difference between the desired and actual knee extension angle was 5.38°.

Muscle exhaustion was defined as a mismatch of only one LED between the right and left LED columns for three consecutive knee extensions, despite strong verbal encouragement. This effectively meant that exhaustion was associated with an inability to complete the last five degrees of knee extension contraction--from 155° to 160° --- at the required contraction rate. Voltages proportional to force and ankle displacement were continuously recorded. Work rate (watts) was determined by multiplying mean force developed per contraction, distance of ankle movement during knee extension from 90° to 160° and rate of knee extension (1 Hz).

To measure the decline in force generating capacity and rate of muscle fatigue, the exercise device allowed performance of MVCs of the knee extensor muscles during brief (≤ 5 sec) pauses in dynamic knee extension. This procedure involved rapid disconnection of the ankle harness from the weight-pulley system, connection to a force transducer dedicated to measurement of MVC force, actual measurement of MVC force, and reconnection to the weight-pulley system.

a. Determination of MVC Force: During the preliminary and definitive testing phases, the subjects performed three or more pre-exercise knee extensor MVCs with the right leg. To minimize duration variability among MVCs, each MVC triggered an audible sound that lasted exactly 2.5 sec. The subject was instructed to provide maximal force during each MVC. At least 1 min of rest followed each MVC. MVC force (strength or rested MVC force) of the leg was then measured immediately prior to, at the end of every 2 min during and immediately following dynamic knee extension. A knee angle of 90° was used for all MVCs.

b. Submaximal Constant Work Rate Knee Extension Exercise: For each subject, 1-leg dynamic knee extension at a frequency of 1 Hz was performed to exhaustion at the same constant work rate (~25 ± 2 watts) during all definitive exercise

bouts at sea level and altitude. The time course of fatigue was determined from MVC force measurements during pauses of $\leq$ 5 sec at the end of every two min of exercise until the point of exhaustion. Subjects were not allowed access to elapsed exercise time.

7. Heart Rate

Heart rate was determined by 3-lead electrocardiograph (Cardiovit AT-6; Schiller, Nepean, ON) for 10 min prior to, and every two minutes during, the $\dot{V}O_2$peak tests (DKE and bicycle ergometry), and all four DKE bouts during the definitive phase.

8. Respiratory Gas Exchange

Resting (10 min) and exercise respiratory data (minute ventilation, oxygen consumption, etc) were collected continuously until exhaustion using a Sensormedics Metabolic Cart (model 2900) during the VO_2peak tests (DKE and bicycle ergometry), and all four DKE bouts during the definitive phase.

9. Ratings of Perceived Exertion

Ratings of perceived exertion localized to the active muscles were obtained before and every two minutes during dynamic knee extension exercise (15 seconds prior to each MVC) using the Borg 6 to 20 scale. RPE data were collected during the $\dot{V}O_2$peak tests (DKE and bicycle ergometer), and all four DKE bouts during the definitive phase.

10. Arterial Oxygen Saturation

Oxygen Saturation was monitored continuously via noninvasive finger pulse oximetry (model N-200, Nellcor, Pleasanton, CA) during all four DKE bouts during the definitive phase.

STATISTICAL ANALYSES

The overall study hypothesis was that acetazolamide would impair exercise performance at sea level and altitude, and that the impairment would occur at sea level but not altitude. The key dependant variable of exercise performance compared among testing sessions was endurance time to exhaustion. Two-way (acetazolamide treatment vs no treatment and sea level vs altitude) analysis of variance (ANOVA) with repeated

measures on both factors was used for data analyses. Neuman-Keuls post-hoc test was used to evaluate significant main effects when they were detected. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

$\dot{V}O_2$ PEAK DURING CONVENTIONAL CYCLE ERGOMETRY

Peak oxygen consumption was 3038 ± 629 ml/min (range: 2325 to 4050 ml/min) or 39 ± 7 ml/min/kg (range: 30 to 48 ml/min/kg) at a workload of 230 ± 31 watts. At exhaustion, heart rate was 180 ± 9 beats/min and rating of perceived exertion was 18 ± 1.

