Adaptation to altitude as a vehicle for experiential learning of physiology by university undergraduates

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Weigle DS, Buben A, Burke CC, Carroll ND, Cook BM, Davis BS, Dubowitz G, Fisher RE, Freeman TC, Gibbons SM, Hansen HA, Heys KA, Hopkins B, Jordan BL, McElwain KL, Powell FL, Reinhart KE, Robbins CD, Summers CC, Walker JD, Weber SS, Weinheimer CJ. Adaptation to altitude as a vehicle for experiential learning of physiology by university undergraduates. Adv Physiol Educ 31: 270–278, 2007; doi:10.1152/advan.00122.2006.—In this article, an experiential learning activity is described in which 19 university undergraduates made experimental observations on each other to explore physiological adaptations to high altitude. Following 2 wk of didactic sessions and baseline data collection at sea level, the group ascended to a research station at 12,500-ft elevation. Here, teams of three to four students measured the maximal rate of oxygen uptake, cognitive function, hand and foot volume changes, reticulocyte count and hematocrit, urinary pH and 24-h urine volume, athletic performance, and nocturnal blood oxygen saturation. Their data allowed the students to quantify the effect of altitude on the oxygen cascade and to demonstrate the following altitude-related changes: 1) impaired performance on selected cognitive function tests, 2) mild peripheral edema, 3) rapid reticulocytosis, 4) urinary alkalization and diuresis, 5) impaired aerobic but not anaerobic exercise performance, 6) inverse relationship between blood oxygen saturation and resting heart rate, and 7) regular periodic nocturnal oxygen desaturation events accompanied by heart rate accelerations. The authors learned and applied basic statistical techniques to analyze their data, and each team summarized its results in the format of a scientific paper. The students were uniformly enthusiastic about the use of self-directed experimentation to explore the physiology of altitude adaptation and felt that they learned more from this course format than from a control group of students felt that they learned from a physiology course taught by the same instructor in the standard classroom/laboratory format.

Excitement fosters the learning of any discipline, and physiology is no exception. Many undergraduate physiology courses include a laboratory component designed to engage and excite students by allowing them to observe responses of living organisms to experimental manipulations. The most effective laboratory exercises permit students to choose the goal of their investigations and play a role in the design of their experiments. However, open-ended projects of this nature are challenging to develop, particularly if the intent is to illustrate principles of human physiology. Here, we describe a month-long laboratory exercise in which excitement was generated by allowing students to be both the experimental subjects and the investigators in a study of the effects of high altitude on multiple organ systems. A critical component of the experience was allowing the students to freely join teams, each of which performed a specific inquiry-driven investigation. It would be possible to adapt our approach for any university curriculum offering a 3- to 4-wk block of unstructured time for experiential learning activities.

Our activity, entitled “Your Body at 12,000 Feet: What Can Altitude Teach Us About Human Physiology?”, was offered as a tuition-supported University of Washington Exploration Seminar from August 21 to September 15, 2006. Nineteen students, representing a variety of undergraduate majors and career plans, participated in the activity. Although most students had previously taken an introductory physiology course, no specific preparation was required or assumed. The goals of the activity were fourfold: 1) to provide a necessary background in cardiopulmonary, neurological, and renal physiology through didactic lectures and student-led discussions; 2) to gain experience with the experimental techniques required to perform student-generated investigations; 3) to learn the basic statistical techniques required to analyze experimental data; and 4) to write up the results of each team’s investigation in the format of a scientific paper.

The first two goals were accomplished in Seattle, WA, during the first 2 wk of the course. Student engagement in the didactic portion of the course was enhanced by guest lectures from an internationally known mountaineer and two high-altitude researchers with experience in the Himalayas and the Alps. During this time, each student underwent maximal treadmill exercise testing and body composition analysis. The results of these tests, as well as the tests devised by each student team, provided the baseline sea level data against which the effects of altitude were assessed.

Weeks 3 and 4 of the course were spent at the White Mountain Research Station (WMRS) in Bishop, CA. This facility, run by the University of California, is available to university groups for research and teaching purposes at a reasonable cost. WMRS maintains laboratory space at elevations of 4,000, 10,200, 12,500, and 14,250 ft. The clear-cut
Table 1. Student characteristics

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23.0 (3.0)</td>
<td>21.4 (0.9)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>182 (7)</td>
<td>169 (10)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>86.1 (19.3)</td>
<td>63.1 (7.3)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.9 (5.4)</td>
<td>22.1 (1.6)</td>
</tr>
<tr>
<td>Fat, %</td>
<td>21.2 (11.1)</td>
<td>29.5 (5.0)</td>
</tr>
</tbody>
</table>

Data are expressed as means (SD); n, no. of students.

Symptoms of hypoxia experienced by all participants at the higher elevations greatly enhanced the impact of the course and provided numerous teaching opportunities. For example, students were intrigued by the realization that the impairment they felt at a mean arterial oxygen saturation (SO₂) of 88% on the mountain was comparable with what an emphysema patient with the same saturation would feel on a daily basis at sea level.

This article, coauthored by all student participants, provides a complete description of the course structure, student investigations, and outcomes. Data are taken directly from the papers written by students as their final course activity. A student opinion survey performed at the end of the course indicated that students felt they learned more than other groups of students felt they learned from a physiology course taught by the same instructor in the standard classroom/laboratory format. Additional indicators of program success included the desire of students to publish their work in a peer-reviewed journal and the presentation of an abstract describing their work at the 15th International Hypoxia Symposium in 2007.

