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Three field studies were conducted under the award. The purpose of the first year's studies was to examine the effect of the menstrual cycle on acclimatization to high altitude (4300 m) in healthy, normally menstruating women. Twenty women were studied in the follicular and luteal phases of the menstrual cycle while residing at sea level and again in either the follicular or the luteal phase during the course of a sojourn in the US Army Research Institute of Environmental Medicine (USARIEM) laboratory on the summit of Pikes Peak, CO (4300 m). The second year's studies were conducted at USARIEM to determine the role of alpha-1 adrenergic activity and its interaction with menstrual cycle phase in early altitude acclimatization. Fifteen women were exposed to an effective altitude of 4300 m in a hypobaric chamber for 52 hr on two occasions, once while being treated with an alpha -1 blocker (prazosin) and once while taking a placebo. Cycle phase was the same (follicular or luteal) during the blocked and unblocked studies for each subject. Sea level studies were performed prior to the altitude exposure. In year three, the purpose of the study was to determine the role of alpha-1 adrenergic activity and its interaction with cycle phase during altitude acclimatization. Sixteen women were divided into two groups, half treated with an alpha-1 blocker (prazosin) and the remaining half with placebo.

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Lona Moore 10-14-99

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INTRODUCTION

Three field studies were conducted under the award. The purpose of the first year's studies was to examine the effect of the menstrual cycle on acclimatization to high altitude (4300 m) in healthy, normally menstruating women. In year one of this three-year project, the period covered in this report, 20 healthy women were studied in the follicular and the luteal phases of the menstrual cycle while residing at sea level and again, in 16 of these women, in either the follicular or the luteal phase of the menstrual cycle during the course of 12 days sojourn in the US Army Research Institute of Environmental Medicine (USARIEM), Maher Memorial Research Laboratory at the summit of Pikes Peak, CO (4300 m). The second year's studies were conducted in order to determine the role of alpha -1 adrenergic activity and its interaction with menstrual cycle phase in early altitude acclimatization. Fifteen healthy young women with normal menstrual cycles were exposed to an effective altitude of 4300 m in a hypobaric chamber for 52 hr on two occasions, once while being treated with an alpha -1 blocker (prazosin, 1 mg every eight hours or 3 mg in a 24-hr period) and once while taking a placebo. The study was of a randomized, double blind design. Menstrual cycle phase was constant (either follicular or luteal) during the blocked *vs.* unblocked studies for each subject. Sea level comparison studies were performed prior to the altitude exposure. The studies were performed at the US Army Research Institute of Environmental Medicine, Natick, MA. In year three, the purpose of the study was to determine the role of alpha-1 adrenergic activity and its interaction with menstrual cycle phase during altitude acclimatization. In year three, sixteen healthy young women with normal menstrual cycles were divided into two groups. Half the subjects were treated with an alpha-1 blocker (n=8, prazosin, 2 mg every eight hours or 6 mg in a 24-hr period) and remaining half were given placebo tablets (n=8). One additional alpha-1 blocked subject completed the sea-level studies, but did not go to high altitude. The study was randomized, double-blind in design. Sea level studies were performed between April 20 and May 30, 1998 at the Aging Study Unit at the Palo Alto, California Veterans Affairs Health Care System. High-altitude studies were performed between July 6 and August 7, 1998 at the US Army Research Institute of Environmental Medicine's Maher Memorial Laboratory on the summit of Pikes Peak, Colorado (4300 m).

BODY

In each of the three years of the project "Women at Altitude", the statement of work consisted of study preparation (project planning, subject recruitment, shipment of equipment), conduct of sea-level studies, conduct of high-altitude studies, analysis of results and preparation of manuscripts or other forms of presentation of study findings.

Study preparation (project planning, subject recruitment, shipment of equipment)

1.) In year one (9/95-9/96), work began in September 1995 to finalize human volunteer consent forms, and preparations for advertising and screening prospective volunteers. During the course of the fall and winter months, decisions were made as to the amount of various supply items, types of instrumentation and costs of items required for the conduct of the sea level and high altitude phase of testing. By the end of February, more than 100 volunteers had been screened. Twenty-one volunteers consented to participate and of these, twenty completed the sea level series of tests. All women resided in the Palo Alto - San Jose, California area. None were native to altitudes greater than 2500 m and none had been at altitudes above 1500 m for the 6 months prior to the sea level phase of testing. Nearly all the volunteers were undergraduate or graduate students at Stanford University. Dr. Gail Butterfield, co-investigator on this project, was in charge of volunteer recruitment.

The characteristics of the volunteers are shown below:

Volunteer characteristics

SL n=20, HA n=16	mean ± SE
Age (yr, range)	22.6 ± 0.8 (19-34)
Height (cm, range)	167 ± 1 (159-175)
Weight (kg, range)	63.4 ± 1.7 (53.6-85.1)
Resting HR (bpm)	66 ± 3
Resting MAP (mmHg)	80 ± 2
VO_2 max (ml/kg/min)	42.8 ± 1.3 (33.2-56.0)

2). In year two (September, 1996-September, 1997), beginning in the fall, protocols and consent forms were prepared, supplies were purchased, equipment was organized, and volunteers were recruited. The recruiting process was carried out by personnel at the USARIEM, Thermal and Mountain Division. The total number of volunteers screened was in excess of 100. Fifteen (15) women were selected for the study, all of whom were residents of sea level and lived in the vicinity of Boston, MA. Supplies and equipment were shipped from the research groups in Denver, CO and Palo Alto, CA to Natick immediately prior to the beginning of the study in June, 1997. The characteristics of the volunteers are shown in the table below (mean ± SEM):

Age (yr)	24.7 ± 1.18
Height (cm)	169.2 ± 1.96
Weight (kg)	70.8 ± 2.49
Resting HR (bpm)	79.1 ± 2.45
Resting MAP (mm Hg)	75.2 ± 1.84
VO2 max (ml/kg/min)	33.7 ± 1.94

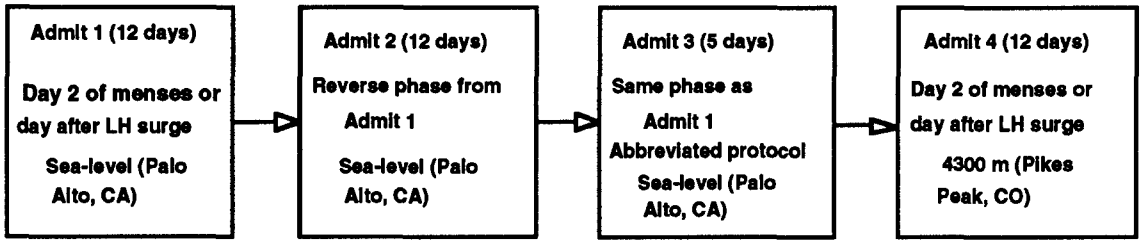
3). During year three (September, 1997-September, 1998), protocols and consent forms were prepared, supplies purchased, equipment organized, and volunteers recruited during the fall. Winter months were again used to recruit volunteers by personnel at the Aging Study Unit at the Palo Alto Veterans Affairs Health Care System. The total number of volunteers screened was in excess of 75. Sixteen (16) women were selected and eventually completed both the sea level and high altitude phases of the study, all of whom were residents of sea level and lived in the vicinity of Palo Alto, CA. Supplies and equipment were shipped from the research groups in Denver, CO, Natick, MA and Dallas, TX to Palo Alto immediately prior to the beginning of the study in April, 1998.
The characteristics of the volunteers as measured at sea level are shown in the table below (mean ± SEM):

Age (yr)	23.3 ± 1.03
Height (cm)	166.6 ± 2.12
Weight (kg)	68.7 ± 3.17
VO2 max (ml/kg/min)	35.1 ± 5.0

Conduct of sea-level and high-altitude studies

1) During year one, formal testing of volunteers commenced following final approval of the first year study protocol by the institutional review committees from the University of Colorado, Stanford University, USARIEM, and the US Army Surgeon General's Human Use Review for Research and Development (HURRAD). Each volunteer underwent extensive studies during three separate time periods, once in both the follicular and the luteal phase of her menstrual cycle and one final time in either the follicular or luteal phase of her cycle. The testing took place with the volunteers residing in a metabolic ward at the Palo Alto VA Medical Center to facilitate dietary control and measurement of metabolic parameters. For that reason, each testing period was designated as an "admission" to the metabolic ward (i.e., Admission #1-3.) To be consistent, each volunteer's 12-day residence at high altitude was designated "Admission 4". The schedule of tests for each woman volunteer during the sea-level and high altitude phases is shown diagramatically below.

Research Design



- Each woman was her own control for sea-level follicular vs. luteal phases
- Women were measured in either follicular or luteal phase at altitude
- A controlled diet was maintained throughout the studies

The controlled diet was maintained during each of the sea-level admissions and during high altitude testing in order to assure that the women were receiving the same proportions of calories from carbohydrates, fats and proteins during each test phase. In addition, diet was adjusted during the first admission to assure that the volunteers were in nitrogen balance.