$\dot{V}O_2$ PEAK DURING DYNAMIC KNEE EXTENSION

Peak oxygen consumption was 975 ± 223 ml/min (range: 689 to 1273 ml/min) at a workload of 34 ± 5 watts. At exhaustion, heart rate was 127 ± 16 beats/min and rating of perceived exertion of the legs was 15 ± 3. Dynamic knee extension peak oxygen consumption was 32 ± 4% of conventional cycle ergometry peak oxygen consumption.

BODY WEIGHT, TOTAL BODY WATER AND ARTERIALIZED BLOOD VALUES

Tables 1 and 2 show that fasting body weight and total body water, respectively, tended ($P>0.05$) to be lower during acetazolamide treatment compared to placebo treatment in each environment. Moreover, body weights and total body water values were nearly identical between sea level and altitude for the same treatment. Collectively, these data indicate that there was only a mild diuretic response to the dose of acetazolamide provided and that overall body hydration was similar prior to exercise in both environments.

TABLE 1: Body Weight (kgs)

	Sea level	Altitude
Placebo	77.3 ± 5	77.3 ± 5
Acetazolamide	75.8 ± 5	76.1 ± 5

Values are means ± SD

TABLE 2: Total Body Water (liters)

	Sea level	Altitude
Placebo	38.7 ± 4	38.5 ± 4
Acetazolamide	36.7 ± 4	36.7 ± 4

Values are means ± SD

Tables 3 and 4 show that acetazolamide treatment lowered arterialized blood pH and HCO_3^{-1}, respectively, in each environment. Therefore, acetazolamide treatment was successful in causing an expected and similar metabolic acidosis in each environment.

TABLE 3: Arterialized Blood pH

	Sea level	Altitude
Placebo	7.427 ± 0.023	7.478 ± 0.070
Acetazolamide	7.339 ± 0.019*	7.369 ± 0.028*

Values are means ± SD *P < 0.05 from placebo.

TABLE 4: Arterialized Blood HCO_3^{-1} (mmol/L)

	Sea level	Altitude
Placebo	28.4 ± 1	27.7 ± 6
Acetazolamide	19.7 ± 2*	19.0 ± 2*

Values are means ± SD *P < 0.05 from placebo

ENVIRONMENTAL SYMPTOMS QUESTIONNAIRE

The Environmental Symptoms Questionnaire (ESQ) results presented in Table 5 indicate that the total score in each environment during acetazolamide treatment was nearly identical to the total score during placebo treatment. However, the total score at altitude was greater than the total score at sea level (main effect, P < 0.05).

Results in Tables 6a and 6b show that the two-weighted sickness scores AMS-c and AMS-r, respectively, were little affected by acetazolamide treatment in either environment. The weighted sickness scores also did not increase at altitude compared to sea level. Thus, during either placebo or acetazolamide treatment at altitude, the subjects did not suffer from acute mountain sickness (To indicate sickness, AMS-c must be greater than 0.70 and AMS-r must be greater than 0.60).

Collectively, the ESQ data indicate that acetazolamide treatment compared to placebo treatment did not induce a change in well being in either environment.

TABLE 5: ESQ Total Score

	Sea level	Altitude
Placebo	6 ± 3	12 ± 7
Acetazolamide	6 ± 2	11 ± 5

Values are means ± SD $^{\#}P < 0.05$ main effect, altitude compared to sea level

TABLE 6a: AMS-C

	Sea level	Altitude
Placebo	0.00 ± 0.0	0.13 ± 0.13
Acetazolamide	0.00 ± 0.0	0.20 ± 0.25

Values are means ± SD

TABLE 6b: AMS-R

	Sea level	Altitude
Placebo	0.05 ± 0.04	0.12 ± 0.11
Acetazolamide	0.06 ± 0.07	0.13 ± 0.11

Values are means ± SD

PRE-EXERCISE RESTING MEASUREMENTS

All measures in Tables 7 to 13 were obtained over a ten-min resting collection period while subjects were comfortably seated on the dynamic knee extension device just prior to the beginning of endurance exercise.