MATERIALS AND METHODS

Participants

Nineteen junior- and senior-level university students enrolled in this graded five-credit course in response to advertisements for the University of Washington Exploration Seminars program and invitations by the course director. An early organizational meeting and precourse communications with the students emphasized the importance of intellectual rigor, teamwork, and commitment (the “expedition attitude”) to the success of the course. The only requirements for participation were a normal medical evaluation performed by a licensed physician and an active health insurance policy. Students represented a wide range of academic preparation, body mass index, athletic experience, fitness level, and prior exposure to high altitude.

Student anthropometric data are summarized in Table 1. All data were recorded using a three-digit code rather than student names to preserve confidentiality. Students provided informed written consent for publication of data resulting from their participation, and all activities were approved by the University of Washington Risk Management and Human Subjects Offices.

Course Structure

Week 1. The first week of the course was intended to provide students with the basic information needed to design projects to investigate adaptation to altitude and to perform reference sea level measurements of fitness and body composition. Mornings were devoted to lectures by the course director, presentations by students working in groups of two to four, and guest lectures. During one morning session, students participated in a demonstration of the acute poikilocapnic hypoxic ventilatory response (HVR), as described below. The major topics covered during these didactic sessions are summarized in Table 2. Afternoons were spent in an exercise physiology laboratory, where groups of 3–4 students/day underwent body composition analysis by dual-energy X-ray absorptiometry scanning (DXA) and performed maximal treadmill exercise testing with indirect calorimetry. Students alternately acted as subjects by running on the treadmill, investigators by operating the equipment, and coaches by encouraging maximal effort from their peers.

Week 2. The didactic portion of the course was completed during week 2, and student learning was verified by means of a written test that included questions about the quantitative effect of acute hypoxia on arterial PO₂ and hemoglobin saturation, ventilatory drives, role of carbonic anhydrase in the kidney, high-altitude pulmonary edema, ventilatory and metabolic pH changes, HVR, and interpretation of maximal exercise testing data. Students then selected which of five investigational teams, as shown in Table 3, they wanted to join. Each team either developed de novo, or received instruction in, the techniques required to perform its specific investigation (Table 3). Using these techniques, the teams collected baseline sea level data on all students over the course of 2 days. Additional baseline data included heart rate and respiratory rate upon awakening, fasting body weight, blood pressure, SO₂, and the Lake Louise Consensus acute mountain sickness score (7).

Weeks 3 and 4. Students traveled to the Owens Valley Laboratory at 4,000-ft elevation for the first of 11 consecutive days of fieldwork at WMR. Maximal exercise testing was repeated here using a bicycle ergometer and gas analysis equipment available for subsequent transport to the Barcroft Laboratory at 12,500-ft elevation. Time at Owens Valley was also used to introduce students to basic statistical methods and the software required to analyze their data. The elevation profile of all fieldwork days is shown in Table 4. During day 3, the group rapidly ascended to 12,500 ft for measurements of acute altitude-induced changes in heart rate, respiratory rate, blood pressure, SO₂,

Table 2. Topics covered during week 1 and 2 didactic sessions

<table>
<thead>
<tr>
<th>Lectures by the Course Director</th>
<th>Student Presentations</th>
<th>Guest Lectures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen transport from atmosphere to the mitochondria and assessment by VO₂ max</td>
<td>Molecular mechanisms of hypoxia sensing</td>
<td>Life of a mountain guide (Lou Whittaker, Director, Rainier Mountaineering)</td>
</tr>
<tr>
<td>Red blood cell production and allosterism of the hemoglobin molecule</td>
<td>Fuel utilization during exercise</td>
<td>High-altitude pulmonary edema (Dr. Erik Swenson, University of Washington)</td>
</tr>
<tr>
<td>Control of alveolar ventilation (including a demonstration of the acute poikilocapnic hypoxic ventilatory response)</td>
<td>Physiological adaptations of the best human and animal athletes</td>
<td>Altitude-induced sleep and cognitive disturbances (Dr. Gerald Dubowicz, University of California-San Francisco)</td>
</tr>
<tr>
<td>Acid-base physiology</td>
<td>High-altitude training to enhance athletic performance</td>
<td></td>
</tr>
<tr>
<td>Physiological adaptations to altitude</td>
<td>Acute mountain illnesses</td>
<td></td>
</tr>
<tr>
<td>Hypoxic pulmonary vasoconstriction and the pathophysiology of altitude</td>
<td>The American Medical Research Expedition to Everest and Operation Everest II</td>
<td></td>
</tr>
</tbody>
</table>

VO₂ max, maximum rate of oxygen consumption.
and cognitive function. Students then descended to the Crooked Creek Laboratory at 10,200-ft elevation to spend the night and allow some time for acclimatization. Days 4–7 were devoted to team-specific measurements and maximal exercise testing on the bicycle ergometer at 12,500-ft elevation. Days 8 and 9 were recreational days during which 12 students spent the night at 14,250-ft elevation in the Summit Laboratory. Final team-specific measurements reflecting a full week of acclimatization were made on day 10, and students finished writing the papers describing their work on day 11.