A testing schedule was designed for ensuring timely completion of the requisite tests in each subject and in keeping with the protocol requirements for each type of study. Approximately half the women were in their follicular phase during admission #1 with the remainder in their luteal phase. Follicular phase was detected by the onset of the menses; luteal phase was detected by a positive ovulation predictor test which was based on the detection of the LH surge in twice daily urine samples. Virtually all the above tests were carried out successfully. Only the "doubly labeled water", alpha- and beta-adrenoreceptor measurements, and venous tone studies described in the original proposal were discontinued for reasons of high cost, blood sample volume required and the possibility that the substrate utilization studies carried out on day 10 would interfere with the results of the doubly-labeled water tests.

Study methods employed are briefly summarized here for the measurements conducted in year one:

a) Ventilation. Forced vital capacity (VC) was measured in standing volunteers. In seated volunteers, ventilation (VE) was monitored while breathing room air. Measured were minute ventilation, breathing frequency, tidal volume, end-tidal PO_2 and PCO_2, arterial O_2 saturation, and heart rate. End-tidal PCO_2 is a measure of alveolar ventilation per unit CO_2 production and is the classic indicator of acclimatization to altitude. The measurement of hypoxic ventilatory response (HVR) was based on a progressive, isocapnic hypoxic test used frequently in our and other's studies.

b) Sympathetic nervous system activation. Sympathetic nervous system activity was assessed by measurement of heart rate (HR) variability, and circulating and urinary catecholamine (catechols) concentrations. Heart rate variability was analyzed by power spectrum analysis during 24-hour Holter monitor recordings in order to determine the relative sympathetic and parasympathetic dominance as previously described (16). Daily 24-hour urinary collections were collected for analysis of catecholamine levels with samples divided between daytime and nocturnal collections. Plasma catecholamine was analyzed in arterial samples obtained from venous samples and, on day 10, from arterial samples using high-performance liquid chromatography as described by Hallman *et al* (1).

c) Hemodynamic studies. Blood pressure was measured from the arterial line and routinely from arm cuff sphygmomanometer. Cardiac output during year 1 was measured non-invasively by acetylene rebreathing at rest and during exercise (2). Systemic vascular resistance was calculated from the cardiac output and blood pressure measurements.

d) Blood volume and volume regulatory hormones. Red cell mass was measured using carbon monoxide rebreathing and was used to calculate plasma volume and total blood volume from hematocrit using previously published methods (3,4) on the days indicated. Plasma renin activity, serum aldosterone, plasma atrial natriuretic peptide and ADH were measured from venous samples using radio-immunoassay to monitor volume regulatory hormones (5,6,7). ADH and aldosterone were measured from 24-hr urine collections.

e) Lean body mass. Body composition, *i.e.* the relative proportions of lean and fat tissue were determined by DEXA (dual x-ray absorptiometry) scan.

f) Total body water. During blood volume studies, body water was measured using a deuterium-based technique and extracellular fluid volume was measured using sodium bromide.

g) Resting and exercise O_2 uptake. Resting measurements were made after an at least 4 hour fast in seated volunteers. Exercise measurements were made during cycle ergometry using conventional techniques for measurement of O_2 uptake, CO_2 production and volume. During exercise, maximum O_2 uptake was defined as the value obtained during progressive cycle exercise (200 kg*m/min every 2 min) where O_2 consumption fails to increase with an increase in workload (3).

h) Basal metabolic rate and dietary control. In order to differentiate fluid loss from loss of body mass, diet was controlled and maintained at a composition approximating 12-14% of calories from protein, 32-40% from fat and the remainder from carbohydrates. Sea level energy requirement was determined by

feeding volunteers a standard diet, adjusted over 10 days for changes in body weight. Adequacy of energy intake was validated by determination of N_2 balance, a more sensitive measure of the adequacy of energy intake than body weight (8). Energy intake on day 1 at high altitude was matched to energy requirement at sea level, and adjusted on subsequent days based on changes in basal metabolic rate (BMR), as previously done in men. BMR was determined by indirect calorimetry in the morning before rising every other day at altitude and energy intake was matched to energy need daily. Body weight was monitored daily upon rising and after voiding. Fluid intake was prescribed and monitored.

i) Substrate utilization. Glucose, glycerol and palmitate kinetics were measured at rest and during moderate steady state exercise at 50% of the sea level on admissions #1 and #2 and at approximately 65% of the sea level VO_2max on admission #3. The palmitate was included to monitor production, utilization and oxidation of FFA. Glucose is included to monitor glucose kinetics. Use of the tracers allowed estimation of rates of appearance, disappearance, and clearance of glucose, glycerol, leucine and palmitate as previously published (9,10). It should be noted that although the use of the stable isotopes was expensive, it avoided the exposure of the volunteers to radioactive substances. The proposed studies were performed using a mixture or "cocktail" which contained all metabolites. Total body water was measured before the metabolite kinetic studies, as to not influence the isotopic enrichment of deuterium labeled isotopes infused during exercise. Volunteers were studied after a 12-hour fast and 36 hr of limited exercise. A flexible catheter (20 gauge) was inserted in a vein of one arm or hand for the constant infusion of the metabolic cocktail of ^{2}H-glucose (9 mg/kg/min), ^{2}H-glycerol (9.2 mg/kg/min), and ^{13}C-palmitate (5.1 mg/kg/min). The infusion began with a bolus and then at the rate indicated above for 90 min before the start of exercise to ensure equilibrium with body pools. Total dose of fuels for a 60 kg volunteer did not exceed 0.6 gm glucose and 0.11 gm glycerol.

j) Hematologic and coagulation factors. Samples were obtained for measurements of plasma erythropoietin concentration by radio-immunoassay and reticulocyte counts by standard laboratory techniques. Fibrinogen was measured by a quantitative determination of fibrinogen based on the Clauss method (Organon Teknika Fibriquik) (11). Fibrinolytic activity was screened using the euglobulin clot lysis time, ECLT (12). Other fibrinolytic tests included: 1) tissue plasminogen activator (tPA) using Asserachrom tPA, an enzyme immunoassay (Diagnostica Stago/American Bioproducts) and 2) plasminogen activator inhibitor-1 (PAI-1) using Stachrom PAI, a quantitative determination of PAI by a synthetic chromogenic substrate method (13). Von Willebrand factor antigen is measured to indicate acute phase activation using Laurell rocket immunoelectrophoresis (14).

k) Ovarian hormones. Estrogen and progesterone levels were measured in 24-hour urine samples and in venous samples drawn periodically (Table 2) in order to quantify hormonal status and menstrual cycle phase using radio-immunoassay (15).

l) Acute Mountain Sickness (AMS). Symptoms of acute mountain sickness were assessed daily using two different validated, self-assessment instruments: the Environmental Symptoms Questionnaire (ESQ) and the Lake Louise AMS Scoring System questionnaire (LLS).

2) During year two, formal testing of volunteers began following final approval of the second-year study protocol by the institutional review committees from the University of Colorado Health Sciences Center, Stanford University, USARIEM, and the US Army Surgeon General's Human Use Review of Research and Development (HURRAD). The study testing schedule included three phases, each lasting approximately one month. During the first month, the volunteers documented their menstrual cycles, received required hypobaric chamber orientation and safety training and were familiarized with testing procedures. The second phase consisted of a six-week period of experimental testing, during which the volunteers continued to document their menstrual cycles. This testing consisted of studies at sea level and at 4300 m altitude performed on two occasions, once while receiving prazosin or once while being treated with a placebo. The third phase was a repetition of the second. During either phase two or three, the subject was treated with prazosin and during the other with placebo. The subject was tested in the same phase (either follicular or luteal) during two consecutive menstrual cycles on these occasions. The following table shows the study testing schedule:

Preliminary Phase (sea level, 1 mo)	Experimental Phase (sea level & altitude, 6 wks)	Follow-up Phase (sea level, 1 mo)
Pregnancy screen	Pregnancy screen	Pregnancy screen
Ovulatory & menstrual cycle assessment	Ovulatory & menstrual cycle assessment	Ovulatory & menstrual cycle assessment
Altitude chamber training	a-blockade documentation	
Procedure training	Sea-level/altitude testing	
Preliminary testing		

A controlled diet was maintained for seven days during each of the experimental study periods in order to assure that the women were receiving the same proportions of calories from carbohydrates, fats and proteins during each test phase. The testing schedule which each volunteer underwent is shown in Appendix A. In this diagram an "X" designates a day on which the measurements were made. The echocardiogram studies and DEXA measurements were not done during this study due to time, cost and space constraints. All other tests were successfully carried out. The methods employed were described in the year 2 protocol description, and are briefly described as follows:

Study methods employed are briefly summarized here for the measurements conducted in year two:

a). Menstrual cycle documentation included documentation of non-pregnant status by blood test for human chorionic gonadotrophin (HCG), a record of cycle length, and assessment of ovulatory status by urine test for the presence of luteinizing hormone (LH). Blood samples were collected on two sea level and two altitude days for evaluation of serum ovarian hormone levels.

b). Environmental Background Survey (EBS) consisted of a 57-item questionnaire completed one time during the preliminary phase to elicit information on the volunteer's previous experience in stressful climatic conditions as well as epidemiological, medical and menstrual history data.