Resting heart rate was not affected by acetazolamide treatment in either environment but did increase from sea level to altitude during acetazolamide treatment (Table 7). Resting values for minute ventilation (Table 8), oxygen consumption (Table 9), carbon dioxide production (Table 10), and ventilatory equivalents for oxygen (Table 11) and carbon dioxide (Table 12) were not statistically significantly affected by either acetazolamide treatment or altitude exposure.

TABLE 7: Resting Heart Rate (beats/min)

	Sea level	Altitude
Placebo	66 ± 7	71 ± 7
Acetazolamide	64 ± 10	76 ± 11$^{\#}$

Values are means ± SD; $^{\#}P < 0.05$ from SL

TABLE 8: Resting Minute Ventilation (L/min)

	Sea level	Altitude
Placebo	9.6 ± 1	10.9 ± 1
Acetazolamide	10.1 ± 1	10.8 ± 2

Values are means ± SD

TABLE 9: Resting Oxygen Consumption (ml/min)

	Sea level	Altitude
Placebo	280 ± 30	322 ± 74
Acetazolamide	271 ± 39	291 ± 114

Values are means ± SD

TABLE 10: Resting Carbon Dioxide Production (ml/min)

	Sea level	Altitude
Placebo	251 ± 31	281 ± 79
Acetazolamide	229 ± 34	257 ± 114

Values are means ± SD

To normalize ventilation to metabolic rate, ventilation was expressed as ventilatory equivalents for oxygen (i.e., minute ventilation / oxygen consumption, Table 11) and carbon dioxide (i.e., minute ventilation / carbon dioxide production, Table 12). Both resting ventilatory measures were not altered by either environment or acetazolamide treatment.

TABLE 11: Resting Ventilatory Equivalent for VO2 (L/min)

	Sea level	Altitude
Placebo	34 ± 2	35 ± 6
Acetazolamide	38 ± 6	39 ± 7

Values are means ± SD

TABLE 12: Resting Ventilatory Equivalent for VCO2 (L/min)

	Sea level	Altitude
Placebo	38 ± 3	41 ± 9
Acetazolamide	45 ± 6	45 ± 9

Values are means ± SD

Table 13 indicates that resting arterial oxygen saturation was lower at altitude compared to sea level for both the placebo and acetazolamide treatments. However, while there was no difference in arterial oxygen saturation between treatments at sea level, arterial oxygen saturation was higher during acetazolamide treatment than during placebo treatment at altitude.

TABLE 13: Resting Arterial Oxygen Saturation (%)

	Sea level	Altitude
Placebo	99 ± 1	75 ± 5[#]
Acetazolamide	99 ± 1	82 ± 4[*,#]

Values are means ± SD; *P < 0.05 from placebo; # P<0.05 from sea level

MAXIMAL VOLUNTARY CONTRACTION FORCE BEFORE AND DURING DYNAMIC KNEE EXTENSION EXERCISE

Maximal voluntary contraction (MVC) force prior to (Table 14) and at exhaustion (Tables 15 and 16) from dynamic knee extension exercise was not altered by either acetazolamide treatment or environment. At exhaustion, the level of MVC force was similar among treatment and environment whether expressed as absolute values (i.e., newtons, Table 15) or as a percentage of rested MVC force (Table 16). Approximately 41% of MVC force was lost from the start of exercise to point of exhaustion regardless of treatment or environment.

TABLE 14: Rested Maximal Voluntary Contraction Force (newtons):

	Sea level	Altitude
Placebo	672 ± 196	623 ± 142
Acetazolamide	632 ± 182	635 ± 173

Values are means ± SD.