Technical Methods

Maximal exercise testing. In Seattle, the maximal oxygen consumption rate (VO₂ max) was measured using a Quinton Q4500 treadmill and electrocardiographic monitor (Quinton, Bothell, WA). Subjects breathed room air via mouthpiece into a two-way nonrebreathing valve (Hans Rudolf, Kansas City, MO), and fractional percentages of exhaled oxygen and carbon dioxide were measured with a TrueOne 2400 metabolic cart (Parvomedics, Salt Lake City, UT). The metabolic cart was calibrated daily using a 3.0-liter gas syringe and standardized gas mixture. Subjects began the exercise protocol at a walking pace of 3.5 mile/h, with an increase of 0.5–1.0 mile/h every 2 min until a prespecified maximum pace was reached. Every 2 min thereafter, the treadmill incline was increased by 4%. Subjects exercised to exhaustion, the point at which VO₂ max was taken. At WMRS, exercise took place on a frictionally braked Schwinn stationary bicycle ergometer (Pacific Cycle, Madison, WI), on which subjects cycled at an individually chosen constant cadence with an increase in resistance every 2 min. Subjects breathed room air via mouthpiece into a nonrebreathing valve, and mixed expired gas was collected into a latex weather balloon for 15 s at the end of every stage of exercise. Gas was expelled into a Harvard Dry Gas Meter (Harvard Apparatus, Hollister, MA) to measure gas volume, and fractional oxygen and carbon dioxide content were measured with a Beckman OM-11 gas analyzer (Beckman Coulter, Fullerton, CA) and a Sensormedics LB-2 Medical Gas Analyzer (Siemens Healthcare, Conshohocken, PA), respectively. These instruments were calibrated daily using a 3.0-liter gas syringe and standardized gas mixture. Calculated flow rate was converted from water-saturated atmospheric temperature and pressure to water-saturated body temperature and pressure using standardized conversion factors, and VO₂ was calculated using the Fick equation. The 15-s gas sample collected during the exercise stage at which exhaustion occurred was used to calculate VO₂ max.

Body composition analysis. The fat, nonfat, and mineral mass of subjects were measured using a fan-beam Lunar Prodigy DXA scanner (General Electric Healthcare, Waukesha, WI). A trained clinical DXA technician performed all measurements at the University of Washington General Clinical Research Center.

Poikilocapnic HVR. The baseline ventilatory rate (VE; in l/min) was calculated while breathing room air for 10 min by having subjects exhale through a nonrebreathing valve into a Wright respirometer (Grace Medical, Kennesaw, GA). Subjects then breathed a 12% oxygen mixture for 10 min, and VE was recalculated. So₂ was measured continuously using a pulse oximeter, and the mean So₂ during the last 3 min was calculated for both conditions. Poikilocapnic HVR (in l·min⁻¹·%⁻¹) was calculated as the change in Ve (12% oxygen minus room air) divided by the change in So₂ (room air minus 12% oxygen).

Continuous pulse oximetry. Measurements of heart rate and blood So₂ levels were taken every 2 s throughout the night using a WristOx 3100 (Nonin Medical, Plymouth, MN). This is a small, lightweight pulse oximeter and fingertip sensor designed to be worn comfortably on the subject’s wrist while sleeping. The accompanying nVision Data Management software was used to download and interpret readings obtained each night.

Cognitive function tests. Students chose four tests to assess cognitive function at altitude. In the first test of visual motor reaction time, subjects were instructed to grasp a plastic 12-in. ruler as quickly as possible after it was released between the subject’s thumb and index finger, which were separated by a standardized distance (10). The edge of the ruler was held at the top of the fingers and released at the investigator’s discretion. Mean reaction acuity was measured over three repetitions as the distance the ruler dropped to the top of the subject’s thumb. This distance was then converted into a corresponding reaction time using the following formula: $d = \frac{1}{2}gt^2$, where $d$ is the distance the ruler dropped, $g$ is the gravitational constant, and $t$ is time (10). The second test was the Stroop color-word test, a classic measure of selective attention that requires the subject to first read aloud color names printed in matching ink color and then name the ink color in which incongruent color names are presented (11). This task requires selective processing of visual stimuli in the presence of distracters through active inhibition of automatic responses. The third test measured reasoning ability as the latency and accuracy of subject responses to a verbally presented problem set consisting of eight questions (9). The questions tested simple mathematical, spatial, and verbal analytic skills. Subjects randomly chose one of six different problem sets during each testing period to prevent the memorization of correct responses. The fourth test used sentence repetition to assess memory (9). In this test, the investigator dictated a multiphase sentence to the subject, who was then instructed to repeat it as quickly and accurately as possible. Five sentences, increasing in length, complexity, and overall memory load, were presented. Again, multiple sentence sets were employed to prevent the memorization of correct responses. Both response time and number of errors were used to quantify performance on the Stroop test, the verbal reasoning test, and the sentence repetition test.

