Cytokine Response at High Altitude: Effects of Exercise and Antioxidants at 4300 m

TODD A. HAGOBIAN[1,2], KEVIN A. JACOBS[1,3], ANDREW W. SUBUDHI[1,4], JILL A. FATTOR[3], PAUL B. ROCK[5], STEPHEN R. MUZA[6], CHARLES S. FULCO[6], BARRY BRAUN[2], ANN GREDIAGIN[6], ROBERT S. MAZZEO[7], ALLEN CYMERMAN[6], and ANNE L. FRIEDLANDER[1]

[1]Veterans Affairs Palo Alto Health Care System, Palo Alto, CA; [2]University of Massachusetts, Amherst, MA; [3]University of California, Berkeley, CA; [4]University of Colorado, Colorado Springs, CO; [5]Oklahoma State University Center for Health Sciences, Tulsa, OK; [6]US Army Research Institute of Environmental Medicine, Natick, MA; and [7]University of Colorado, Boulder, CO

ABSTRACT

HAGOBIAN, T. A., K. A. JACOBS, A. W. SUBUDHI, J. A. FATTOR, P. B. ROCK, S. R. MUZA, C. S. FULCO, B. BRAUN, A. GREDIAGIN, R. S. MAZZEO, A. CYMERMAN, and A. L. FRIEDLANDER. Cytokine Responses at High Altitude: Effects of Exercise and Antioxidants at 4300 m. *Med. Sci. Sports Exerc.*, Vol. 38, No. 2, pp. 276–285, 2006. **Purpose:** This study tested the hypothesis that antioxidant supplementation would attenuate plasma cytokine (IL-6, tumor necrosis factor (TNF)-α), and C-reactive protein (CRP) concentrations at rest and in response to exercise at 4300-m elevation. **Methods:** A total of 17 recreationally trained men were matched and assigned to an antioxidant (N = 9) or placebo (N = 8) group in a double-blinded fashion. At sea level (SL), energy expenditure was controlled and subjects were weight stable. Then, 3 wk before and throughout high altitude (HA), an antioxidant supplement (10,000 IU β-carotene, 200 IU α-tocopherol acetate, 250 mg ascorbic acid, 50 μg selenium, 15 mg zinc) or placebo was given twice daily. At HA, energy expenditure increased approximately 750 kcal·d^{-1} and energy intake decreased approximately 550 kcal·d^{-1}, resulting in a caloric deficit of approximately 1200–1500 kcal·d^{-1}. At SL and HA day 1 (HA1) and day HA13, subjects exercised at 55% of $\dot{V}O_{2peak}$ until they expended approximately 1500 kcal. Blood samples were taken at rest, end of exercise, and 2, 4, and 20 h after exercise. **Results:** No differences were seen between groups in plasma IL-6, CRP, or TNF-α at rest or in response to exercise. For both groups, plasma IL-6 concentration was significantly higher at the end of exercise, 2, 4, and 20 h after exercise at HA1 compared with SL and HA13. Plasma CRP concentration was significantly elevated 20 h postexercise for both groups on HA1 compared to SL and HA13. TNF-α did not differ at rest or in response to exercise. **Conclusion:** Plasma IL-6 and CRP concentrations were elevated following exercise at high altitude on day 1, and antioxidant supplementation did not attenuate the rise in plasma IL-6 and CRP concentrations associated with hypoxia, exercise, and caloric deficit. **Key Words:** IL-6, TNF-α, C-REACTIVE PROTEIN, HYPOXIA, ENERGY DEFICIT

Athletes and military personnel who exercise or work at high altitude experience hypoxia and, very often, caloric deficit. Evidence indicates that the stressors (i.e., hypoxia, exercise, caloric deficit) may initiate an acute phase response (2,8,9,11,13,24), characterized by alterations in immune and inflammatory responses (11,13,21), including increases in plasma interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) concentrations. Elevations in plasma IL-6, TNF-α, and CRP concentrations may contribute to altitude-associated illnesses (e.g., acute mountain sickness and high altitude pulmonary edema), which can compromise physical work capacity (8,24). Therefore, preventing or attenuating the cytokine rise and the acute phase response may be beneficial during prolonged or intense physical work at high altitude.

Antioxidant supplementation has been shown to reduce the plasma cytokine response to exercise at sea level (23,27). Vassilakopoulos et al. (27) showed that an antioxidant cocktail blunted the plasma IL-6 and TNF-α responses to 45 min of exercise in untrained individuals, whereas Phillips et al. (23) demonstrated that supplementation reduced plasma IL-6 and CRP after eccentric exercise. In theory, antioxidant supplementation may counter the rise in free radical production, measured by markers of oxidative stress, which underlies the acute phase response (10,27). Based on results from an *in vitro* study (10), Vassilakopoulos et al. (27) proposed a theory that the exercise-induced cytokine increase is a reactive oxygen species (ROS)–dependent pathway. In contrast, others have shown that the exercise-induced plasma cytokine increase is not attenuated with antioxidant supplementation (17,21) and is not induced by oxygen free radicals (21). Because the oxidative stress response is

Address for correspondence: Anne L. Friedlander, GRECC, 182B, Building MB2B, VA Palo Alto Health Care System, 3801 Miranda Ave., Palo Alto, CA 94304-1290; E-mail: friedlan@stanford.edu.

sensitive to various exercise parameters, differences in the mode, duration, and intensity of exercise could explain previous contradictory findings. At high altitude, few studies (11,13) have evaluated the plasma cytokine and CRP responses to a controlled exercise bout and none have tried to attenuate these responses with antioxidant supplementation.

Therefore, the primary purpose of this investigation was to evaluate the efficacy of an antioxidant supplement in reducing plasma cytokine and CRP concentrations associated with the common conditions that occur at high altitude (i.e., hypoxia, exercise, caloric deficit). To mimic the daily total caloric deficit that is typically observed in individuals living and working at high altitude, a lower energy intake and higher energy expenditure were imposed. We hypothesized that (a) resting plasma IL-6, TNF-α, and CRP concentrations would be elevated throughout the 14-d stay at high altitude, (b) plasma IL-6, TNF-α, and CRP concentrations would increase during and after prolonged submaximal exercise at high altitude compared with sea level, and (c) antioxidant supplementation would attenuate the rise in plasma IL-6, TNF-α, and CRP concentrations associated with hypoxia, exercise, and caloric deficit.

METHODS

Approach. A prospective, double-blind, placebo-controlled design was used to determine the efficacy of antioxidant supplementation in reducing plasma cytokines and CRP concentrations in response to the common conditions that can occur at high altitude (i.e., hypoxia, exercise, caloric deficit). Testing phases included 5 d at sea level and 14 d at high altitude, with subjects ingesting an antioxidant supplement or placebo during the high altitude phase. The main outcome variables were plasma cytokines (IL-6 and TNF-α) and CRP concentrations. Details of the study are outlined below.