TABLE 15: Maximal Voluntary Contraction Force at Exhaustion (newtons):

	Sea level	Altitude
Placebo	386 ± 124	360 ± 116
Acetazolamide	393 ± 164	376 ± 160

Values are means ± SD

TABLE 16: Maximal Voluntary Contraction Force at Exhaustion (% of Rested MVC force):

	Sea level	Altitude
Placebo	58 ± 7	58 ± 9
Acetazolamide	62 ± 13	58 ± 14

Values are means ± SD

Endurance time to exhaustion was reduced at altitude compared to sea level by 63 ± 18% (P<0.01) during placebo treatment and by 43 ± 22% (P<0.01) during acetazolamide treatment (Table 17). Endurance time to exhaustion at sea level also was

reduced during acetazolamide treatment by 26 ± 24% (P<0.02) compared to placebo treatment. However at altitude, for the same subjects, there was no reduction in endurance time between treatments.

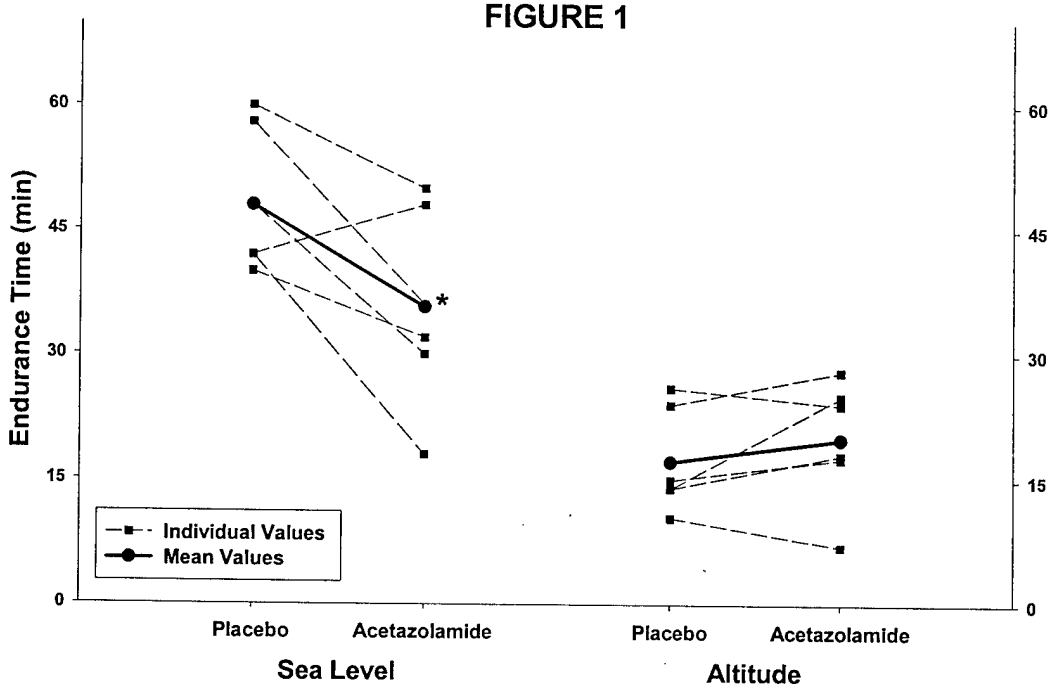
TABLE 17: Endurance Time to Exhaustion (min)

	Sea level	Altitude
Placebo	48.3 ± 9	17.0 ± 6#
Acetazolamide	35.7 ± 12*	19.9 ± 8#

Values are means ± SD. *P < 0.05 from placebo; P < 0.05 from sea level

Individual endurance times for sea level and altitude during placebo and acetazolamide treatments are illustrated in Figure 1. At sea level, endurance time declined for five subjects (range: -8 min to -24 min) and improved for one (+6 min) during acetazolamide treatment (overall mean change: -12 ± 10 min, P<0.02). In sharp contrast, endurance times at altitude declined for only two subjects (-2 min and -3 min) but improved for four (range: +3 min to +11 min) during acetazolamide treatment (overall mean change: +3 ± 5 min, n.s.).

FIGURE 1



STEADY-STATE EXERCISE MEASUREMENTS

Each subject exercised to exhaustion at identical submaximal work rates (25 ± 2 watts or 72 ± 5% of sea-level DKE peak work rate) during placebo and acetazolamide treatments at sea level and altitude. For Tables 18 to 25, comparisons among treatments and environments were made during exercise "steady-state" at 50% of endurance time.