Table 3. Student investigational teams

<table>
<thead>
<tr>
<th>Team No.</th>
<th>Area of Investigation</th>
<th>Techniques Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of altitude on cognitive function</td>
<td>Four student-selected psychometric tests</td>
</tr>
<tr>
<td>2</td>
<td>Effect of altitude on extremity volume</td>
<td>Water displacement plethysmography</td>
</tr>
<tr>
<td>3</td>
<td>Hematological adaptation to altitude</td>
<td>Measurement of hematocrit and reticulocyte counts</td>
</tr>
<tr>
<td>4</td>
<td>Renal compensation for respiratory alkalosis and altitude-induced diuresis</td>
<td>Measurement of 24-h urine volume, specific gravity, and pH</td>
</tr>
<tr>
<td>5</td>
<td>Effect of altitude on athletic performance</td>
<td>Four student-selected field exercise tests</td>
</tr>
</tbody>
</table>

Table 4. Elevation profile of week 3 and 4 fieldwork days

<table>
<thead>
<tr>
<th>Day No.</th>
<th>WMRS Facility</th>
<th>Overnight Elevation, ft</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2</td>
<td>Owens Valley Laboratory</td>
<td>4,000</td>
</tr>
<tr>
<td>3</td>
<td>Crooked Creek Laboratory*</td>
<td>10,200</td>
</tr>
<tr>
<td>4–7</td>
<td>Barcroft Laboratory</td>
<td>12,500</td>
</tr>
<tr>
<td>8</td>
<td>Summit Laboratory</td>
<td>14,250</td>
</tr>
<tr>
<td>9–10</td>
<td>Barcroft Laboratory</td>
<td>12,500</td>
</tr>
<tr>
<td>11</td>
<td>Owens Valley Laboratory</td>
<td>4,000</td>
</tr>
<tr>
<td>12</td>
<td>Return to Seattle</td>
<td></td>
</tr>
</tbody>
</table>

WMRS, White Mountain Research Station. *Two hours were spent on day 3 at Barcroft Laboratory to measure vital signs and administer cognitive function tests prior to descending to Crooked Creek Laboratory.
Plethysmography. Hand and foot volumes were measured by water displacement plethysmography (2, 3) using a low-cost apparatus designed and validated by the students. For each extremity, a polyurethane tub of appropriate size was prepared with a water-tight aperture at a nominal height of 20 cm from the base of the container. A short section of 3/4-in. Proline tubing was attached to the aperture fitting to direct displaced water into a beaker for collection. Tubs were placed on elevated platforms and filled to the point of overflow with water at a temperature of 20–22°C measured by thermometer on the morning of the testing session. Measurements were made after all water motion and drainage from the outflow tube had ceased. For the measurement of hand volume, the subject’s right hand was slowly inserted into the water with the forearm vertical. The hand was lowered until the tip of the middle finger came into light contact with the bottom of the tub without supporting any of the arm weight to ensure reproducible placement and pressure. For the measurement of foot volume, the subject’s right foot was lowered slowly into the filled tub. The subject then stood, supporting full body weight on the foot to assure reproducible placement and pressure. For both hand and foot measurements, displaced water was collected until the wave motion of the water in the tank stopped and dripping from the outflow tube ceased. The displaced water was then transferred into graduated cylinders of the appropriate size for accurate volume measurement. All measurements were made during the morning prior to students undertaking any significant physical activity.

Hematological measurements. Hematocrit was measured using capillary blood obtained from a clean warmed finger by means of a 2.25-mm 23-gauge spring-loaded lancet (no. 366583, Becton Dickenson, Franklin Lakes, NJ). Freely flowing blood was collected into an EDTA-coated Microtainer tube (no. 365974, Becton Dickenson) to prevent clotting. Approximately 50 μl blood was drawn from the Microtainer tube into a microhematocrit capillary tube (no. 22-362-566, Thermo Fisher Scientific, Pittsburgh, PA), which was sealed with Crites sealant (no. 02-676-20, Thermofisher) and spun for 5 min in a capillary microcentrifuge. Hematocrit was determined using a standard capillary tube reading card. A reticulocyte count was performed by mixing the blood remaining in the Microtainer tube with 2–3 volumes of methylene blue stain and incubating at room temperature for 10 min. The methylene blue stain was prepared by dissolving 0.5 g of new methylene blue and 1.2 g of oxalic acid in 100 ml of water and filtering the solution at a pore size of 0.45 μm. Reticulocytes were visualized using a ×100 oil-immersion objective. Two observers determined the percentage of reticulocytes in 500–600 red blood cells each. If these percentages agreed within a factor of two, they were averaged to give a final reticulocyte count. If the percentages did not agree within a factor of two, a third count of 500–600 cells was made, and the two percentages agreeing most closely were averaged to give a final reticulocyte count.

Urine measurements. Urine volume over 24 h was measured in 3-liter calibrated jugs by collecting all urine produced after the morning void at the start of the 24-h period through the morning void at the end of the 24-h period. Urine collection by female participants was facilitated by using Speci-Pan urine collection toilet inserts with pour spouts (Medline Industries, Mundelein, IL). The second morning void was collected separately to allow pH measurement on the fresh specimen using an Oakton double-junction pH meter (no. S65289, Thermo Fisher). Following pH measurements, the second morning void was added to the collection jug for volume determination and specific gravity measurement of the mixed 24-h sample using a Midget Urinometer (no. 22-274209, Thermofisher).

Field tests of athletic performance. Four field tests were chosen by the students to measure the impact of altitude on athletic performance: maximal number of pushups (not timed), maximal number of jumping jacks in 1 min, the faster of two timed 50-m sprints, and a timed 800-m run. Subjects were allowed to rest between activities until they felt they could give a maximum effort during the next test. Times were recorded using stopwatches accurate to 0.01 s, and distances for the running tests were measured using a 12-in. surveyor’s wheel (no. 77-194, Stanley Works, New Britain, CT). The running surfaces in Seattle and at the Barcroft Laboratory were gravel roads with minimal grade.