c). Documentation of alpha 1-adrenergic blockade (agonist challenge) was employed to demonstrate the extent of a-blockade induced by prazosin by evaluating the blood pressure response to increasing dosages of phenylephrine administered as an intravenous infusion (16,17). This test lasted approximately a one hour and was done twice at sea level, once while taking prazosin and once while taking placebo. Test results showed that in the 15 subjects taking 1 mg prazosin t.i.d. when studied at sea level, the PD_{20} changed from 2.0 in the placebo subjects to 10.7 in the blocked subjects. This confirms a high degree of blockade, though some of the subjects were more densely blocked than others.

d). Fluid status assessment. Since altered fluid-volume regulation is thought to play a role in altitude acclimatization, body fluid status was assessed using 24-hour fluid intake; 24-hour urinary volume; daily body weight, bio-impedance measurements for total body water and intra- and extra-cellular fluid; urinary and plasma sodium, potassium, chloride, and osmolality; and calculated percent change in plasma volume.

e). Basal metabolic rate- same as in year one.

f). Carbohydrate regulation. Since insulin secretion and glucose production are regulated by alpha-1 adrenergic receptors, we measured glucose tolerance once at sea level and once at altitude during each experimental test phase (once on prazosin, once on placebo). Following a 12-hour overnight fast, an intravenous catheter was placed, a fasting blood sample was taken, then a standard 75 gm carbohydrate meal was eaten. Blood samples were taken at 30, 60, 90 and 120 minutes following meal ingestion. The blood samples were analyzed for insulin, glucose and C-peptides. These analyses are presently in progress.

g). Bio-impedance Testing. A bioelectric-impedance technique was used to estimate changes in water intra-and extra-cellular body water. Subjects were tested after 2-hour fast from food and liquid and after being supine without moving for 10 minutes. An electric current (800 uA maximum at 50 kHz) was passed through the body from electrodes on the hand to similar electrodes on the ankle. The testing

required approximately 15 minutes to complete and was performed once at sea level and once at altitude during each sea-level/altitude exposure.

h). Ventilation. same as in year one with the addition that blood pressure, heart rate and arterial saturation were also measured during the hypoxic and hypercapnic challenges using non-invasive methods.

i). Blood pressure and heart rate. Since blood pressure is greatly influenced by alpha 1-adrenergic activity, blood pressure and heart rate were measured on multiple occasions. Blood pressure and heart rate were measured during the following tests: twice daily during the assessment of acute mountain sickness, during the ventilatory control tests, during the orthostatic response testing, during the exercise test, and during the phenylephrine challenge testing period. During some of the studies, blood pressure was measured manually, and during others using an automated blood pressure device.

j). Orthostatic response testing was done to determine how each experimental test phase (once on placebo, once on placebo) modifies the cardiovascular response to an orthostatic challenge during early acclimatization to altitude. A "tilt-test" was performed which required the volunteer to lie supine while blood pressure and heart rate were measured for 20 minutes. Then, the subject was rotated to a 60-degree "head-up" position and blood pressure and heart rate were measured every two minutes for 12 minutes. Volunteers were tested once at sea level and once at altitude during both prazosin and placebo phases.

k). Exercise testing was performed to provide an integrated measure of many of the physiologic components of altitude acclimatization. An incremental, progressive exercise bout to volitional exhaustion on a bicycle ergometer was used to assess each volunteer's peak oxygen consumption. A metabolic cart was used for measurement of O_2 uptake, CO_2 production and respiratory volume. To determine possible shifts in fuel utilization with a-1 blockade, a catheter was placed in a warmed hand vein and blood was sampled at rest, at the end of each work load, and as close to exhaustion as possible for analysis of glucose, insulin and free fatty acids. In addition, the Borg Relative Perceived Effort scale was used to quantitate each volunteer's subjective assessment of her physical effort (18).

l). Assessment of acute mountain sickness (AMS)- same as in year one.

m). Intraocular pressure (IOP). IOP may provide a marker for altitude acclimatization and may be affected by adrenergic activity. Measurements were made with a hand held tonometer (TONO-PEN^{TM}XL) or the equivalent and were taken after the cornea was anesthetized with a topical solution. Measurements were made at rest once at sea level and daily at altitude during each sea-level/altitude exposure. These measurements were done to determine whether intraocular pressure is affected by altitude-induced changes in retinal blood flow, and what relation those changes might have to AMS.

3) During year three, formal testing of volunteers again began following final approval of the third-year study protocol by the institutional review committees from the University of Colorado Health Sciences Center, Stanford University, USARIEM, and the US Army Surgeon General's Human Use Review of Research and Development (HURRAD). The study testing schedule included three phases, each lasting approximately one month. The initial phase was the screening process which consisted of an initial interview and a bicycle exercise trial. Formal documentation of menstrual cycles began after admission to the study and an phenylephrine challenge was administered, once while on prazosin and once on placebo. Subjects were assigned to either the prazosin (alpha-1 adrenergic blocked) or placebo (unblocked) groups for further testing. The second phase consisted of a period of experimental testing at sea level, during which the volunteers continued to document their menstrual cycles. The third phase was the high-altitude study phase. During phases two or three, each subject was treated with prazosin or placebo, according to the assignment done following phase one. To the extent possible, each subject was studied in the same menstrual cycle phase during study phases two and three. A controlled diet was begun three days before study phases two and three and was maintained throughout each phase in order to assure that the women were receiving the same proportions of calories from carbohydrates, fats and proteins during each test phase.

A complicated schedule of tests was constructed. Considerations were to group studies requiring catheters onto as few days as possible to limit the number of venipunctures, to properly interleave rest and exercise studies and to allow for scattered days with a light schedule to provide relaxation time for the test volunteers. All tests were performed both at sea level and at altitude on the same study day. The methods, briefly, were as follows:

a). Menstrual cycle documentation - same as in year two.

b). Documentation of alpha-1 adrenergic blockade - same as in year two. Test results showed that in women given prazosin 2 mg t.i.d., the PD20 changed from 1.5 mg on placebo to 9.5 mg on prazosin. Thus, the blockade was about the same on 1 mg ('97 study) or 2 mg ('98 study), although there was less variability in the degree of blockade between the subjects in 1998. (Wolfel FIGURES 10,11)

c). Assessment of fluid status and body fluid volume distribution (total body water, blood volume, extracellular fluid volume) - same as in years one and two.

d). Basal metabolic rate - same as in years one and two.

e). Ventilation - same as in years one and two with the addition of single breath flow-volume loop tests, performed to measure maximal lung capacities and flows at sea level and at altitude.

f). Cardiac output during rest and exercise. Cardiac output was measured at quasi-steady state at the end of 3 min of a given exercise level by an acetylene rebreathing method. From end-expiration, the subject inspired (up to total lung capacity) a breath of gas containing 0.3% CH_4, 0.8% C_2H_2, 0.3% CO, 40%O_2 in balance N_2. The subject rebreathed this mixture for 16 sec while expired gas concentrations were measured continuously at the mouth by a rapid response infrared gas analyzer. Cardiac output was calculated from the exponential uptake of acetylene. Measurements were obtained at rest on days 1, 2, 5, 9, and 12 at high altitude and during exercise on days 2, 5, and 9 at high altitude at successive, pre-selected workloads up to the highest workload that could be sustained for 3 minutes.

g). Resting venous tone and forearm blood flow was measured non-invasively using forearm plethysmography.

h). Whole body exercise testing was performed to provide an integrated measure of the physiologic components of altitude acclimatization. An incremental, progressive exercise bout to volitional exhaustion on a bicycle ergometer was used to measure each test volunteer's peak oxygen consumption. A metabolic cart was used for measurement of O_2 uptake, CO_2 production and respiratory volume. Measurements of fuel utilization were also carried out at rest and while the subject exercised at 50% of their previously measured peak O_2 consumption for 50 min.

i). Fuel (carbohydrate) utilization. To determine fuel utilization with a-1 adrenergic blockade, a catheter was placed in a hand vein, solutions containing stable isotopes were infused, and blood was sampled multiple times during rest and exercise for analysis of free fatty acids, catecholamine, insulin, glucagon, lactate, glucose, pyruvate, and glycerol.

j). Static muscle contraction (thumb exercise endurance) was evaluated using the adductor pollicis muscle of the hand (19). Each volunteer was tested with her hand and forearm secured and with the thumb coupled to a force transducer by means of a strap looped around the thumb. Maximal voluntary contraction (MVC) was determined and submaximal exercise then consisted of intermittent static contractions at 50% of the initial MVC followed by five sec of rest. The cycle of MVC measurements and submaximal contractions was continued until maintenance criteria could not be met. Rate of fatigue was quantified as decline in MVC force and endurance time was defined as time to target contraction failure.

k). Tests of sympathetic reactivity ("tilt", SNS reactivity, acute hypoxia/hypercapnia, heart rate variability). Blood pressure and heart rate were recorded using an ambulatory blood pressure monitor and

Holter monitor during two, 24-hour periods while at sea level and altitude as well as during the various tests described below.
"Tilt" (orthostatic response) tests involved measuring blood pressure and heart rate while the test volunteer lay supine for 20 min and after the subject was rotated to a 60-degree "head-up" position for a period of 12 min.