Exercise heart rate (Table 18) tended (P>0.05) to be higher at altitude than at sea level. Exercise heart rate was not affected by acetazolamide treatment in either environment.

TABLE 18: Exercise Heart Rate (beats/min) at 50% Endurance Time

	Sea level	Altitude
Placebo	109 ± 16	113 ± 11
Acetazolamide	108 ± 11	117 ± 14

Values are means ± SD

Ratings of perceived exertion at 50% of endurance time were not affected either by environment or acetazolamide treatment (Table 19). In all treatments, leg exercise was perceived to be "hard" to "very hard".

TABLE 19: Ratings of Perceived Exertion at Exhaustion at 50% Endurance Time

	Sea level	Altitude
Placebo	16.3 ± 1	14.7 ± 1
Acetazolamide	15.2 ± 1	16.0 ± 1

Values are means ± SD

Exercise minute ventilation increased (P<0.05) from sea level to altitude during placebo and acetazolamide treatments (Table 20). At altitude, ventilation was higher during acetazolamide treatment compared to placebo treatment.

TABLE 20: Minute Ventilation (L/min) at 50% Endurance Time

	Sea level	Altitude
Placebo	25.9 ± 5	35.8 ± 8$^{\#}$
Acetazolamide	29.5 ± 6	42.2 ± 10$^{*,\#}$

Values are means ± SD; * P <0.05 from placebo, # P<0.05 from sea level

Oxygen consumption (Table 21) and carbon dioxide production (Table 22) during exercise were not significantly affected by either acetazolamide treatment or altitude exposure.

TABLE 21: Oxygen Consumption at 50% Endurance Time

	Sea level	Altitude
Placebo	759 ± 172	772 ± 148
Acetazolamide	708 ± 99	787 ± 146

Values are means ± SD

TABLE 22: Carbon Dioxide Production at 50% Endurance Time

	Sea level	Altitude
Placebo	778 ± 174	826 ± 149
Acetazolamide	698 ± 118	851 ± 156

Values are means ± SD

Ventilatory equivalents for oxygen (Table 23) and carbon dioxide (Table 24) were higher during acetazolamide treatment at sea level and altitude. They also were higher at altitude compared to sea level for both the placebo and acetazolamide treatments ($P<0.05$).

TABLE 23: Ventilatory Equivalent for VO2 (L/min) at 50% Endurance Time

	Sea level	Altitude
Placebo	34 ± 3	47 ± 4$^{\#}$
Acetazolamide	42 ± 6*	52 ± 5$^{*,\#}$

Values are means ± SD; * $P<0.05$ from placebo, # $P<0.05$ from sea level

TABLE 24: Ventilatory Equivalent for VCO2 (L/min) at 50% Endurance Time

	Sea level	Altitude
Placebo	34 ± 2	43 ± 5$^{\#}$
Acetazolamide	42 ± 5*	50 ± 5$^{*,\#}$

Values are means ± SD; * $P<0.05$ from placebo, # $P<0.05$ from sea level

Arterial oxygen saturation was reduced from sea level to altitude for both treatments (Table 25). At altitude, arterial oxygen saturation was higher during acetazolamide treatment compared to placebo treatment.