Study design. Subjects were first studied at sea level (Palo Alto, CA, 15-m elevation, atmospheric pressure 748–762 torr) during a 7-d diet stabilization period, followed by a 5-d testing period during which they resided in the Clinical Studies Unit at the Veterans Affairs Palo Alto Health Care System (VAPAHCS). At sea level, energy expenditure was controlled and subjects were fed a weight-stabilizing diet. Cytokine and CRP concentrations were measured at rest and in response to exercise at sea level day 1 (SL). At 3 wk before departure for high altitude, subjects began taking an antioxidant or placebo supplement twice a day and continued throughout their 2 wk stay at high altitude. Approximately 6 wk after the sea-level phase, subjects were flown to Colorado Springs, CO (1850 m), where they spent the night in an apartment while breathing supplemental oxygen to maintain sea-level oxygen saturation (≥97%). The next morning, subjects were driven to the summit of Pikes Peak, CO (4300 m, atmospheric pressure 458–464 torr) to the U.S. Army Research Institute of Environmental Medicine (USARIEM) facilities, while still breathing supplemental oxygen. Cytokine and CRP testing was repeated before and after exercise on high altitude day 1 (HA1) and day 13 (HA13), and fasting CRP was measured on days 3 (HA3), 5 (HA5), and 10 (HA10). While living at high altitude, energy intake and energy expenditure was altered to cause a 30–40% energy deficit (see below for details). The subjects resided on Pikes Peak in the USARIEM laboratory facility for the entire 14-d study. Figure 1 illustrates a timeline of the activities at sea level and high altitude.

Subjects. A total of 18 healthy men (mean ±SD; 25.1 ± 4.9 yr, 77.7 ± 8.2 kg, 178.1 ± 4.2 cm) were recruited to participate in the study from Stanford University and the surrounding areas (Palo Alto or San Jose, CA) using flyers and local newspaper advertisements. To participate in the current investigation, potential volunteers had to be participating in >6 h·wk^{-1} of regular endurance exercise, have a

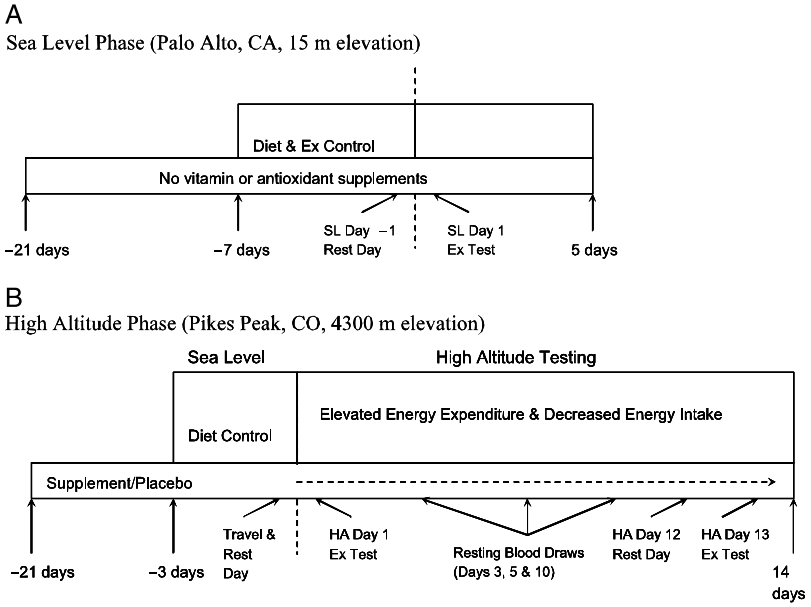

FIGURE 1—Timeline of activities at sea level (A) and high altitude (B). SL, sea level; HA, high altitude; Ex, exercise.

peak oxygen uptake ($\dot{V}O_{2peak}$) ≥45 mL·kg^{-1}·min^{-1}, be able to complete a endurance cycle test for 1 h at 70–75% of energy output for peak oxygen consumption, have a body fat percentage between 5 and 16% measured by seven-site skinfold, and have no history of residence at altitudes >1500 m. All participants were in good overall health, nonsmokers, and disease free as determined by health history questionnaire and medical evaluation. Data of one individual were omitted because of a medical emergency unrelated to the study (final N = 17). Written informed consent was obtained from all individuals, and the institutional review boards at Stanford University and USARIEM approved the study.

Screening tests. On admission to the Clinical Studies Unit at the VAPAHCS, a resting electrocardiogram (ECG), and a standard blood and urine chemistry panel was conducted. Following medical clearance, body composition was evaluated by seven-site skinfold test. Subjects then performed a continuous progressive exercise test to volitional exhaustion on a cycle ergometer (Sensormedics 800, Yorba Linda, CA). An initial workload of 100 W was increased 30 W every 2 min until oxygen consumption failed to increase or the subject stopped, despite strong encouragement. During the test, expired air was analyzed using an online system (Parvomedics TrueMax 2400, Consentius Technologies, Sandy, UT), and $\dot{V}O_{2peak}$ was determined to be the highest 30-s value obtained. In addition, heart rate (Polar Electro Inc. A1, Woodbury, NY), rating of perceived exertion, and an ECG were continuously monitored during the test.

Antioxidant supplementation. Three weeks before going to high altitude, subjects were matched on age, body mass, $\dot{V}O_{2peak}$, body fat percentage, partial pressure of end tidal volume CO_2 ($P_{ET}CO_2$), and hypoxic ventilatory response (HVR), and assigned to either an antioxidant supplement (AO; N = 9) or placebo (PL; N = 8) group in a double-blinded fashion (Table 1). The AO consisted of a capsule of ascorbic acid (250 mg), β-carotene (10,000 IU), α-tocopherol acetate (200 IU), selenium (50 μg), and zinc (15 mg), and the PL was an identical-looking and -tasting cellulose capsule. Subjects were instructed to ingest the supplement twice daily, once in the morning and once in the evening, continuing throughout the high-altitude phase. As reported previously by Subudhi et al. (26), the antioxidant protocol elevated blood concentrations in the AO group at high altitude compared with sea level for α-tocopherol (14.9 ± 5.5, 10.0 ± 3.8, respectively) and β-carotene (0.58 ± 0.31, 0.26 ± 0.17, respectively). The PL group had similar α-tocopherol and β-carotene concentrations to the AO group at sea level, with no subsequent change at high altitude. Based on previous research that has shown a reduction in cytokine concentrations and markers of oxidative stress in response to an antioxidant supplement (22,23,27), we formulated a broad-based antioxidant cocktail composed of vitamins and minerals to maximize the possible benefits of the supplement.

Energy intake, energy expenditure and body composition. Subjects were fed a standardized diet throughout the study composed of whole foods (e.g., pasta, bagels, cereal) containing modest levels of antioxidants (just below the RDA). Energy intake was initially estimated before the first day of sea-level testing with the Harris–Benedict equation, using an activity factor between 1.5 and 1.9, and, if necessary, was adjusted during the next 7 d to maintain body mass. After the weight-stabilization period, energy intake was maintained for the next 4 d with the diet composed of 63% carbohydrate, 23% fat, and 13% protein. Energy expenditure was estimated from self-reported daily activity logs in which the subjects recorded all activities for each 24-h period in 15-min intervals using the ACSM compendium of physical activities (1). On several occasions for each subject, heart rate was monitored to assess energy expenditure. While at sea level, subjects maintained their normal exercise routine.

Military personnel exposed to field training exercises and prolonged excursions (including high altitude) typically experience a caloric deficit ≥1000 kcal·d^{-1} mainly because of elevated energy expenditure (5). The goal of the high-altitude phase of this study was to mimic the typical undernutrition observed during these field training exercises and prolonged excursions at high altitude, therefore, subjects were asked to increase their energy expenditure to 1200–1500 kcal·d^{-1} more than at sea level (40% of caloric intake) while maintaining sea-level energy intake values. At high altitude, energy expenditure was estimated as described above. For all subjects, total daily energy expenditure was adjusted with basal metabolic rate (BMR), which was assessed by indirect calorimetry on high-altitude days 2 through 6 and days 10 through 13. To elevate energy expenditure, subjects had unlimited access to treadmills, cycle ergometers, and rowing and ski machines, and were required to participate on a hike for 1–3 h (>3650 m of elevation) at least three times throughout the high-altitude phase. If subjects were unable to achieve their targeted energy expenditure each day at high altitude, energy intake was adjusted to reach a total energy deficit of approximately 1200–1500 kcal·d^{-1}. Subjects were given continuous feedback on a daily basis to ensure that the overall caloric deficit at high altitude was approximately 40% of their sea-level energy intake.