SNS reactivity was evaluated during cold pressor and isometric hand grip challenges. Each test raises blood pressure transiently but does so by a different mechanism. For the isometric hand grip test, maximum strength of the preferred hand was determined using a calibrated handgrip dynamometer. Then the subject was asked to squeeze the dynamometer at 30% of her maximal ability for 3 min. The cold pressor test required the subject to immerse her non-preferred hand in cold (4°C) water for 3 min.

During acute hypoxia or hypercapnia, forearm and hand blood flow, peripheral blood flow responses of the left forearm and hand were assessed by thermographic, impedance, and laser Doppler procedures performed in conjunction with the HVR and HCVR tests. The outputs of the thermographic, impedance, and laser Doppler devices were digitized, displayed and recorded (AT-Codas, Dataq Inc) on a PC. Thermographic images were made using a AGEMA TIC-8000 Infrared System consisting of a AGEMA ThermovisionR camera, a PC and a CATS E 1.00 software package. Forearm electrical impedance data were collected using a Minnesota (model 304B) impedance cardiograph. Finger tip blood perfusion was measured from the ventral surface of the left middle finger using a laser blood perfusion monitor (TSI, Model 403A).

Heart rate variability analyses were performed to assess the balance of sympathetic vs. parasympathetic autonomic tone on two occasions at sea level and at altitude (20,21). ECG data were recorded while the subject lay supine as well as after rising to upright posture, under controlled and spontaneous breathing conditions.

l). Assessment of acute mountain sickness (AMS) - same as in years one and two.

Analysis of results and preparation of manuscripts or other forms of presentation of study findings

Given that each of the three study years terminated at approximately the same time as data collection was completed, most of the analysis and preparation of manuscripts took place in the following year or in the current, fourth year of the project which has been an extension of the original study period with no additional funds. The process of analysis and preparation of study results has occurred and is still in occurring in several forms.

1) Periodic meetings of study investigators. These meetings took place at monthly intervals in the Cardiovascular Pulmonary Research Lab Conference Room at the University of Colorado Health Sciences Center throughout the 3-year study period. The Colorado investigators were physically present and phone connections were made with the California and Massachusetts groups. These meetings were designed to ensure the smooth conduct of each year's studies and to exchange requisite data required by individual investigators for the analysis of their section of the project.

2) Yearly meetings held by study investigators at key points in the overall study period. During each year, face-to-face meetings were arranged with as many study investigators as possible and that involved key persons from each of the geographic groups (Colorado, California, Massachusetts, Texas). These were held in December 1995 in Denver, October 1996 in Palo Alto, October 1997 in Denver and February 1999 in Jasper, Canada. The Jasper meeting was a day-long session held before the 1999 International Hypoxia Symposium in which each of the study co-investigators presented their principal findings to the entire group.

3) Regular exchange of manuscript drafts among co-authors. This process is continuing, as attested to by the number of manuscripts in preparation below.

KEY RESEARCH ACCOMPLISHMENTS

The purpose of the research conducted for the "Women at Altitude" contract was determine the effects of the menstrual cycle, and alpha 1-adrenergic blockade on the response to high altitude in four areas: 1) ventilatory, 2) blood oxygenation and blood volume, 3) cardiovascular, 4) metabolic, 5) exercise performance, and 6) Acute Mountain Sickness (AMS) symptoms. Since the majority of previous studies of high-altitude acclimatization were performed in men, the comparison of our results with those obtained previously permits us also to examine the extent to which differences exist between women and men as well Thus, the key research accomplishments are organized below by in relation to these study goals. Numbered figure and tables referred to appear in the appendix.

• 1. Gender Differences in Acclimatization to High Altitude

Ventilatory acclimatization, as judged by the time-dependent changes in end-tidal PCO_2 (a measure of effective alveolar ventilation) during the course of 12 days exposure to 4300 m, was similar in the 30 women participating in the present studies as found previously in studies conducted by the same investigative team in 37 men. The sea-level values in the men were higher and more variable (Figure 1).

Blood oxygenation, as measured by SaO_2 was similar in women as observed previously in men studied under the same altitude conditions. Variation among individual subjects related to erythropoietin levels similarly in women and men, with higher SaO_2 found in combination with lower the erythropoietins (Figure 2).

Cardiovascular. Resting cardiac index decreased from sea level to day 4 at altitude, then returned to sea level baseline by day 10 at altitude. Compared with previous data acquired in men under the same altitude conditions, women have a higher cardiac index and appear to recover sea-level cardiac outputs more rapidly than men (Figure 3). An elevation in blood pressure occurs during the initial days of high-altitude exposure, as has been observed previously in men (Figure 4). Women like men demonstrate a rise in urinary norepinephrine levels at rest and during submaximal exercise.

Metabolic. The relative energy requirement to maintain body weight at altitude was 10% higher in women than men. Basic metabolic rate (BMR) rose more modestly in women than men during the initial period and, unlike men, fell to near sea-level values by day 10 at 4300 m. The rise in BMR was unrelated to changes in thyroid hormone (Figure 5). Total carbohydrate oxidation is lower in women after 10 days at altitude, suggesting that there is a decrease in the rate of muscle glycogen utilization (Figure 6). This is unlike men in whom carbohydrate oxidation is enhanced at high altitude. High-altitude exposure increased insulin sensitivity similar to the effects seen previously in men (Figure 7). No altitude-associated changes occurred in vitamin E levels, indicating that previous recommendations (made in men) for vitamin E supplementation at altitude are not unwarranted in women (Figure 8).

Exercise. In women and men matched for muscle strength at sea level, the adductor pollicis muscle fatigued more slowly, muscle endurance was greater and after a similar degree of muscle exhaustion, early force recovery was faster for women than for men (Figure 9). In contrast to men, women's endurance time to exhaustion of the adductor pollicis muscle did not decline at altitude when compared with sea level (Figure 10).

AMS symptoms. There were no noticeable differences in the occurrence of AMS symptoms in women as seen previously in men. Women, like men, show a correlation between intraocular pressure and AMS scores (Figure 11).

• 2. Effects of Menstrual Cycle on Acclimatization to High Altitude.

The rise in estradiol and progesterone during the menstrual cycle was unaffected by high altitude (Figure 12-13).

Ventilatory. Resting ventilation, hypoxic ventilatory response, and hypercapnic were similar in the follicular and luteal phases in women studied both at sea level and during 12 days exposure to 4300 m. The changes from sea level to altitude in these variables was also similar in the two cycle phases (Table 1).

Blood oxygenation and blood volume. Blood oxygenation (SaO_2) did not differ in the two menstrual cycle phases. Aldosterone and plasma renin activity were higher in the luteal than follicular phase of the cycle (Figure 14). Whereas blood volume fell after 3 days of high-altitude exposure in both phases, there was a tendency for luteal phase women to have a greater increase in plasma volume by day 10 (Figure 15). There was an overall enhancement of fibrinolysis at high compared with low altitude, with values being higher in the follicular than luteal phase at sea level and 4300 m. There was evidence of endothelial cell activation at high altitude, as demonstrated by a rise in von Willebrand factor, which was not affected by menstrual cycle phase (Table 2).

Cardiovascular. There were no differences in the cardiac output response to altitude in the two cycle phases nor was there any cycle phase effect on the increase in sympathetic or parasympathetic nervous system activation, the rise in blood pressure, or the early increase in heart rate at high altitude at sea level or high altitude (Figure 16-18). Menstrual cycle phase had no effect on urinary or plasma catecholamines under resting conditions or submaximal exercise conditions either at sea level and during acute or chronic exposure to 4,300 m (Figure 19).

Metabolic. There was no effect of cycle phase on the BMR or carbohydrate oxidation responses to high altitude (Figure 20). At sea level and altitude the glucose response to a meal was greater (less insulin-sensitive) in the luteal than follicular phase of the menstrual cycle and blood glucose returned to baseline more slowly after a meal in the luteal phase of the cycle at sea level (Figure 7)

Exercise performance. Muscle strength and endurance of the adductor pollicis and quadriceps muscles were not affected by cycle phase either at sea level or at altitude (Table 3).

AMS symptoms. AMS prevalence and severity were lower in the luteal than follicular phase during the first 60 hours at 4300 m. When assessed by resolution of AMS, women in the luteal phase acclimatized more rapidly during 12 days at 4300 m. However, the differences in AMS between cycle phases are too small to be clinically relevant or to impact military operations (Figure 21).

• 3. Effects of Alpha-Adrenergic Blockade on Acclimatization to High Altitude:

Ventilatory and blood oxygenation. Alpha 1 blockade did not alter ventilatory acclimatization as measured by the time-dependent changes in resting ventilation, $PETCO_2$, SaO_2, HVR or HCVR or the ventilatory response to exercise (Figure 22-23).