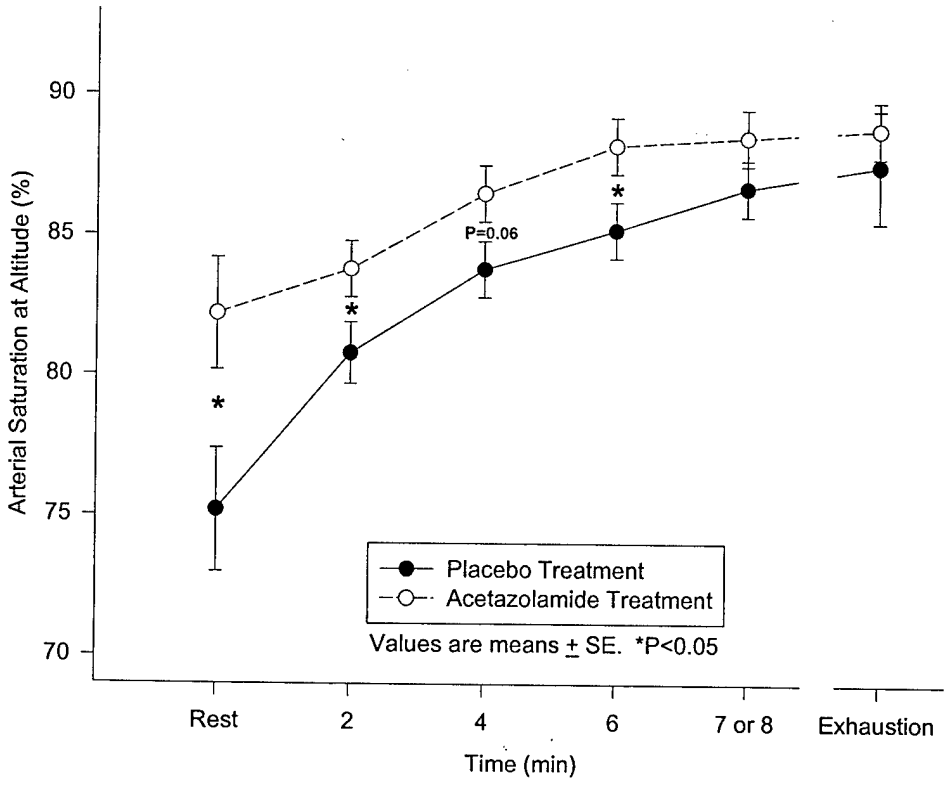
TABLE 25: Arterial Oxygen Saturation at 50% Endurance Time

	Sea level	Altitude
Placebo	97.7 ± 1	86.3 ± 2$^{\#}$
Acetazolamide	97.5 ± 1	88.5 ± 3$^{*,\#}$

Values are means ± SD *P < 0.05 from placebo; # P<0.05 from sea level

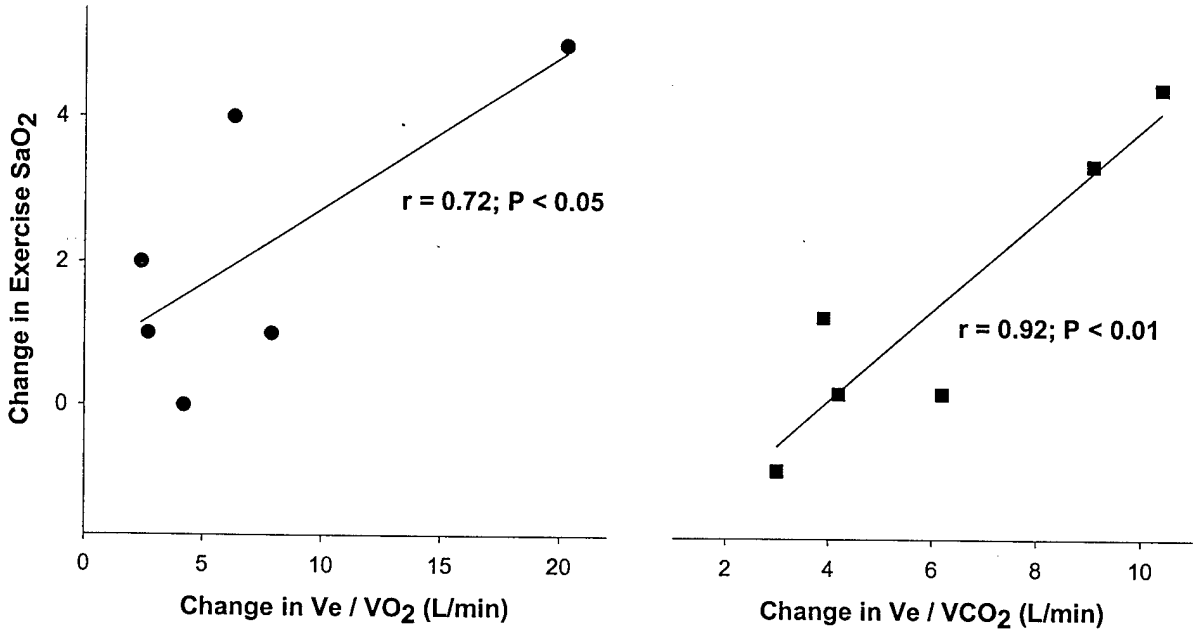
Figure 2 shows arterial oxygen saturation at altitude during rest, the first 7 or 8 min of exercise (i.e., one subject was able to complete only 7 min at altitude), and exhaustion. Arterial oxygen saturation was higher during acetazolamide treatment compared to placebo treatment during exercise in general (main effect: P < 0.001) and at indicated time points.