Statistical Methods

Students were given basic instruction in descriptive statistics, the use of histograms to examine the spread of data and identify outliers, normalization of data for differences in body size, univariate regression to examine relationships between two variables, two-group hypothesis testing, and ANOVA for comparisons among more than two groups. The greater power of within-subject than between-subject study design and the appropriate use of paired and unpaired t-tests were stressed. JMP version 6.0 statistical software (SAS Institute, Cary, NC) was chosen for this course due to its ease of use, quality of graphics, and compatibility with commonly used spreadsheets.

RESULTS

The course started with a discussion of gas partial pressure and the multiple steps by which oxygen molecules in the atmosphere ultimately reach the mitochondria of cells (the oxygen cascade). It was stressed that the driving force for the oxygen cascade is the gradient between atmospheric and tissue PO2 and that altitude ultimately affects human performance by diminishing this gradient. Measurement of VO2max was introduced as a method to assess fitness at sea level and to quantify the impact of altitude on the oxygen cascade. Students found that their VO2max was 45.8 (9.0) ml·kg−1·min−1 [mean (SD), n = 17] using a maximal treadmill exercise protocol and metabolic cart in a well-standardized research laboratory in Seattle. In addition to providing reference sea level data for subsequent analyses, this activity taught students how to operate clinical exercise testing equipment and introduced the concepts of respiratory quotient and anaerobic threshold. In examining histograms of the VO2max data, students noticed that several females and heavier individuals were outliers with very low values. This prompted a discussion of how to normalize physiological data to account for differences in body size and composition. Students found that normalizing VO2max data for kilograms of lean body mass (determined by DXA scanning) rather than total body mass eliminated the outliers and produced a tighter data set [VO2max = 63.0 (7.6) ml·kg (lean)−1·min−1, mean (SD), n = 17].

Because the treadmill and metabolic cart could not be transported to WMRS, it was necessary for the students to learn how to perform and validate a field test of VO2max. This was accomplished at the Owens Valley Laboratory using a bicycle ergometer as described in MATERIALS AND METHODS. Students reasoned that although VO2max measurements made at Owens Valley would not be identical to Seattle measurements due to technical differences and the 4,000-ft elevation difference, the two data sets should bear a consistent relationship to one another. Regression analysis supported this assumption with an R2 value of 0.87 for the comparison of the two techniques (P < 0.001). Thus validated, bicycle ergometry revealed the mean VO2max of the group at 4,000 ft to be 4.11 (0.24) l/min and at 12,500 ft to be 3.31 (0.18) l/min [mean (SE), n = 19 in both locations, P < 0.0001; Fig. 1]. This 19% drop in VO2max nicely reflected the effect of altitude on the oxygen cascade and helped the students to understand their breathlessness with even minimal exertion at the Barcroft Laboratory.
Another topic that lent itself to experimental demonstration was the role of HVR (measured with the poikilocapnic technique) in adapting to altitudes that cause the arterial PO2 to fall below ~60 mmHg. Breathing a 12% oxygen mixture was sufficient to elicit this degree of hypoxemia at sea level and produce an increase in VO2 that was easily measured, as described in MATERIALS AND METHODS. The median acute poikilocapnic HVR measured in seven students (2 men and 5 women) was 1.4 (range: 0.1–8.4) 1·min⁻¹·%⁻¹. This wide range agreed with the reported variability in acute poikilocapnic HVR, which is felt to have a strong genetic component (8). The students were intrigued by the observation that the two subjects with the lowest values of poikilocapnic HVR were competitive swimmers. This led to an interesting discussion about whether a genetically low HVR could favor swimming as an athletic event for some individuals or whether HVR could be altered by specific types of training.

The centerpiece of the course was the inquiry-driven investigation performed by each student team. Some of these investigations addressed questions that the students generated themselves based on their limited familiarity with the literature. Other investigations were designed to demonstrate physiological phenomena previously described to occur at altitude. Each team had to choose, develop, or learn the necessary techniques for their project, collect reliable baseline data at sea level, and make repeated measurements at WMRS to assess the effects of acute exposure and subsequent acclimatization to high altitude. The projects designed by each team prior to departure for WMRS are shown as the first five entries in Table 5. Outcomes of each team’s investigation are also listed in Table 5 and described briefly below. Subsequent entries in Table 5 reflect some of the investigations designed to answer questions that occurred to one or more students as the course progressed and that could be evaluated using the group data set. This ability to address new student questions as they arose added greatly to the interest and excitement generated by the course.

**Team Investigations**

**Team 1.** Members of this investigational team were impressed by the frequently fatal errors in judgment made by even the most experienced mountaineers at high altitude (6, 12). Their goal was to determine whether the hypoxic cognitive impairment responsible for these errors could be demonstrated at an elevation as low as 12,500 ft. To maximize the likelihood of detecting cognitive changes, team members selected tests of four neural processing networks and performed these tests immediately after arriving at 12,500 ft on day 3 at WMRS, before any acclimatization could occur. As shown in Table 6, two of the four tests demonstrated significant impairment of each team's investigation are also listed in Table 5 and