Cardiovascular: alpha 1-adrenergic blockade. As noted above, the phenylephrine challenge administered during year 3 showed that effective alpha 1-adrenergic blockade was achieved at sea level as well as at high altitude (Figure 24-25). At high altitude, a greater dose of phenylephrine was required to raise blood pressure 20 mmHg in both the placebo and prazosin groups, suggesting downregulation of vascular alpha 1-adrenergic receptors with prolonged hypoxia as a result of persistently elevated sympathetic activity. Consistent with this, prasozin-treated subjects had a greater rise in plasma catecholamines than did placebo-treated subjects at 4300 m (Figure 26). Lower daytime and nocturnal heart rates with alpha-blockade at high altitude may indicate that prasozin also attenuated cardiac alpha-1 adrenergic receptor activity (Figure 27-28). There was no change in arterial baroreceptor function, as measured by the systolic BP/heart rate relationship with progressive doses of phenylephrine, with alpha 1-adrenergic blockade (Table 4).

Cardiovascular: arterial and venous tone. Alpha 1-adrenergic blockade with prazosin attenuated the rise in SNS activity at 4,300 m and prevented the increase in PNS activity in the supine position (Figure 29). Alpha 1-blockade decreased blood pressure and heart rate and their responses to brief hypoxia or

hypercapnia (Figure 30). Forearm vascular resistance was decreased and blood flow increased at high altitude. These values returned towards sea level by day 10, but that return was less marked in prazosin-treated women. Venous compliance fell during the course of 10-days exposure to 4300 m in placebo- but not prasozin-treated subjects (Figure 31) and was associated with plasma epinephrine levels. Women who maintained better oxygenation (greater hematocrit, higher arterial oxygen saturation) on day 3 at 4300 m had less marked changes in blood flow and venous compliance (Figure 32). Forearm vascular resistance was lower and venous compliance higher in alpha 1-blocked compared with unblocked subjects across all measurement times (Figure 33). Overall, these comparisons suggest: 1) alpha 1-adrenergic receptor stimulation contributes to the maintenance of blood pressure at high altitude and to the return of peripheral arterial vascular resistance to sea-level values following acclimatization; and 2) alpha 1-adrenergic stimulation, presumably via epinephrine, contributes to the decline in forearm venous compliance that occurs during acclimatization.

Cardiovascular: cardiac output. Alpha 1-adrenergic blockade did not alter the cardiac output rise during exercise at sea level or high altitude. It tended to reduce arterial -venous O_2 content difference during exercise, likely due to redistribution of the cardiac output (Figure 34).

Metabolic. There was no acute effect of prazosin on the rise in BMR with altitude exposure (Figure 35). Alpha-blockade exaggerates the increase in carbohydrate metabolism response to a meal seen in women acutely exposed to high altitude (Figure 36).

Exercise performance. Alpha 1-blockade lowered VO2 peak at sea level and altitude but did not alter the altitude-associated (Figure 37). Muscle strength and endurance in women was unaffected by alpha 1-adrenergic receptors at sea level or altitude (Figure 38).

AMS symptoms. Alpha-1 adrenergic blockade did not affect AMS in women at 4300 m.

REPORTABLE OUTCOMES

a. Manuscripts, abstracts and presentations

a.1. Manuscripts, published or in press

Braun, B., L.G. Moore, S.D. Dominick, S. Zamudio, R.G. McCullough, P.B. Rock, and G.E. Butterfield. Women at Altitude: Changes in carbohydrate metabolism at 4300 m elevation and across the menstrual cycle. J. Appl. Physiol. 85:1966-1973, 1998.

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a. 2. Manuscripts, in preparation

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a.3. Technical reports

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Muza, S.R., B. Braun, G.E. Butterfield, P.B. Rock, C.S. Fulco, R.S. Mazzeo, and L.G. Moore. Women at Altitude: Effect of alpha-blockade on exercise ventilation at sea level and high altitude. Med. Sci. Sports Exerc. 31:S219, 1999.

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White D.B., R.E. McCullough, J.Grillo, J.T. Reeves, E.E. Wolfel, and L.G. Moore. Women at Altitude: The Effect of Altitude and the Menstrual Cycle on Sympathetic and Parasympathetic Inputs to the Heart. presented at the 10th International Hypoxia Symposium, Lake Louise, Canada, February, 1997.

Wolfel E.E., J.T. Reeves, J. Lindenfeld, P. Rock, C. Fulco, S.Muza, G.E. Butterfield, B. Braun, S. Zamudio, and L.G. Moore. Women at Altitude: Reduction in peak exercise systemic blood pressure with alpha-adrenergic blockade upon exposure to simulated altitude. FASEB J. 12: A56, 1998, presented at the Experimental Biology Meeting, San Francisco, CA, April, 1998.

Wolfel E.E., P.Rock, R.E. McCullough, R.G. McCullough, S. Zamudio, B. Braun, G.E. Butterfield, and L.G. Moore. Diminished vascular response to phenylephrine infusion in women after nine days at 4,300 m altitude; presented at the 11th International Hypoxia Symposium, Jasper, Alberta, Canada, February-March, 1999.

Wolfel E.E., J. Tokeshi, M. Meertens, R.G. McCullough, P. Rock, G.E. Butterfield, and L.G. Moore. Alpha adrenergic blockade limits the increase in ambulatory blood pressure observed in young women

after short-term residence at 4,300 m altitude. The FASEB J. 13 (Part II): A784, 1999; presented at the Experimental Biology meeting, Washington D.C., April, 1999.

Wolfel, E.E., J.T. Reeves, J. Lindenfeld, P. Rock, C. Fulco, S. Muza, G.E. Butterfield, B. Braun, S. Zamudio, and L.G. Moore. Women at Altitude: Reduction in peak exercise systemic blood pressure with alpha-adrenergic blockade upon exposure to simulated altitude. FASEB Journal, April, 1998.

Wolfel E.E., P. Rock, R.E.McCullough, R.G. McCullough, S. Zamudio, B. Braun, G.E. Butterfield and L.G. Moore. Diminished vascular response to phenylephrine infusion in women after nine days at 4300 m altitude. Proceedings of the International Hypoxia Symposia, p 174, 1999.

Wolfel, E.E., J. Tokeshi, M Meertens, R.E. McCullough, P. Rock, G.E. Butterfield, and L.G. Moore. Alpha -adrenergic blockade limits the increase in ambulatory blood pressure observed in young women after short-term residence at 4300 m altitude. FASEB J. 13:A784, 1999.

Zamudio S., M. Douglas, R.Rathod, R.E. McCullough , R.G. McCullough, E.E. Wolfel, S. Muza, P.Rock, G. Butterfield and L.G. Moore. Forearm hemodynamics during acclimatization to 4300 m with and without alpha-adrenergic blockade. Proceedings of the International Hypoxia Symposia, p 182 1999.

b. Degrees obtained with support by the award

Barken, Randi BA, Stanford University
Bradford, Susan, BA, Stanford University
Carroll, Joy, MS, University of Colorado at Boulder
Cesario, Karrie, BA , Stanford University
Child Avon, MS, University of Colorado at Boulder
DeLee, Ryan, BA, Stanford University
Dubay, Andrea, MS, University of Colorado at Boulder
Kirsh, Jennifer, BA, University of Colorado at Boulder
Mawson, Jacinda, MA, Stanford University
Meertens, Maaike, M.D. degree; Maastricht University, Netherlands
Rathod, Raj, BA, Stanford University
Tamhane, Rahul. M.Sc. Biomedical Engineering, Joint Program, Univ of Texas at Arlington and Southwestern Medical School.
Tokeski, Jay, BA, Stanford University
White, Doug, MD, University of California at San Francisco
Wojtkoski, Dorothy, BA, Stanford University

c. Funding applied for based on work supported by the award

Gail Butterfield, VA-DOD grant (performance enhancement in troops)

d. Employment or research opportunities applied for and/or received on experiences/training supported by this award.

Bradford, Susan - Tufts Medical School
Braun, Barry - Assistant Professor, University of Mass at Amherst
Cesario, Karrie - University of California Davis graduate school
DeLee, Ryan - University of Chicago Medical School
Dominick, Shannon - graduate study, Stanford University
Douglas, Matthew - University of Colorado Medical School research rotation
Friedlander, Anne - Fellowship, NIH
Matthews, Amanda K. - Stanford University Medical School research rotation
Mawson, Jacinda - Luce Scholarship, graduate study at Bryn Mawr University

Meertens, Maaike - Maastricht Univeristy (Holland) research rotation
White, Douglas - Univesity of California at San Francisco research rotation
Wojtkowski, Dorothy - University of Washington dental school

CONCLUSIONS

An ambitious series of three field studies were successfully conducted over a period of four years by a team comprised of investigators at the University of Colorado Health Sciences Center and Denver Campuses, Palo Alto Veteran's Administration Hospital / Stanford University, and USARIEM to investigate the menstrual cycle phase, and alpha 1-adrenergic blockade on acclimatization to high altitude. In addition, this information was used to compare with that obtained from prior studies in men to identify the effects of gender on altitude acclimatization. Gender did not affect ventilatory, blood oxygenation, or blood pressure response to high altitude. Women compared with men demonstrated a faster return to sea-level for cardiac output, greater small muscle (adductor pollicis) endurance, a higher energy requirement to maintain BMR, a lesser altitude-associated rise in BMR, and a decreased reliance on glucose during exercise. The menstrual cycle did not influence ventilatory acclimatization, blood oxygenation, cardiac output, sympathetic or parasympathetic activity, catecholamine levels, BMR, glucose utilization, or exercise performance. During the luteal compared with follicular phases of the menstrual cycle, aldosterone and plasma renin were elevated, fibrinolytic activity was diminished, and AMS symptoms were lessened. Alpha 1-adrenergic stimulation occurred during acclimatization to high altitude and contributed importantly to the rise in sympathetic nervous system activity, the rise in blood pressure and vascular resistance, and the decline in venous compliance.