Fasted body mass was measured every morning at SL and HA, and body fat percentage was evaluated at SL and at HA1, HA3, HA5, HA10, and HA14 using skinfold calipers. Fat-free mass was calculated as body mass minus body fat mass.

TABLE 1. Physical characteristics of matched groups.

Variable	AO	PL
N	9	8
Age (yr)	24.6 (5.5)	24.8 (4.0)
Body mass (kg)	77.5 (7.8)	79.6 (8.7)
Ht (cm)	180.6 (4.2)	176.8 (4.2)
Body fat (%)	7.6 (2.9)	9.3 (3.8)
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	57.0 (1.5)	53.9 (10.1)
L·min^{-1}	4.4 (0.6)	4.3 (0.8)
$P_{ET}CO_2$ (torr)	40.4 (1.7)	40.8 (2.4)
HVR	0.9 (0.8)	1.0 (0.7)

Values are mean (SD) for AO and PL. AO, antioxidant supplement group; PL, placebo group; $\dot{V}O_{2peak}$, peak oxygen uptake; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; HVR, hypoxic ventilatory response.

Prolonged submaximal exercise cycling test. Prolonged submaximal exercise cycling (SEC) tests were performed on SL day 1 (after 7 d of diet stabilization), and HA1 and HA13 on an electromagnetically braked cycle ergometer (Sensormedics). Exercise was prohibited 36 h before all trials, and energy intake was adjusted to account for the lower energy expenditure the day before the SEC test at HA. After a 12-h overnight fast, subjects consumed a standardized breakfast (478 kcal) composed of 74% carbohydrate, 14% fat, and 12% protein. Within 2 h after consumption of the meal, subjects were required to consume at least 250 mL of water. A catheter was then inserted into a forearm vein and a venous blood sample was taken at rest 2 h after eating. Subjects then rode the cycle ergometer at a pedal speed between 70 and 90 rpm. At sea level, subjects exercised at 55.0% ± 4.1 of $\dot{V}O_{2peak}$ until approximately 1500 kcal was expended. At high altitude, subjects exercised at the same relative intensity as sea level (i.e., same percent of high altitude $\dot{V}O_{2peak}$), and the same absolute workload on HA1 and HA13, again until an energy expenditure of approximately 1500 kcal was achieved. Previous research has shown that $\dot{V}O_{2peak}$ at 4300 m is reduced by approximately 25% compared with sea level (6); therefore, the relative intensity level at high altitude was estimated using the sea-level $\dot{V}O_{2peak}$ values and the predicted decrement. The actual average reduction in $\dot{V}O_{2peak}$ determined on day 2 at HA was 27% (26), meaning that the actual relative intensity during the SEC test was 57.0% ± 2.9 of $\dot{V}O_{2peak}$. Because of this reduction during the SEC test, the absolute workload decreased compared with sea level and the duration of exercise at high altitude increased compared with sea level (193 ± 25 vs 135 ± 25 min, respectively). Oxygen consumption was measured during the initial 5 min of rest and 15 min of exercise, and then the last 5 min of every hour completed to ensure the required exercise intensity was being maintained. During exercise the same volume of water was consumed during all tests. Heart rate and oxygen saturation were recorded every 15 min. After completion of exercise, subjects consumed a standardized lunch (738 kcal) composed of 53% carbohydrate, 17% fat, and 29% protein. Blood samples were collected at rest, at the end of exercise, and 2, 4, and 20 h after exercise.

Resting C-reactive protein. Resting CRP was determined after a 10- to 12-h overnight fast at SL and HA3, HA5, and HA10. Exercise was prohibited a minimum of 14 h before obtaining the blood draw.

Biochemical and acute mountain sickness analyses. Blood samples were collected in sterile syringes and transferred to EDTA tubes, then immediately spun in a refrigerated centrifuge (3,000 ×←*g*) for 15 min. The plasma was aliquotated into polystyrene tubes and stored at −80°C until analyzed. Plasma concentrations of IL-6 and TNF-α were determined by high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits using 96-well plates (R&D Systems, Minneapolis, MN), and plasma CRP concentrations were analyzed by the Palo Alto VA Laboratory using a high-sensitivity assay. Catecholamine concentrations were determined by high-performance liquid chromatography (HPLC; BioRad Model 1330 pump, Model 1340 electrochemical detector) with electrochemical detection as previously described (14). Acute mountain sickness scores (AMS), assessed by Lake Louise and Environmental Symptoms questionnaires (ESQ), were determined at SL and throughout the HA stay (data published elsewhere). In the present investigation, AMS scores were used for correlations with plasma cytokine and CRP concentrations.

Statistical analysis. Data were analyzed using SAS Inc. software (Cary, NC). A mixed model three-way ANOVA with one between factor (group) and two within factors (day and time) was used to determine the group ×← day ×←time interaction using a compound symmetric covariate structure. Pearson product moment correlations were used to assess the relationship of plasma catecholamines with plasma IL-6 or CRP concentrations, and AMS with plasma IL-6 and CRP concentrations. When appropriate, *post hoc* tests of significance were performed with a Tukey HSD test. By convention, the *a priori* level of significance was set at $\alpha < 0.05$.

RESULTS

Energy intake, energy expenditure, and body composition. By design, energy intake and energy expenditure were equivalent at SL (mean ±SD; 3895 ± 348 kcal·d^{-1} and 4078 ± 531 kcal·d^{-1}, respectively). At HA, the planned dietary restriction and increased physical activity resulted in a decreased energy intake and increased energy expenditure of 3357 ± 579 kcal·d^{-1} and 4723 ±

TABLE 2. Body composition at sea level and high altitude.

	SL	HA Day 1	HA Day 3	HA Day 5	HA Day 10	HA Day 14
Body mass (kg)						
AO	77.5 (7.8)	77.5 (7.7)	77.0 (7.6)	76.1 (7.6)*†	75.2 (7.5)*†	73.9 (7.5)*†
PL	79.6 (8.7)	80.3 (8.9)	80.3 (9.0)	79.1 (8.7)*†←	78.0 (9.1)*†←	76.5 (8.8)*†←
Fat mass (kg)						
AO	6.0 (2.5)	6.2 (2.6)	6.2 (2.8)	5.9 (2.3)	5.3 (2.3)*	5.0 (2.3)*
PL	7.6 (3.7)	8.0 (3.8)	7.4 (5.4)	8.1 (4.6)	7.5 (3.8)	7.1 (4.3)*
Fat-free mass (kg)						
AO	71.6 (7.3)	71.2 (7.3)	70.2 (7.4)	69.9 (7.5)*†	69.0 (7.6)*†	68.6 (7.5)*
PL	72.0 (6.8)	72.9 (7.4)	72.2 (7.1)	70.6 (6.5)*†←	69.5 (7.6)*†←	69.2 (7.2)*

Values are mean (SD) for AO and PL. * Significantly different than SL and HA day 1 ($P < 0.05$). †Significantly different than previous measurement ($P < 0.05$). AO, antioxidant supplement group; PL, placebo group; SL, sea level; HA, high altitude.