**Table 5. Student inquiry-driven investigations**

<table>
<thead>
<tr>
<th>Topic of Investigation</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance on cognitive function tests with acute exposure to an elevation of 12,500 ft</td>
<td>Cognitive impairment and recovery were test specific, suggesting that neural networks supporting specific functions have differing sensitivities to hypoxia.</td>
</tr>
<tr>
<td>Changes in hand and foot volume with altitude as measured by water displacement plethysmography</td>
<td>Hand and foot volumes increased with ascent and returned to baseline values by day 6 at WMRS.</td>
</tr>
<tr>
<td>Time course of changes in reticulocyte counts and hematocrit with altitude</td>
<td>Reticulocyte count progressively increased, beginning within 24 h of ascent. Hematocrit also increased over 3 days, likely due to an acute decrease in plasma volume.</td>
</tr>
<tr>
<td>Changes in urine pH and 24-h volume with altitude as indicators of increased bicarbonate excretion to compensate for respiratory alkalosis</td>
<td>Urine pH rose within 24 h of ascent, but changes in urine volume were more delayed and variable, possibly due to unmeasured fluctuations in fluid intake.</td>
</tr>
<tr>
<td>Differential effect of altitude on aerobic and anaerobic exercise performance</td>
<td>The percentage increase in time required to run 800 m was greater than the percentage increase in time required to run 50 m at altitude compared with sea level.</td>
</tr>
<tr>
<td>Changes in cardiac output (inferred by heart rate) and minute ventilation (inferred by respiratory rate) in relationship to decreased blood oxygen saturation at altitude</td>
<td>An increase in mean resting heart rate, but not mean resting respiratory rate, VO2max accompanied the decrease in blood oxygen saturation at altitude.</td>
</tr>
<tr>
<td>Relationship between the time required to run 800 m and the percentage decrease in VO2max at altitude</td>
<td>The decline in VO2max going from 4,000 to 12,500 ft did not predict the increase in time required to run 800 m.</td>
</tr>
<tr>
<td>Change in frequency of desaturation events measured by continuous nocturnal pulse oximetry over the course of 1 wk at altitude</td>
<td>No clear trend in nocturnal desaturation events was seen in the limited number of subjects studied; however, episodes of periodic breathing at a cycle length of 16.7 s were clearly observed.</td>
</tr>
</tbody>
</table>
Table 6. Cognitive function test outcomes

<table>
<thead>
<tr>
<th>Test</th>
<th>Sea Level</th>
<th>WMRS</th>
<th>WMRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual motor reaction time, ms</td>
<td>185 (3)</td>
<td>191 (4)*</td>
<td>191 (3)</td>
</tr>
<tr>
<td>Stroop test, s</td>
<td>8.7 (1.0)</td>
<td>11.1 (0.9)†</td>
<td>9.6 (0.8)</td>
</tr>
<tr>
<td>Verbal reasoning, s</td>
<td>11.4 (1.0)</td>
<td>12.5 (1.2)</td>
<td>10.7 (1.0)</td>
</tr>
<tr>
<td>Sentence repetition, s</td>
<td>5.9 (0.4)</td>
<td>5.9 (0.3)</td>
<td>5.7 (0.4)</td>
</tr>
</tbody>
</table>

Data are expressed as means (SE). *P = 0.033 vs. sea level; †P = 0.044 vs. sea level and P = 0.049 vs. WMRS on day 6.

relative to sea level performance. The initial 3.2% impairment in visual motor reaction time persisted with repeated measurements on day 6 at WMRS. Interestingly, this impairment was seen only in male subjects. The initial 27% increase in adjusted time to complete the Stroop test of conflicting visual stimulus suppression could no longer be demonstrated by day 6 at WMRS. Tests of verbal reasoning and sentence repetition showed no significant impairment at 12,500 ft relative to sea level. Team 1 concluded that the hypoxia produced by a rapid ascent to 12,500 ft was not severe enough to cause global cognitive impairment and that visual motor reaction time might be a particularly sensitive indicator of the altitude effect in males.

Team 2. This team chose to determine whether ascent to 12,500 ft would cause peripheral edema that could be detected by using water displacement plethysmography to measure hand and foot volume. This edema has been variously ascribed to increased capillary permeability, increased central venous pressure, and sodium retention (13). The possibility that changes in extremity volume were due to changes in blood flow was reduced by performing all tests in the morning prior to any significant physical activity and consistently using water at 20–22°C. The major challenge faced by this team was to design a technique sufficiently sensitive and reproducible to measure the anticipated small volume changes. As described in MATERIALS AND METHODS, it was ultimately possible to measure hand volume with a coefficient of variation (CV) of 1.6% and foot volume with a CV of 2.3% using simple water tanks and graduated cylinders. Measurements were always performed early in the morning to prevent confounding by dependent edema associated with prolonged standing and exercise. Team 2 was able to demonstrate a 5.4 (2.9)-ml increase in hand volume [mean (SE), n = 19, P < 0.05] and a 41.6 (17.3)-ml increase in foot volume [mean (SE), n = 19, P < 0.0001] by day 5 at WMRS (Fig. 2). These increases in volume resolved by day 6. Plethysmography performed on the final day at 12,500 ft, following a day of aggressive climbing and hiking by the group, showed an increase in foot, but not hand, volume (data not shown). This recurrence of increased volume only in the feet was consistent with increased lower extremity venous pressure and sympathetically mediated sodium retention in the setting of strenuous exercise.