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3. Wolfel E.E. , B.M. Groves, G.A. Brooks, et al. Oxygen transport during steady-state submaximal exercise in chronic hypoxia. J Appl Physiol 1991;70(3):1129-36.

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APPENDICES

Figures 1 - 38 and Tables 1-4 appear on the following pages

Figure 1

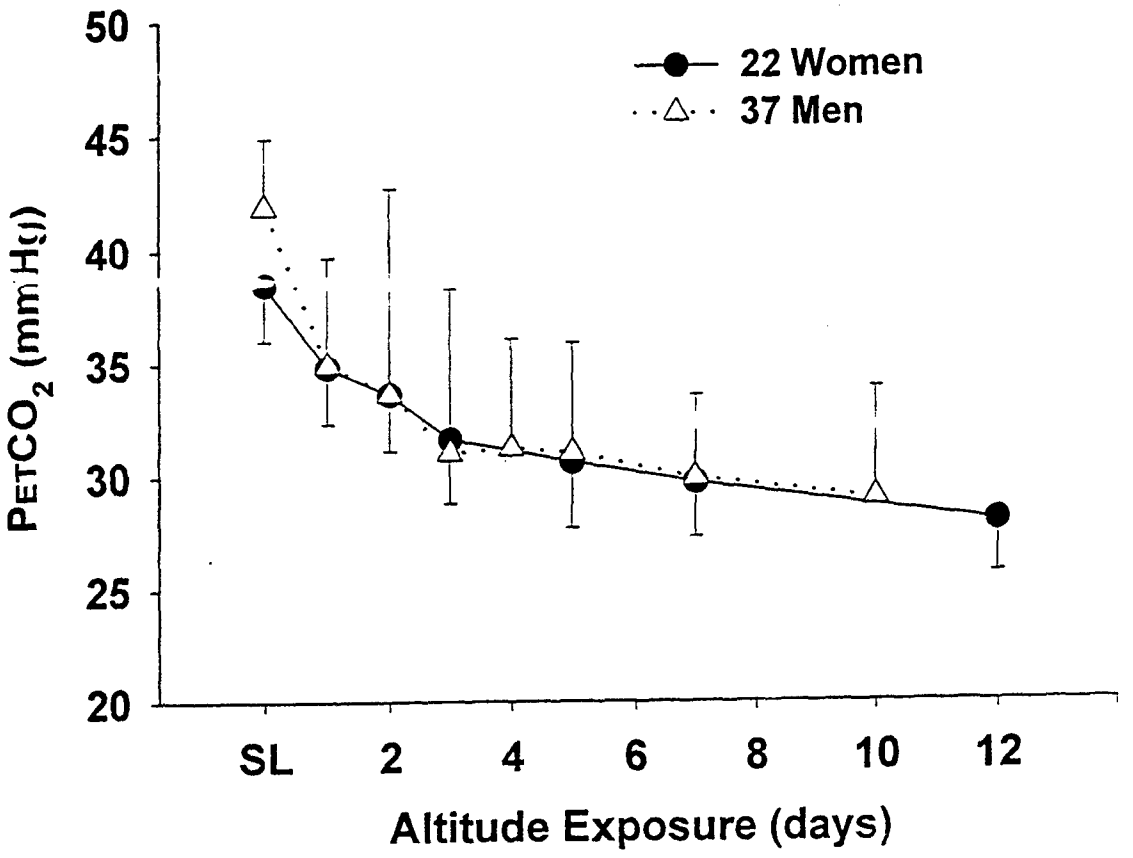

50
45
40
35
30
25
20
$P_{ET}CO_2$ (mm Hg)
22 Women
37 Men
SL
2
4
6
8
10
12
Altitude Exposure (days)