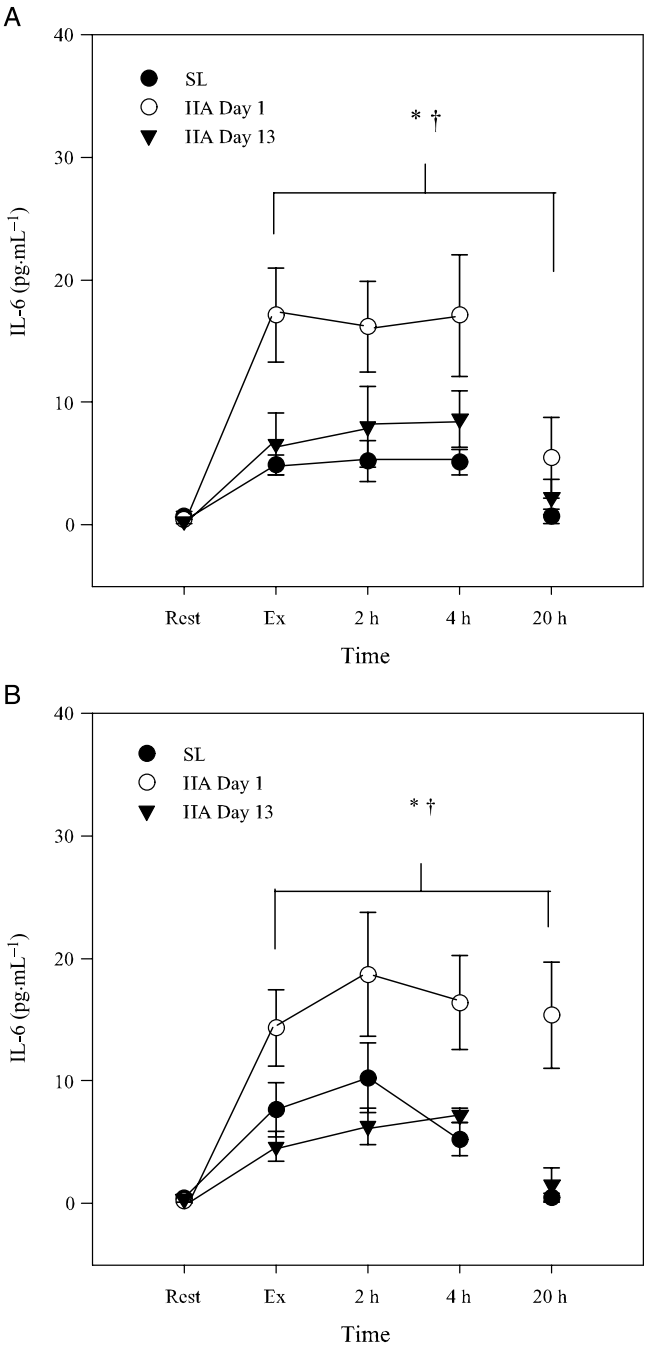

FIGURE 2—Plasma IL-6 concentrations at rest, at the end of exercise (Ex), and after exercise (2, 4, and 20 h) for AO (A) and PL (B) groups. Values are mean (SEM). * Significantly different than rest for SL, HA day 1, and HA day 13 ($P < 0.001$); † HA day 1 significantly different than SL and HA day 13 ($P = 0.035$). AO, antioxidant supplement group; PL, placebo group; IL-6, interleukin-6; SL, sea level; HA, high altitude; Ex, end of exercise.

484 kcal·d^{-1}, respectively, for a total energy deficit of 1385 ± 531 kcal·d^{-1}. The total energy deficit for the AO and PL groups was similar at HA (1400 ± 465 kcal·d^{-1} and 1331 ± 613 kcal·d^{-1}, respectively). The resulting changes in body mass, fat-free mass, and fat mass were similar between the groups (Table 2). Body mass and fat-free mass were reduced approximately 4.3 and 3.1 kg throughout the duration of high altitude, respectively. Fat mass was significantly reduced in both groups by HA day 14 compared with sea level. Body composition changes over the course of the study are presented in Table 2.

Prolonged submaximal exercise cycling test. No difference was seen in steady-state $\dot{V}O_2$ during exercise between the AO and PL groups at SL (31.4 ± 0.8 vs 29.7 ± 5.6 mL·kg^{-1}·min^{-1}, respectively), HA1 (21.9 ± 0.8 vs

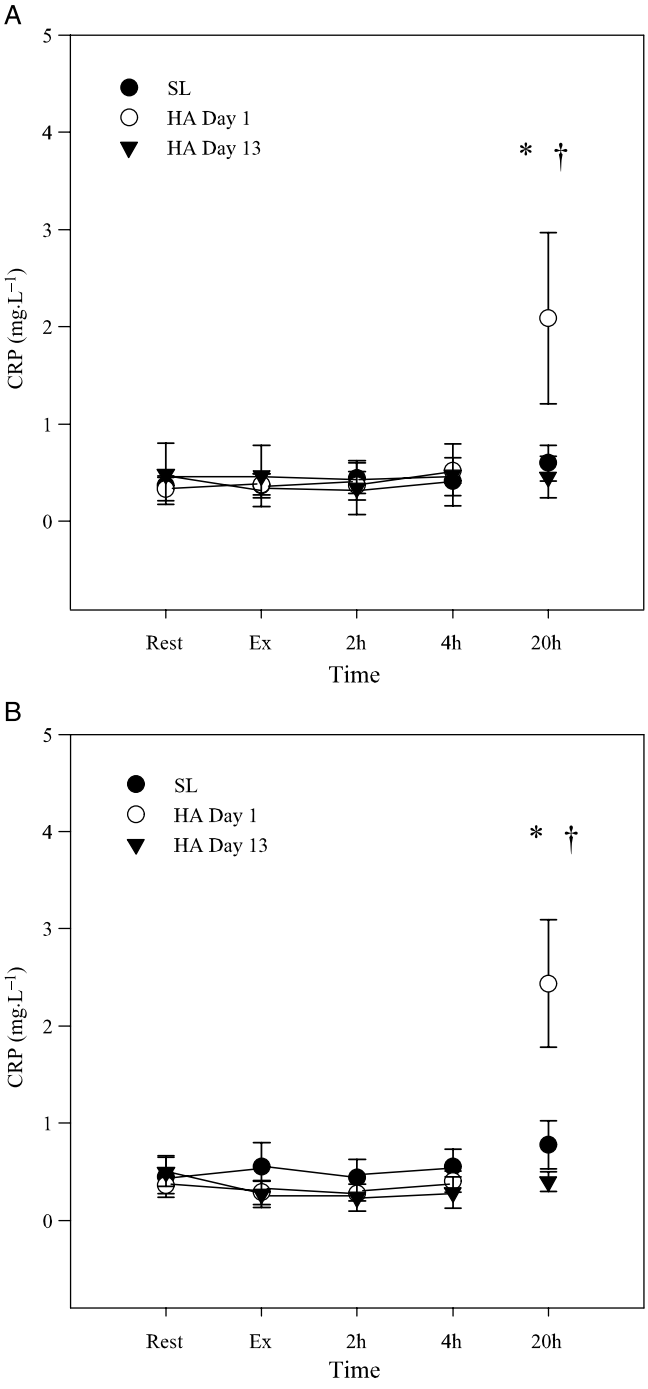

FIGURE 3—Plasma CRP concentrations at rest, at the end of exercise (Ex), and after exercise (2, 4, and 20 h) for AO (A) and PL (B) groups. Values are mean (SEM). * Significantly different than rest ($P < 0.001$) for HA day 1; † HA day 1 significantly different than SL and HA day 13 ($P < 0.001$). AO, antioxidant supplement group; PL, placebo group; CRP, C-reactive protein; SL, sea-level; HA, high altitude; Ex, end of exercise.