FIGURE 2



For each subject, individual values of the difference between acetazolamide and placebo ventilatory equivalents for oxygen ($\dot{V}e/\dot{V}O_2$) and carbon dioxide ($\dot{V}e/\dot{V}CO_2$), and SaO_2 were plotted in Figure 3. The results clearly show that for all subjects at altitude, treatment with acetazolamide augmented $\dot{V}e/\dot{V}O_2$ and $\dot{V}e/\dot{V}CO_2$. Moreover, higher values for $\dot{V}e/\dot{V}O_2$ and $\dot{V}e/\dot{V}CO_2$ were each associated with an increase in exercise SaO_2 during acetazolamide treatment.

FIGURE 3



DISCUSSION

Acetazolamide causes incomplete hydration of carbon dioxide (CO_2) in cells; increased renal excretion of bicarbonate, sodium, potassium, and water; and increased arterial and venous blood hydrogen ion concentrations [H^+] (1;25;26;29). Though acetazolamide has been useful in the prophylaxis of acute mountain sickness (10), the induced metabolic acidosis can impair ability to buffer increases in organic acids during exercise (13;20;24) and thereby adversely affect metabolic processes involved in muscular contraction (15;28). While it is commonly reported that endurance exercise performance is impaired at sea level as a result of induced acidosis (13;15;17;22-24), findings of impaired exercise performance during acetazolamide treatment at altitude are reported in some (11;17;23) but not all (3;22;26) studies.

Conflicting findings may relate to inconsistencies in experimental conditions that make it difficult to reach a consensus regarding the effect of acetazolamide on exercise performance at altitude. Among studies, there have been differences in: drug dose and responsiveness, administration schedule, and diuresis among subjects during treatment; altitude elevation, duration, ascent rate, and degree of altitude acclimatization; variability and intensity of the exercise mode; size of active muscle mass during exercise; physiological limitations to exercise performance; recovery times between consecutive exercise bouts; diet; and degree of altitude sickness (3;5;7;11;17;22;23).

The experimental design of present study attempted to minimize as many of these potentially confounding factors as possible to determine the effect of acetazolamide on exercise performance at sea level and altitude. One key feature was to have subjects exercise at identical power outputs under all experimental conditions using an exercise model that provided a means of detecting small changes in isolated muscle endurance performance (8). Using this exercise model instead of universally employed conventional ergometry (11;17;22;23) eliminated the possibility that central circulatory and pulmonary diffusion limitations would restrict muscle perfusion and oxygen delivery to active muscle at sea level or altitude (9;18;19). For this reason, adverse effects on local muscle performance resulting from induced acidosis could be separated from those due to central circulatory or pulmonary diffusion limitations associated with intense exercise, hypoxia, or both. In addition, unlike exertion during conventional ergometry, exertion

during isolated muscle exercise does not exaggerate the hypoxic stress associated with altitude exposure (as evidenced by an *increase* in SaO_2 from resting values during exercise (9)). By not further exaggerating the hypoxic stress and thereby overriding potentially beneficial secondary responses resulting from the induced acidosis (e.g., increased ventilation), the effect of acetazolamide on muscle endurance performance *per se* at altitude could be more clearly assessed.