Figure 2

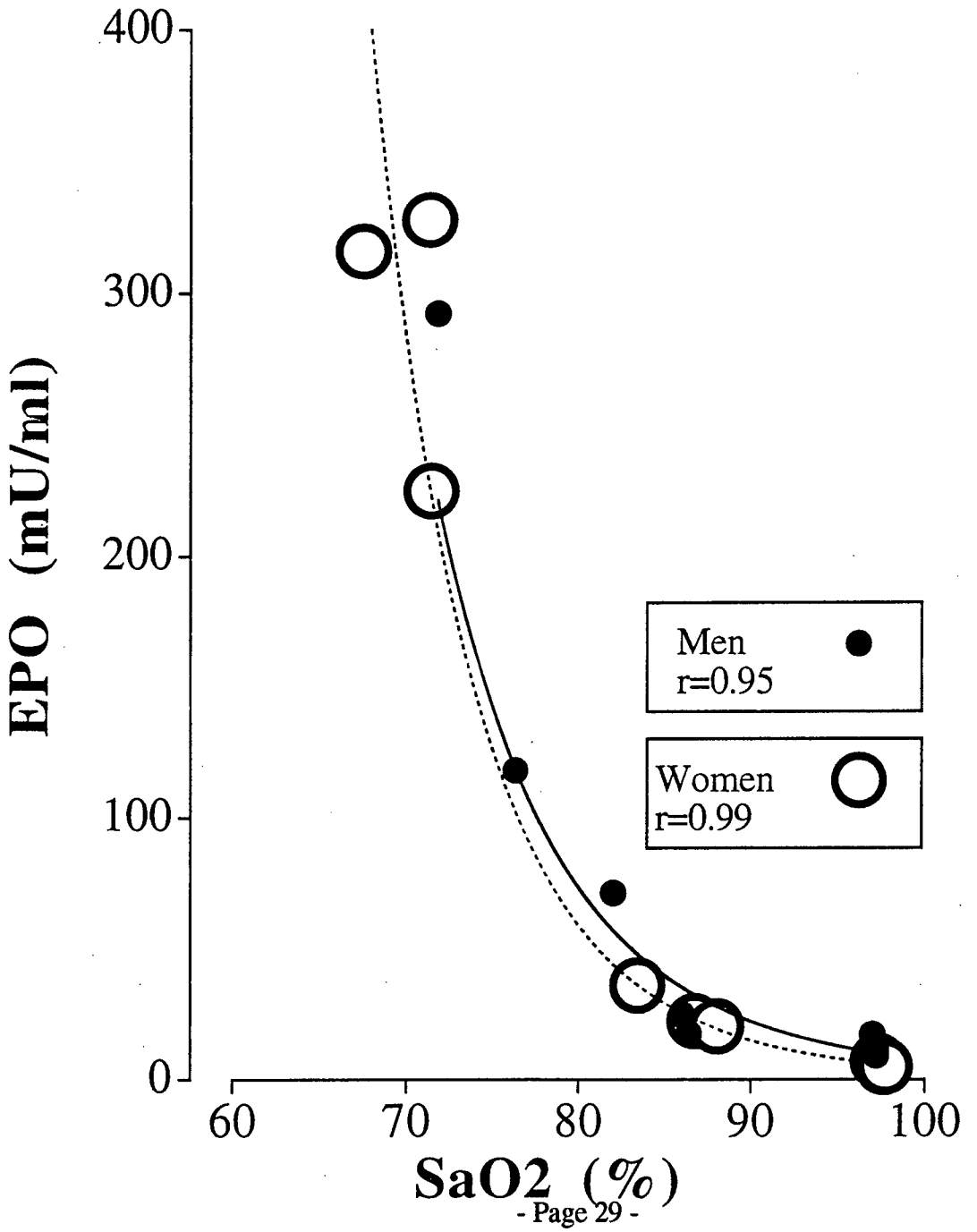

400
300
200
100
0
EPO (mU/ml)
60
70
80
90
100
SaO2 (%)
Men
r=0.95
Women
r=0.99

Figure 3

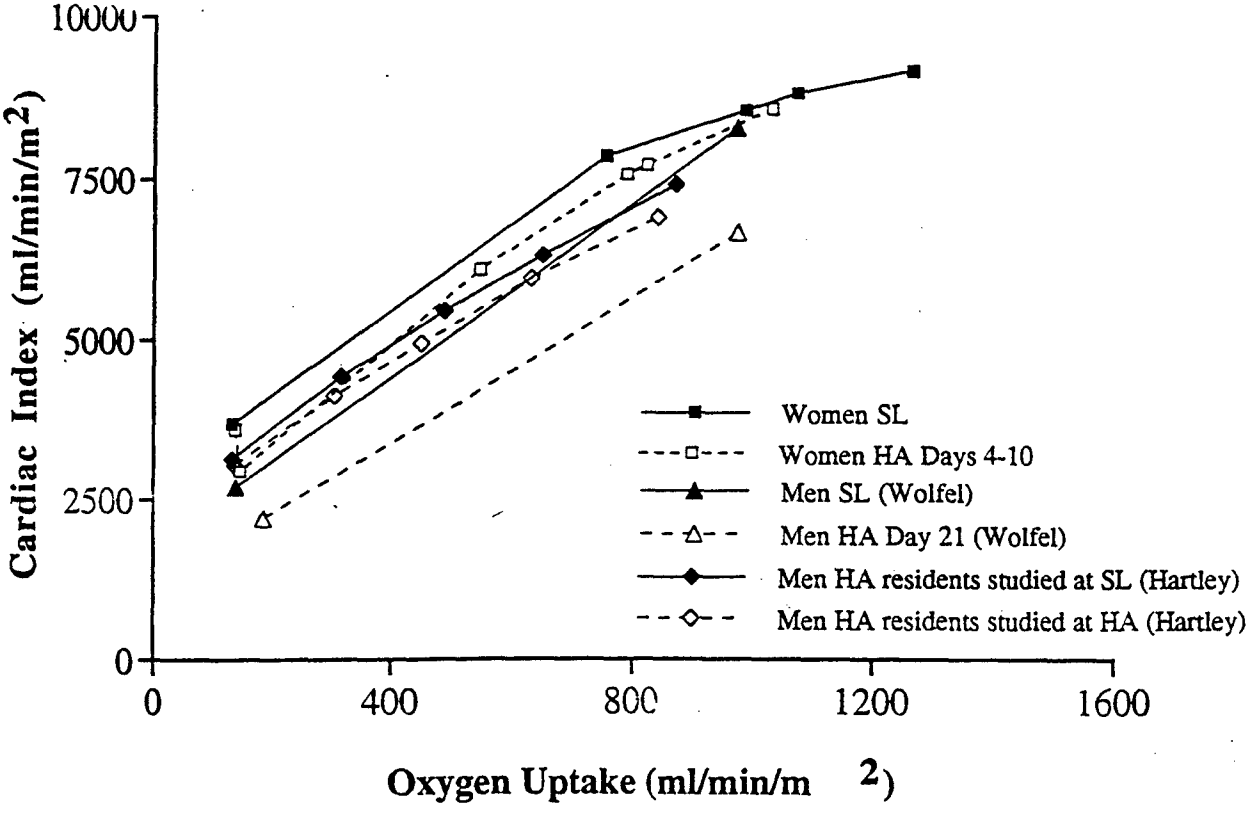

10000
7500
5000
2500
0
Cardiac Index (ml/min/m2)
0
400
800
1200
1600
Oxygen Uptake (ml/min/m 2)
Women SL
Women HA Days 4-10
Men SL (Wolfel)
Men HA Day 21 (Wolfel)
Men HA residents studied at SL (Hartley)
Men HA residents studied at HA (Hartley)

Figure 4

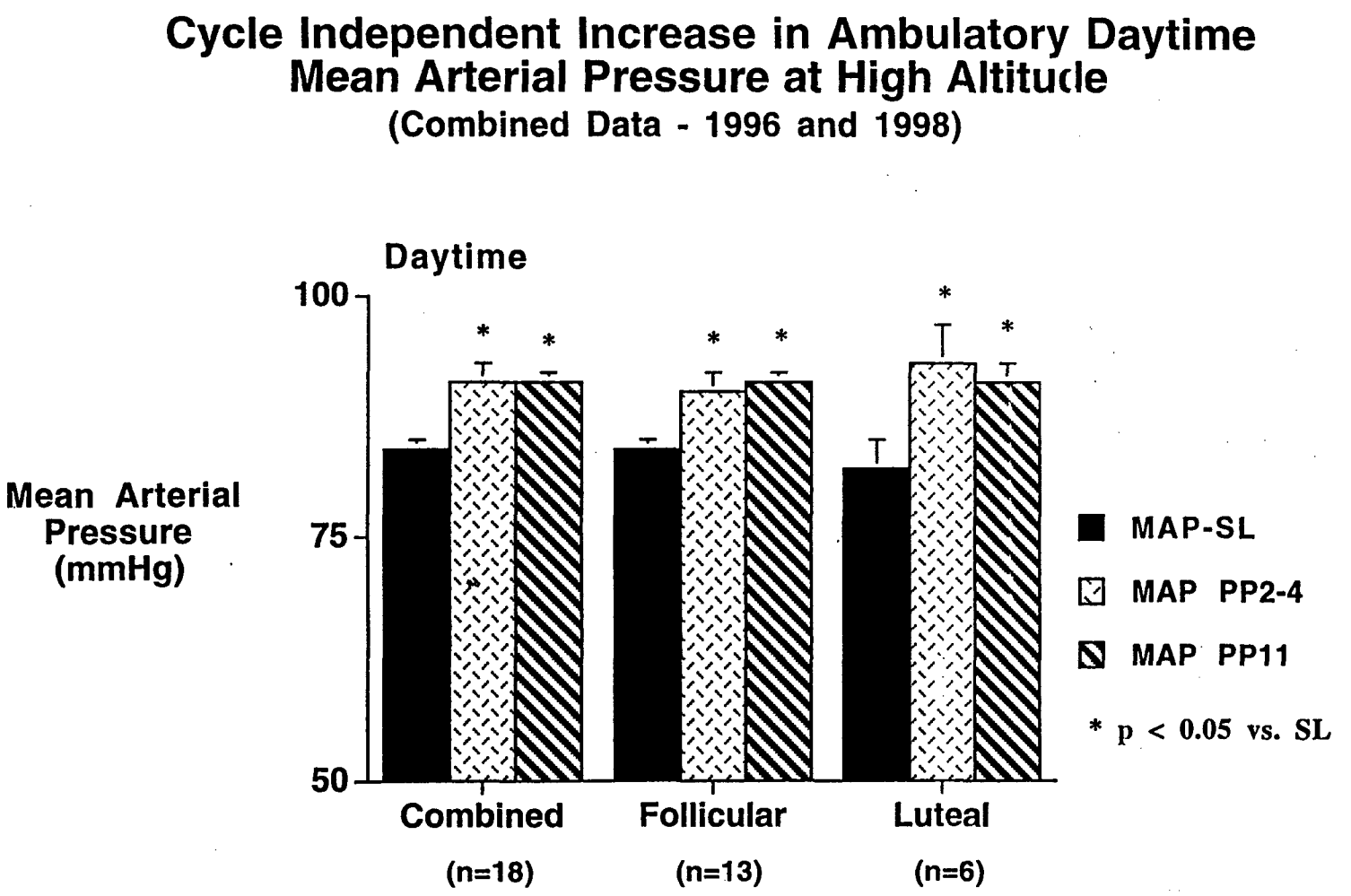

Cycle Independent Increase in Ambulatory Daytime
Mean Arterial Pressure at High Altitude
(Combined Data - 1996 and 1998)
Daytime
Mean Arterial Pressure (mmHg)
100
75
50
Combined
(n=18)
Follicular
(n=13)
Luteal
(n=6)
MAP-SL
MAP PP2-4
MAP PP11
* p < 0.05 vs. SL