20.6 ± 3.9 mL·kg^{-1}·min^{-1}, respectively), or HA13 (21.9 ± 0.8 vs 20.4 ± 4.3 mL·kg^{-1}·min^{-1}, respectively), and the total volume of O_2 consumed for the SEC test (317 ± 173 L) was similar for each group. No difference was noted in oxygen saturation during exercise between groups. Within the AO and PL groups, oxygen saturation was significantly lower at HA1 (72 ± 8 and 75 ± 5, respectively) compared with SL (96 ± 1 and 97 ± 1, respectively) and HA13 (82 ± 3 and 83 ± 4, respectively). Heart rate was not different between groups. Heart rate was significantly lower for AO and PL at HA13 (115 ± 11 and 119 ± 10 bpm, respectively) compared with SL (129 ± 7 and 138 ± 12 bpm, respectively) and HA1 (129 ± 11 and 124 ± 20 bpm, respectively).

Plasma cytokines, C-reactive protein, and catecholamine concentrations. No group differences were found between AO and PL in plasma IL-6, CRP, TNF-α, or catecholamine concentrations. For both groups, plasma IL-6 concentration was significantly higher at the end of exercise, 2, 4, and 20 h after exercise at HA1 compared with SL and HA13 (Fig. 2), with the maximal value for both groups on HA1 approximately 100% greater compared with SL and HA13. At HA1, plasma IL-6 increased approximately 42 times compared with rest for both groups with peak values of 17.1 ± 3.8 and 18.2 ± 5.5 pg·mL^{-1} for AO and PL, respectively. Plasma catecholamine concentrations and AMS scores were not correlated with IL-6 ($P > 0.05$). For both groups, plasma CRP concentrations were significantly elevated 20 h after exercise at HA1 compared with SL and HA13 (Fig. 3). The maximal rise for both groups at HA1 was approximately 200% greater compared with SL and HA13. At HA1, plasma CRP concentration increased approximately sixfold compared with rest for both groups with peak values of 2.1 ± 0.8 and 2.4 ± 0.7 mg·L^{-1} for AO and PL, respectively. Plasma norepinephrine was significantly correlated with CRP on HA1 at 20 h after exercise (Fig. 4; combined data, $r^2 = 0.42$, $P = 0.005$); however,

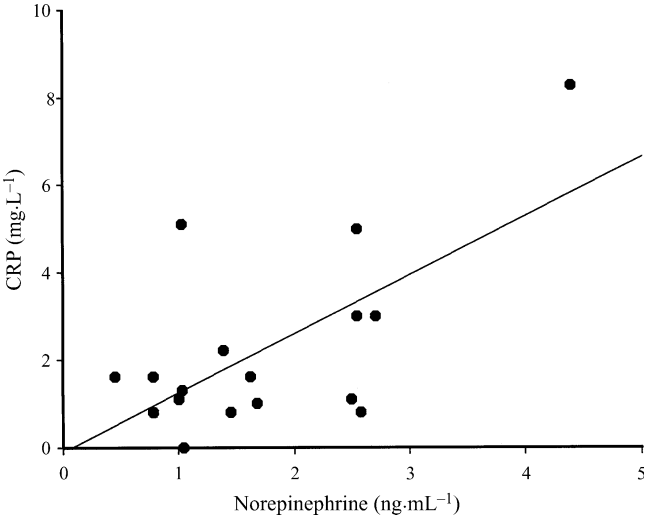

FIGURE 4—Relationship between 20 h after exercise plasma CRP and plasma norepinephrine (data combined; $P = 0.005$, $r^2 = 0.42$). CRP, C-reactive protein.

TABLE 3. Plasma TNF-α (pg·mL^{-1}) response to the prolonged submaximal exercise test for both groups.

Group	Test Day	Rest	End of Exercise	2 h After Exercise	4 h After Exercise
AO	SL	2.1 (1.2)	1.8 (1.1)	1.7 (1.1)	1.7 (0.9)
	HA day 1	1.9 (0.8)	1.7 (1.1)	1.6 (1.0)	1.7 (0.8)
	HA day 13	1.6 (0.4)	1.7 (1.2)	1.9 (1.4)	1.8 (1.2)
PL	SL	1.8 (0.8)	1.7 (0.4)	1.6 (0.8)	1.7 (0.6)
	HA day 1	2.0 (0.8)	2.1 (1.1)	1.8 (1.0)	1.8 (0.9)
	HA day 13	1.9 (0.8)	1.7 (0.6)	1.5 (0.9)	1.3 (0.7)

Values are mean (SD) for AO and PL. AO, antioxidant supplement group; PL, placebo group; TNF-α, tumor necrosis factor α; SL, sea level; HA, high altitude.

plasma epinephrine and AMS scores were not ($P > 0.05$). Plasma TNF-α concentration did not differ between any conditions (Table 3). For both groups (data combined), plasma epinephrine concentrations were significantly elevated at the end of exercise, 2, 4, and 20 h after exercise on HA1 compared with sea level and HA13 (Fig. 6). Plasma norepinephrine concentrations (data combined) tended to be higher at HA1 and HA13 compared with SL, but did not reach statistical significance (Fig. 6).

Resting C-reactive protein. No difference was seen in resting plasma CRP concentration between the groups (Fig. 5). Plasma CRP concentration was significantly elevated at HA3 and HA5 compared with SL for both groups, but returned to similar SL values at HA10. Plasma CRP concentration tended to be higher for the AO group at HA10 compared with the PL group because one subject's CRP value was approximately 560% greater than the group mean (10.1 vs 1.6 mg·L^{-1}, respectively). No significant correlations were found between plasma norepinephrine or epinephrine and resting CRP ($P > 0.05$). Because CRP concentrations initially rose over time at high altitude (days 3 and 5), it is possible that the elevation of plasma CRP

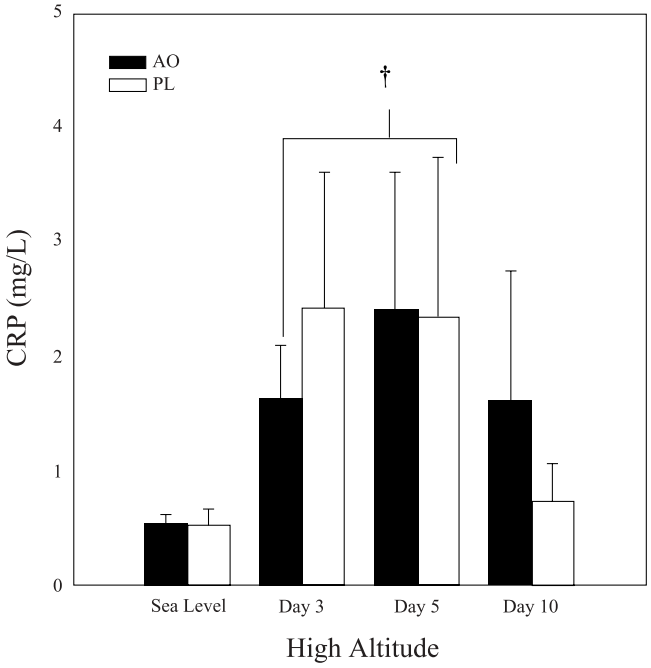

FIGURE 5—Resting plasma CRP concentrations at SL and HA days 3, 5, and 10. Values are mean (SEM). † HA day 3 and HA day 5 significantly different than SL for both groups ($P = 0.028$). AO, antioxidant supplement group; PL, placebo group; CRP, C-reactive protein; SL, sea level; HA, high altitude.