Team 3. Our discussion of the molecular mechanisms of hypoxia sensing and erythropoiesis suggested to the members of this investigational team that reticulocytosis should be detectable shortly after the arrival at altitude but that a measurable increase in red blood cell mass would take 2–3 wk to develop. To test this assertion, the team performed reticulocyte counts and measured hematocrit both at sea level and at 1- to 4-day intervals at WMRS. As predicted, the reticulocyte count tripled over the course of 1 wk at altitude (Fig. 3). However, hematocrit also increased acutely, contrary to expectations. Team 3 attributed the latter observation to a decrease in plasma volume due to altitude-related respiratory and urinary fluid losses rather than an increase in red blood cell mass. Consistent with this explanation, there was a decrease of 1.1 kg in weight accompanied by an increase in 24-h urine volume on day 6 at WMRS, and hematocrit started to decline between days 6 and 10 despite a progressive increase in reticulocyte count (Fig. 3).
Team 4. This investigational team postulated that respiratory alkalosis at altitude would lead to a compensatory increase in renal bicarbonate excretion that would be detectable as an acute rise in urinary pH. They also postulated that in the absence of the sodium-retaining effect of strenuous exercise, they would be able to detect the frequently reported, but incompletely understood, diuresis associated with acute elevation gain (13). As shown in Fig. 4, urinary pH increased as predicted after spending the first night at 10,200 ft and then decreased to its baseline sea level value by day 6 at WMRS. The 24-h urinary volume increased significantly when measured on day 6 at WMRS but decreased to a value slightly below that observed at sea level on day 10. Urinary specific gravity showed no clear trend over time. Team 4 felt that their data were consistent with acute renal compensation for respiratory alkalosis. They reasoned that, because renal base excretion was the product of base concentration and 24-h urine volume, total base excretion might have remained elevated due to increased urine volume even after the urine pH returned to its sea level value on day 6 (Fig. 4). Team 4 further recognized that fluid and acid-base balance could not be accurately assessed in the face of unmeasured fluid intake and respiratory fluid loss.

Team 5. The goal of this investigational team was to assess altitude-related decreases in the performance of four standardized exercise tests. They reasoned that altitude would lead to a greater impairment in performing aerobic than anaerobic exercise. An 800-m run was chosen as the “aerobic” exercise, even though both aerobic and anaerobic metabolism contribute to a run of this length. A longer run would have been impractical in the field. The 50-m sprint was a purely anaerobic event. The clearest data in support of this group’s prediction came from a comparison of sea level run times and times for the first set of running tests performed on day 5 at WMRS (Fig. 5). There was a significant increase of 21.2% in the mean time required to complete the 800-m run at maximal effort but a nonsignificant 3.4% increase in the mean time to complete the 50-m sprint at maximal effort. The mean 800-m run time decreased by day 10 at WMRS, but this decrease did not reach statistical significance (data not shown). There was no significant change from sea level in the maximal number of pushups performed at WMRS, and the number of jumping jacks performed in 1 min actually increased significantly by day 10 at WMRS, possibly as a result of practice. Team 5 concluded that performance on the 800-m run, the most aerobic of the exercise tests, was most impaired at altitude and that a longer period of observation would be required to assess the degree to which performance on this event improved with acclimatization.

The final three entries in Table 5 are examples of investigations designed to answer questions that occurred to students as a result of their personal experience at altitude and their discussions of data obtained by the group. One student speculated that increased cardiac output, as inferred by changes in heart rate, would act to compensate for the decreased blood SO2 at altitude. As shown in Fig. 6, the vital signs data obtained upon awakening every morning supported this student’s prediction. Interestingly, resting respiratory rate increased as blood SO2 fell, but this increase did not reach statistical significance (data not shown). In the absence of tidal volume measurements, this student acknowledged that an increase in minute ventilation could not be excluded as another mecha-
nism attenuating the decrease in blood So2. Indeed, the HVR data obtained at sea level suggested that minute ventilation probably increased in most participants.

Another question that occurred to a student at WMRS was whether the decrement in performance in the 800-m run time between sea level and 12,500 ft could be accurately predicted by the decrement in \( \dot{V}O_2 \text{max} \) measured by bicycle ergometry between 4,000 and 12,500 ft. The student felt that if this were true, it would be possible in future studies to replace ergometry with the technically simpler measurement of an 800-m run time to assess the effect of altitude on the oxygen cascade. The student addressed her question by performing univariate linear regression using the change in \( \dot{V}O_2 \text{max} \) as the independent variable and the change in the 800-m run time as the dependent variable (data not shown). Although the \( P \) value of 0.47 for this regression was inconsistent with the student’s hypothesis, this analysis had only a 50% power to detect a correlation coefficient of 0.45 and an 80% power to detect a correlation coefficient of 0.65.

The last entry in Table 5 illustrates the learning opportunities presented by ongoing projects of other researchers using the WMRS facilities. One student from our group was allowed to use continuously recording pulse oximeters to collect sleeping So2 and heart rate data from four of his colleagues. These devices were obtained by a researcher from another institution in support of a project evaluating the effect of altitude on sleep. The student’s question was whether the nocturnal desaturation events seen in many individuals shortly after arriving at altitude would decrease in frequency over the course of 1 wk at 12,500 ft. The data did not show a clear change in frequency, most likely because of the limited number of subjects who could be studied with only four devices. However, on carefully evaluating his recordings on an expanded time scale, the student was able to demonstrate stretches of regular periodic nocturnal oxygen desaturation with a cycle length of 16.7 s associated with heart rate accelerations (Fig. 7). The student attributed this phenomenon to Cheyne-Stokes respiration caused by an oscillating relationship between hypoxic and hypercarbic ventilatory drives. Presumably, the corresponding heart rate accelerations were driven by the sympathetic response to falling So2.