In the present study, bicarbonate and blood pH levels at sea level and altitude indicated that the degree of induced acidosis during acetazolamide treatment was similar for both environments. Yet, our results unequivocally show that acetazolamide impaired muscle endurance performance at sea level but not at altitude. If the percentage decline in endurance performance during treatment with acetazolamide compared to placebo at altitude matched that at sea level (i.e., -26%), then the mean endurance time at altitude would have been reduced from 17 min to 13 min. Instead, the actual measured mean endurance time at altitude during acetazolamide treatment tended to increase from 17 min to 20 min. This finding indicates that one or more opposing secondary physiological processes affected by acetazolamide became effective or more effective at altitude than at sea level to successfully counteract the direct adverse effect of induced acidosis on isolated muscle during endurance exercise. Our data implicate greater ventilation and a resulting enhanced oxygenation during acetazolamide treatment while exercising at altitude.

Exercise ventilation (expressed as L/min or $\dot{V}e/\dot{V}O_2$) was higher at altitude compared to sea level for both the placebo and acetazolamide treatments. An increase in ventilation during exercise at the same power output at altitude compared to sea level during placebo treatment is mediated by peripheral chemoreceptors sensing both a altitude-induced reduction in PaO_2 and exercise-induced increases in blood $[H^+]$ and PCO_2 (14;22). A further increase in exercise ventilation during treatment with acetazolamide compared to placebo at altitude was likely mediated by the additional combined effects of acetazolamide-induced increases of blood $[H^+]$ and brain cell accumulation of CO_2 (resulting in a higher $[H^+]$) that further stimulated peripheral and central chemoreceptors (2;4;25;26). Some (5;11;22) though not all (16;23) previous reports agree with our finding of an increase in exercise ventilation resulting from acetazolamide treatment at altitude.

The higher exercise SaO_2 at altitude during treatment with acetazolamide compared to placebo was likely a consequence of the acetazolamide-induced increase in exercise ventilation (i.e., $\dot{V}e/\dot{V}O_2$) (22). Results of most previous altitude studies in which both ventilation and SaO_2 were determined during exercise indicate that if ventilation was significantly higher during acetazolamide treatment, then SaO_2 or PaO_2 also was higher (3;22). Moreover, another study indicated that if acetazolamide treatment during exercise did not cause ventilation to increase, then SaO_2 also did not increase (23). Collectively, results from previous studies agree with the results of the present study and are consistent with our postulate of a direct link between an acetazolamide-induced increase in ventilation and SaO2. Exactly why similar acetazolamide treatment at altitude increases exercise ventilation and SaO_2 in some but not all studies is not well understood.

At altitude, enhanced oxygenation during acetazolamide treatment (consistent with our finding of higher SaO_2) would provide a better oxygen delivery gradient from capillary to exercising muscle. In addition, an acidosis-induced rightward shift of the hemoglobin dissociation curve would increase oxygen unloading from capillaries to muscle. These secondary, beneficial changes at altitude apparently were enough to offset the direct, adverse effect on induced acidosis on exercising muscle. In contrast, at sea level, arterial oxygenation was already near maximal levels during placebo treatment and therefore could not meaningfully improve as a result of acetazolamide-induced increase in ventilation. This interpretation is consistent with our finding in the same subjects of a decrease in performance at sea level but no change in performance at altitude despite an increase in ventilation in both environments.

In summary, induced acidosis during acetazolamide treatment caused a similar ventilatory increase during exercise at sea level and altitude. As a result of the increase in ventilation, SaO2 increased, muscle oxygen delivery improved, and performance was not impaired at altitude even though it was impaired at sea level in the same subjects. Thus, during isolated muscle exercise at altitude, partial carbonic anhydrase inhibition via acetazolamide apparently can be overcome by the resulting acidosis-induced increase in ventilation that leads to a better gradient for oxygen delivery to active muscles.

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