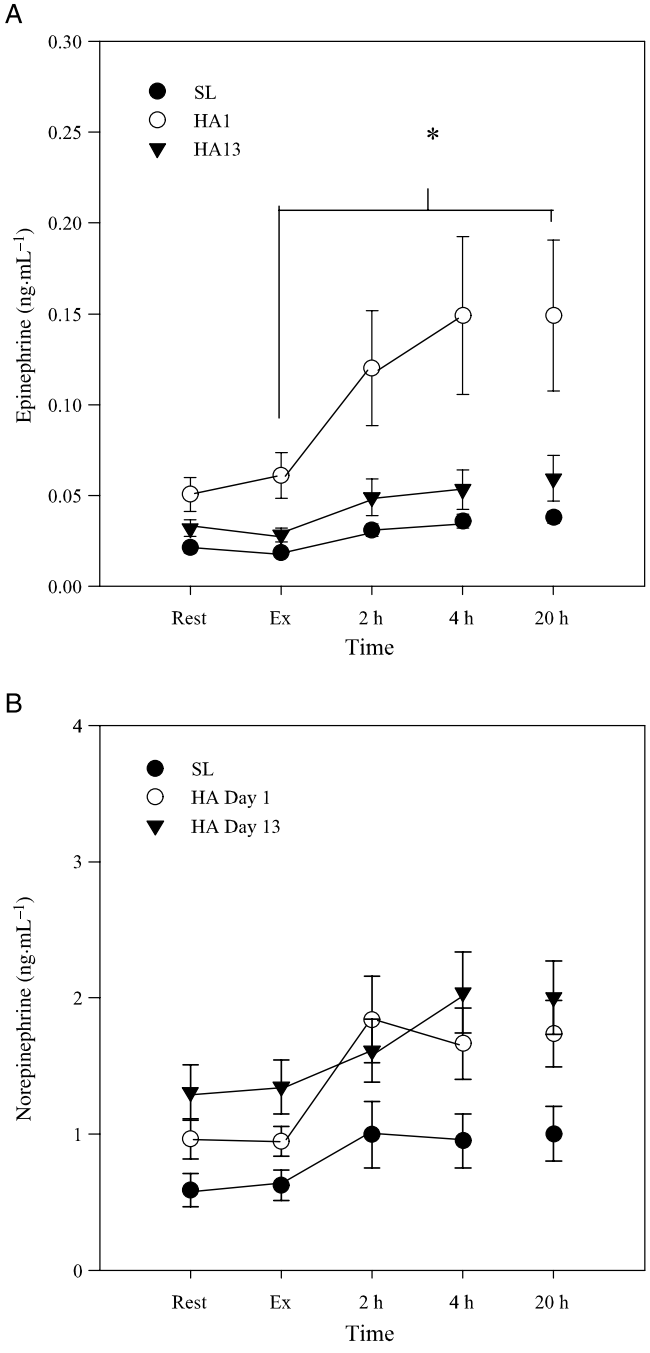

FIGURE 6—Plasma epinephrine (A) and norepinephrine (B) concentrations at rest, at the end of exercise (Ex), and after exercise (2, 4, and 20 h) for AO and PL groups (data combined). Values are mean (SEM). * HA day 1 significantly different than SL and HA day 13 ($P < 0.001$). AO, antioxidant supplement group; PL, placebo group, HA, high altitude; SL, sea level.

concentrations on HA1 at 20 h after exercise is a factor of the duration of altitude exposure rather than the SEC test, *per se*.

DISCUSSION

Military personnel or athletes working at high altitude may experience several stressors (e.g., hypoxia, exercise, energy deficit) that will initiate an acute phase response and contribute to altitude-associated illness (8,9,11,13), which may be detrimental to work capacity or performance over time. In the present investigation, we evaluated the efficacy of an antioxidant supplement in reducing the acute phase response associated with hypoxia, exercise, and caloric deficit at high-altitude exposure (4300-m elevation) by measuring changes in cytokines and CRP responses in subjects given a placebo or antioxidant supplement. We found that plasma IL-6 and CRP concentrations were elevated in response to exercise during acute high-altitude exposure compared with sea level, although plasma TNF-α concentrations were not elevated. The elevation in plasma IL-6 and CRP concentrations in response to exercise, however, did not persist at high altitude. In addition, resting plasma CRP returned to similar sea-level values during the 14-d stay at high altitude. Finally, in contrast to our primary hypothesis, antioxidant supplementation did not attenuate the rise in plasma IL-6 and CRP concentrations associated with hypoxia, exercise, and caloric deficit.

Antioxidant supplementation. Antioxidant supplementation in this investigation did not attenuate the rise in plasma IL-6 or CRP concentrations, which is in agreement with some studies performed at sea level and high altitude (2,17,21), but not others (23,27). A few explanations for the lack of effect of antioxidant supplementation in our data may exist. First, it has been hypothesized that an antioxidant supplement blunts the cytokine response through a ROS-dependent pathway as an increase in oxidative stress markers have been associated with elevated production of cytokines (27). We, however, previously reported that prolonged submaximal exercise at high altitude did not induce any significant increases in oxidative stress markers (i.e., lipid hydroperoxides, reduced glutathione, oxidized glutathione, and glutathione peroxidase) (26), even in the placebo group. The lack of ROS response suggests that either no additional ROS were generated or the endogenous production of antioxidants was sufficient at matching the generation of ROS. Because we saw an increase in cytokines without an elevation in ROS, the contribution of ROS to cytokine production in response to prolonged, submaximal exercise at high altitude may be minimal, with other factors (e.g., skeletal muscle contraction, depleted skeletal muscle glycogen stores, increased sympathetic activity) possibly playing a more significant role in elevating plasma cytokines. This would minimize the effects of antioxidant supplementation on cytokine production. Bailey et al. (2) also found a dissociation between markers of oxidative stress and plasma cytokines in volunteers ascending to 4780 m. Although we saw an elevation in plasma catecholamine concentrations at the end of exercise (+20%), we must note that the intensity of the SEC test (approximately 55% $\dot{V}O_{2peak}$) may not have been sufficiently strenuous to generate any ROS production, diminishing the effects of the antioxidant supplement. We chose the same relative exercise intensity at sea level and high altitude instead of the same absolute exercise intensity because, based on previous unpublished observations, the relative exercise intensity of approximately 55% of $\dot{V}O_{2peak}$ was considered an upper limit for

prolonged, steady state exercise at acute high-altitude exposure. Together, these contradictory studies suggest that the contribution of ROS to cytokine production remains to be determined both at sea level and high altitude.

Second, previous reports have shown a reduction in plasma cytokines with antioxidant supplementation in untrained, but not trained individuals (17,21,23,27). Indeed, resting antioxidant levels in endurance-trained individuals have been shown to be higher than in sedentary individuals (4), and antioxidant concentrations may stay elevated during and after an exercise bout in trained individuals (21). Therefore, the recreationally trained subjects in this investigation may have had a natural antioxidant defense system that was not further enhanced by supplementation. Direct comparison between trained and untrained individuals is difficult to interpret, however, because exercise duration and intensity have been different (17,27). To clarify this issue, future studies should directly compare the impact of antioxidant supplementation on cytokine production in trained and untrained subjects exercising at the same exercise intensity and duration.