DISCUSSION

It is difficult to overstate the impact of a laboratory activity in which the students are themselves the object of investigation. Add to this a physiological stimulus such as altitude, which is perceived strongly by all participants, as well as the flexibility to design one’s own experiments in an environment far removed from the classical laboratory, and a memorable learning experience is virtually assured. Perhaps even more important than the factual knowledge acquired during this course was the excitement generated by the group as a whole for the discipline of physiology and its experimental basis.

A comparison of several of the students’ results with published data revealed that both their experimental techniques and interpretation of their findings were in line with previous studies. For example, their measurement of acute poikilocapnic HVR yielded results comparable with those reported over 20 years ago by Moore and coworkers (8). These investigators also found a wide range in poikilocapnic HVR among 14 normal individuals living at sea level, and their mean value of 0.32 \( \text{l/min/} % \) was reasonably close to our value of 1.4

Table 7. Course evaluations

<table>
<thead>
<tr>
<th>Item Rated</th>
<th>Biology 220</th>
<th>Current Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall quality of the course</td>
<td>4.1 (0.1)</td>
<td>5.0 (0.0)</td>
</tr>
<tr>
<td>Amount learned in the course</td>
<td>4.1 (0.1)</td>
<td>4.8 (0.1)</td>
</tr>
<tr>
<td>Instructor’s contribution</td>
<td>4.6 (0.1)</td>
<td>4.9 (0.1)</td>
</tr>
<tr>
<td>Difficulty of the course</td>
<td>3.9 (0.1)</td>
<td>3.8 (0.2)</td>
</tr>
</tbody>
</table>

Data are expressed as means (SE). Ratings were on a scale of 0 (lowest) to 5 (highest).
Teaching In The Laboratory

I·min⁻¹·%⁻¹ for the 7 students who were studied. The students’ speculation that a low HVR might favor swimming as an athletic event has been examined previously as well. Bjurstrøm and Schoene (1) found the mean HVR in 10 nationally ranked synchronized swimmers to be less than half of the mean value found in control subjects. However, Byrne-Quinn and coworkers (4) found that acute HVR was depressed in a variety of athletes compared with controls, suggesting that this phenomenon may not be unique to swimmers. With regard to the effect of altitude on V̇O₂ max, the students found a 19% drop in this parameter between 4,000 and 12,500 ft. This decrease agrees almost exactly with the decline predicted by Fulco and coworkers based on an extensive review of published results (5).

Because this course was without precedent at the University of Washington, it was difficult to judge its success objectively. However, all students completing Exploration Seminars in the summer of 2006 provided a standardized written evaluation and numeric ratings of multiple facets of their respective courses. We compared these evaluations with those provided over the past 5 years by similar groups of students taking Biology 220 with the same professor who mentored the current course. Biology 220 presents basic animal physiology by means of 19 large classroom lectures and 5 laboratory exercises on campus. Like the present course, Biology 220 is rated at five credits. A total of 9 Biology 220 evaluations, each completed by ~200 students between 2002 and 2006, were treated as single data points for the purpose of this comparison. While no statistical analysis was performed due to the very different sample sizes, the current course was rated more highly than Biology 220 in terms of overall quality and amount learned, despite more similar assessments of instructor’s contribution and difficulty for the two courses (Table 7).

Although we had access to some sophisticated and expensive technologies, such as DXA scanning, continuous pulse oximetry, and low-oxygen gas mixtures, these were not critical to the success of the course. Some of the group’s most interesting results came from using a hand-held pH meter, a stopwatch, and plastic tubs purchased in a home goods store. Indeed, the ability of team 2 to devise a volumetric technique de novo, construct the required apparatus, demonstrate reproducibility of measurements, and apply their technique successfully under field conditions embodied all of the goals of the course. Even the more technically demanding measurement of V̇O₂ max was accomplished in the field without a metabolic cart by using simple pieces of equipment and techniques similar to the original research methods developed by exercise physiologists. Every student came away with a feeling for the patience required to make valid experimental measurements, the need to solve unanticipated problems in the field, the objectivity necessary to evaluate data that did not agree with their predictions, and the statistical techniques required to analyze and present their results. Submitting their work as a scientific paper and discussing what they did at an international meeting completed the experience.

The cost of this course was surprisingly low and easily covered by a modest supplemental tuition payment. Although the WMRS facilities were ideal for physiological studies at altitude and were very reasonably priced, much of what we did could have been accomplished on a mountain field trip with any basic shelter, as long as the altitude was high enough. At an elevation of 12,500 ft, inspired P O₂ is 90 mmHg and arterial S O₂ is ~90%, the point of inflection of the oxygen-hemoglobin dissociation curve where most physiological responses to hypoxia become significant and measurable. The University of Washington General Clinical Research Center provided exercise laboratory time and DXA scans at no charge. Using this facility was in itself an educational experience for the group. The demonstration of HVR on seven students was accomplished in the University of Washington Hospital Pulmonary Function Laboratory during the lunch hour of a technician who kindly volunteered her time to do this. Facilities of this sort are widely available in larger universities, particularly those with medical schools. Faculty members who put in the effort to locate these resources on behalf of their students are frequently well rewarded.

In conclusion, a high-altitude laboratory course that allows students to be both experimental subjects and investigators is an effective and exciting way to teach physiology. We hope that our experience will provide some guidance for others who want to design an activity of this nature.

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GRANTS

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