Finally, research has shown that plasma cytokine concentrations are correlated with levels of plasma catecholamines. More specifically, IL-6 responses at rest and during exercise are dependent on a strong β-adrenergic component, and a β-adrenergic antagonist blocks the IL-6 response (19). Exercise combined with hypoxia further increases the epinephrine response (15), which may enhance the cytokine response at altitude. In this investigation, antioxidant supplementation may not have attenuated the rise in IL-6 and CRP because a strong adrenal release of epinephrine in response to hypoxia may have overridden the efficacy of the antioxidant supplement. In contrast, Lundby et al. (11) found that the catecholamine response to exercise at altitude did not significantly contribute to elevated plasma IL-6 levels. In this investigation, plasma catecholamine concentrations were not correlated with plasma cytokines. In summary, the results of this investigation are not consistent with the hypothesis that antioxidant supplementation attenuates the cytokine and CRP response associated with high-altitude exposure, prolonged submaximal exercise, and caloric deficit in recreationally trained subjects.

Cytokine response. Previous research at high altitude has shown that plasma IL-6 concentrations are elevated during exercise at the same absolute (same work rate), but not relative (same percentage of maximal work rate) exercise intensity compared with sea level (11,13). We, however, did show a twofold increase in plasma IL-6 on acute altitude exposure compared with sea level during and after exercise at the same relative workload (~55% of $\dot{V}O_{2peak}$) (Fig. 2). It may be that the increased duration of exercise in our study at high altitude (resulting in the same total energy flux) was sufficient to induce a greater response in plasma IL-6 despite the similar relative workloads. Indeed, a study by Lundby et al. (11) demonstrated, with similarly recreationally trained subjects working at a comparable work load as in our study, a peak exercise and altitude-induced increase in plasma IL-6 of only about 6 $pg \cdot mL^{-1}$ after 1 h of exercise compared with our observed increase of approximately 18 $pg \cdot mL^{-1}$ after 3 h, suggesting that duration of exercise may be important.

In contrast to the HA1 response, we did not show an increase in plasma IL-6 concentrations in response to exercise at HA13 compared with sea level (Fig. 2), which is in disagreement with others (11,13). Because the subjects in this investigation exercised a minimum of 4 $h \cdot d^{-1}$ for the entire duration at high altitude (~20% increase above sea-level energy expenditure), they may have adapted to the elevated energy expenditure, thereby blunting the plasma IL-6 response during the 3-h exercise test at HA13. Subjects completed the prolonged exercise test more easily and expressed feelings of less weakness and fatigue at HA13 compared with HA1, despite no accompanying increase in $\dot{V}O_{2peak}$ over the 14-d stay at altitude (subjects were still working at the same relative intensity). In addition, subjects displayed higher oxygen saturation during the SEC test at HA13 compared with HA1 (83 vs 73%, respectively; $P < 0.05$), suggesting that acclimatization had occurred during the 14-d stay at high altitude. In summary, our results suggest that acclimatization over a 14-d stay at high altitude, combined with elevated energy expenditure, attenuates the rise in plasma IL-6 concentrations in response to exercise initially observed on HA1.

To our knowledge, this is the first investigation to evaluate the plasma TNF-α response to a controlled bout of exercise at high altitude. Because TNF-α is elevated after a prolonged and intense exercise bout (e.g., marathon race) at sea level (18), we predicted TNF-α would be elevated after exercise at high altitude. In contrast to our hypothesis, plasma TNF-α did not differ at either sea level or high altitude. Both animal and human studies suggest that circulating levels of IL-6 inhibit TNF-α production. Mizuhara et al. (16) demonstrated that rIL-6 administration in mice reduces TNF-α production by 40–50%. In humans, Starkie et al. (25) demonstrated that both exercise and infusion of rhIL-6 blunts circulating plasma levels of TNF-α. In addition, Pedersen et al. (20) proposed that exercise-induced elevations in TNF-α are caused by endotoxemia only after extreme exercise (e.g., marathon race), but other modes of aerobic exercise do not elevate plasma TNF-α. Evidence seems to support this theory as most, but not all, have shown increases after a marathon race, but not more modest exercise (7,18). The lack of change in plasma TNF-α concentration in this investigation at both sea level and high altitude supports the theory that prolonged cycle ergometer exercise is not a sufficient stressor to elevate plasma TNF-α.

C-reactive protein response. Plasma concentration of CRP, an acute-phase protein largely regulated by IL-6, is augmented after exercise at sea level (3). Most studies at high altitude have focused on the CRP response to acute hypoxia (8) and not the combined effects of strenuous exercise and hypoxia. We found that the plasma CRP

response after exercise at HA1 was elevated compared with SL and HA13. It is possible that this elevation is related to the increased plasma IL-6 response and heightened sympathetic activity (Figs. 3 and 4). Research has consistently shown that a rise in plasma IL-6 induces an elevation in the hepatic-derived CRP 3–20 h after exercise (24). Furthermore, the significant correlation in this investigation between plasma norepinephrine and CRP concentrations (Fig. 3) on HA1 at 20 h after exercise suggests a potential link between increased sympathetic activity and a rise in plasma CRP at high altitude. Plasma norepinephrine is a good marker of increased sympathetic activity (14,15), which can remain elevated for up to 14 d of high-altitude exposure (15). We also saw elevated resting norepinephrine concentrations throughout the 14-d stay at high altitude, but no correlation was found between plasma norepinephrine and plasma CRP concentrations during the latter days of altitude exposure.

High-volume exercise training, with insufficient rest, will initiate an acute phase response and is thought to continuously increase resting CRP levels (24); however, research supporting this theory is minimal. In this investigation, the resting plasma CRP response was augmented at high altitude (Fig. 5). Subjects were asked to increase their energy expenditure by approximately 1200–1500 $kcal \cdot d^{-1}$ at high altitude, but we were only partially successful because energy expenditure increased approximately 750 $kcal \cdot d^{-1}$ and energy intake decreased approximately 550 $kcal \cdot d^{-1}$, resulting in an approximately 4.3-kg loss of body mass over a 2-wk period. Nonetheless, the resting plasma CRP response at HA10 (Fig. 5) and HA14 (Fig. 2) returning to similar sea-level values was surprising and contradicted our hypothesis. Our results are consistent with research at sea level, which has shown that after a period of increased training intensity and volume, plasma CRP was either reduced or returned to baseline values (12).

Methodological consideration. In the current investigation, because we altered energy intake and expenditure to induce a total energy deficit of approximately 1500 $kcal \cdot d^{-1}$ at high altitude and not at sea level, it is difficult to determine the altitude influence, *per se*, on the cytokine and CRP responses. We chose to mimic conditions that military personnel or athletes may experience at high altitude during intense physical work periods (e.g., hypoxia, elevated energy expenditure, energy deficit), with the idea that an antioxidant supplement might attenuate the acute phase response commonly seen with these conditions at high altitude. If the goal is to tease out the independent effects of hypoxia, elevated energy expenditure, and decreased energy intake, a more appropriate study design should include a sea-level energy deficit period that would be repeated at high altitude or a nonenergy deficit group at high altitude.

In conclusion, we have demonstrated that plasma IL-6 and CRP concentrations are elevated at acute high-altitude exposure compared with both sea-level and chronic high-altitude exposure in response to exercise at the same relative intensity. Results from this investigation, however, do not support the hypothesis that antioxidant supplementation attenuates the plasma cytokine and CRP response associated with high-altitude exposure, elevated energy expenditure, and decreased energy intake. Future research should focus on determining the independent effects of elevated energy expenditure and decreased caloric intake at high altitude on the acute phase response.

This investigation was supported by the Departments of Veterans Affairs and Defense. Results of the current study do not constitute endorsement of antioxidant supplementation by the authors or ACSM. We would like to thank all the volunteers for their time and participation in this investigation. We would like to thank George A. Brooks for his consultation and help in all aspects of the study. We are grateful to Hamdee Attallah for performing all the medical evaluations, Sharon Moynihan for developing and administering the diets, Kimberly Stone for her help with all aspects of the study, and the nursing staff in the Clinical Studies Unit at the Veterans Affairs Palo Alto Health Care System. We would like to thank the following individuals for their technical assistance: Beth Beidleman, Ph.D., SGT Tommy J. Bruington, SSG Dan T. Ditzler, Vincent A. Forte, Erik Lammi, Sharon K. Lesher, SPC Mona M. Mathow, SPC Jack E. Mazzotti, SGT Dennis M. Rufolo, Tracey J. Smith, Robert Soares, Janet E. Staab, SGT Stephen M. W, and Frank Zirpolo.

REFERENCES

1. Ainsworth, B. E., W. L. Haskell, A. S. Leon, et al. Compendium of physical activities: classification of energy costs of human physical activities. *Med. Sci. Sports Exerc.* 25:71–80, 1993.
2. Bailey, D. M., P. N. Ainslie, S. K. Jackson, R. S. Richardson, and M. Ghatei. Evidence against redox regulation of energy homoeostasis in humans at high altitude. *Clin. Sci. (Lond).* 107:589–600, 2004.
3. Castell, L. M., J. R. Poortmans, R. Leclercq, M. Brasseur, J. Duchateau, and E. A. Newsholme. Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. *Eur. J. Appl. Physiol. Occup. Physiol.* 75:47–53, 1997.
4. Child, R. B., D. M. Wilkinson, and J. L. Fallowfield. Resting serum antioxidant status is positively correlated with peak oxygen uptake in endurance trained runners. *J. Sports Med. Phys. Fitness.* 39:282–284, 1999.
5. Friedl K. E., and R. W. Hoyt. Development and biomedical testing of military operational rations. *Annu. Rev. Nutr.* 17:51–75, 1997.
6. Fulco, C. S., A. L. Friedlander, S. R. Muza, et al. Energy intake deficit and physical performance at altitude. *Aviat. Space Environ. Med.* 73:758–765, 2002.
7. Gannon, G. A., S. G. Rhind, M. Suzui, P. N. Shek, and R. J. Shephard. Circulating levels of peripheral blood leucocytes and cytokines following competitive cycling. *Can. J. Appl. Physiol.* 22:133–147, 1997.
8. Hartmann, G., M. Tschop, R. Fischer, et al. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and c-reactive protein. *Cytokine* 12:246–252, 2000.
9. Klausen, T., N. V. Olsen, T. D. Poulsen, J. P. Richalet, and B. K. Pedersen. Hypoxemia increases serum interleukin-6 in humans. *Eur. J. Appl. Physiol.* 76:480–482, 1997.

10. Kosmidou, I., T. Vassilakopoulos, A. Xagorari, S. Zakynthinos, D. A. Papanicolaou, and C. Roussos. Production of interleukin-6 by skeletal myotubes: role of reactive oxygen species. *Am. J. Respir. Cell. Mol. Biol.* 26:587–593, 2002.
11. Lundby C., and A. Steensberg. Interleukin-6 response to exercise during acute and chronic hypoxia. *Eur. J. Appl. Physiol.* 91: 88–93, 2004.
12. Mattush, F., B. Dufaux, O. Heine, I. Mertens, and R. Rost. Reduction of the plasma concentration of c-reactive protein following nine months of endurance training. *Int. J. Sports Med.* 20:21–24, 1999.
13. Mazzeo, R. S., D. Donovan, M. Fleshner, et al. Interleukin-6 response to exercise and high-altitude exposure: influence of alpha-adrenergic blockade. *J. Appl. Physiol.* 91:2143–2149, 2001.
14. Mazzeo, R. S., A. Dubay, J. Kirsch, et al. Influence of alpha-adrenergic blockade on the catecholamine response to exercise at 4,300 meters. *Metabolism* 52:1471–1477, 2003.
15. Mazzeo R. S., and J. T. Reeves. Adrenergic contribution during acclimatization to high altitude: perspectives from Pikes Peak. *Exerc. Sport Sci. Rev.* 31:13–18, 2003.
16. Mizuhara, H., E. O'Neill, N. Seki, et al. T cell activation-associated hepatic injury: mediation by tumor necrosis factors and protection by interleukin 6. *J. Exp. Med.* 179:1529–1537, 1994.
17. Nieman, D. C., D. A. Henson, S. R. McAnulty, et al. Vitamin E and immunity after the Kona Triathlon World Championship. *Med. Sci. Sports Exerc.* 36:1328–1335, 2004.
18. Ostrowski, K., T. Rohde, S. Asp, P. Schjerling, and B. K. Pedersen. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J. Physiol.* 515(Pt 1):287–291, 1999.
19. Papanicolaou, D. A., J. S. Petrides, C. Tsigos, et al. Exercise stimulates interleukin-6 secretion: inhibition by glucocorticoids and correlation with catecholamines. *Am. J. Physiol. Endocrinol. Metab.* 271:E601–E605, 1996.
20. Pedersen, B. K., A. Steensberg, P. Keller, et al. Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. *Eur. J. Appl. Physiol.* 446:9–16, 2003.
21. Petersen, E. W., K. Ostrowski, T. Ibfelt, et al. Effect of vitamin supplementation on cytokine response and on muscle damage after strenuous exercise. *Am. J. Physiol. Cell. Physiol.* 280: C1570–C1575, 2001.
22. Pfeiffer, J. M., E. W. Askew, D. E. Roberts, et al. Effect of antioxidant supplementation on urine and blood markers of oxidative stress during extended moderate-altitude training. *Wilderness Environ. Med.* 10:66–74, 1999.
23. Phillips, T., A. C. Childs, D. M. Dreon, S. Phinney, and C. Leeuwenburgh. A dietary supplement attenuates IL-6 and CRP after eccentric exercise in untrained males. *Med. Sci. Sports Exerc.* 35:2032–2037, 2003.
24. Smith, L. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med. Sci. Sports Exerc.* 32: 317–331, 2000.
25. Starkie, R. L., S. R. Ostrowski, S. Jauffred, M. A. Febbraio, and B. K. Pedersen. Exercise and IL-6 infusion inhibit endotoxin-induced TNFa production in humans. *FASESB J.* 17:884–886, 2003.
26. Subudhi, A. W., K. A. Jacobs, T. A. Hagobian, et al. Antioxidant supplementation does not attenuate oxidative stress at high altitude. *Aviat. Space Environ. Med.* 75:881–888, 2004.
27. Vassilakopoulos, T., M. A. Karatza, P. Katsaounou, A. Kollintza, S. Zakynthinos, and C. Roussos. Antioxidants attenuate the plasma cytokine response to exercise in humans. *J. Appl. Physiol.* 94:1025–1032, 2003.