Figure 5

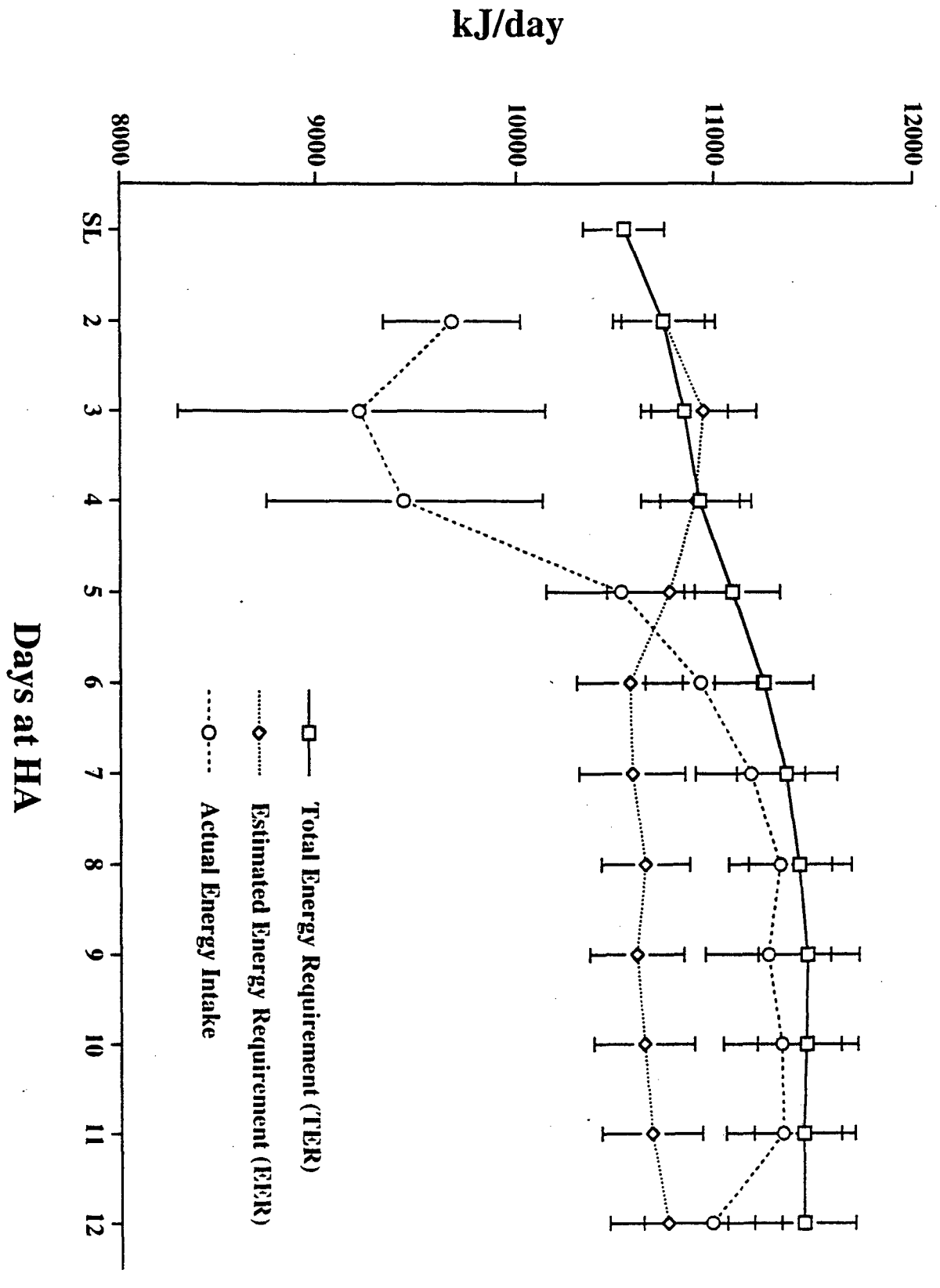
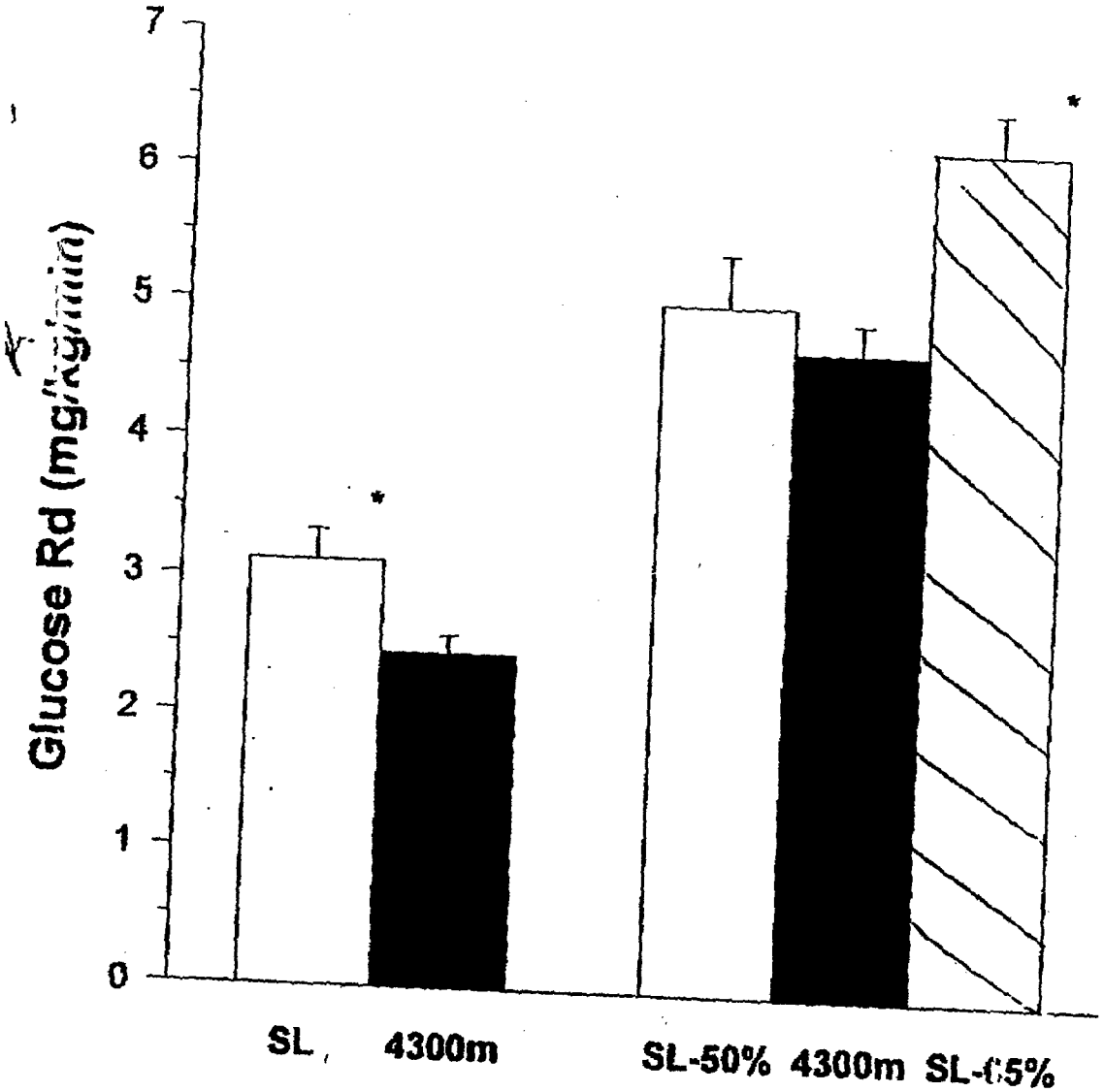

kJ/day
8000
9000
10000
11000
12000
SL
2
3
4
5
6
7
8
9
10
11
12
Days at HA
Total Energy Requirement (TER)
Estimated Energy Requirement (EER)
Actual Energy Intake

Figure 6



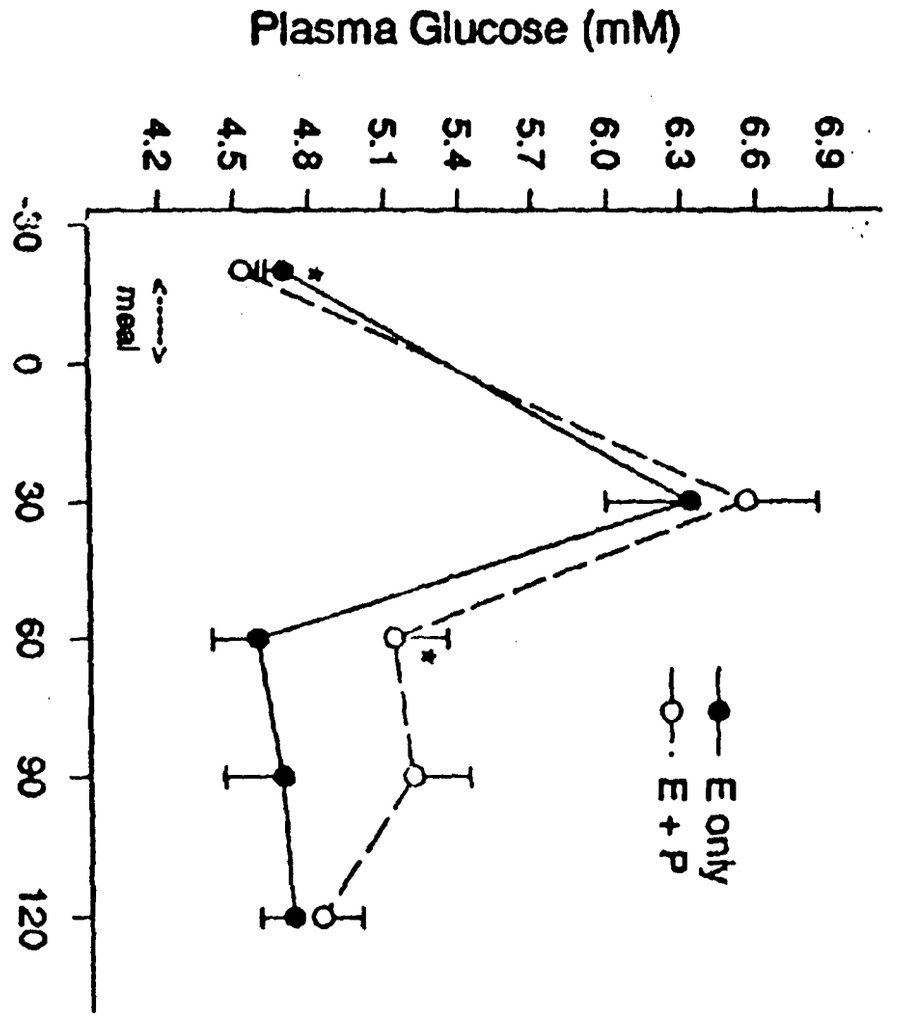

Plasma Glucose (mM)
4.2
4.5
4.8
5.1
5.4
5.7
6.0
6.3
6.6
6.9
-30
0
30
60
90
120
TIME (minutes)
<-----> meal
E only
E + P

Figure 7

Figure 8

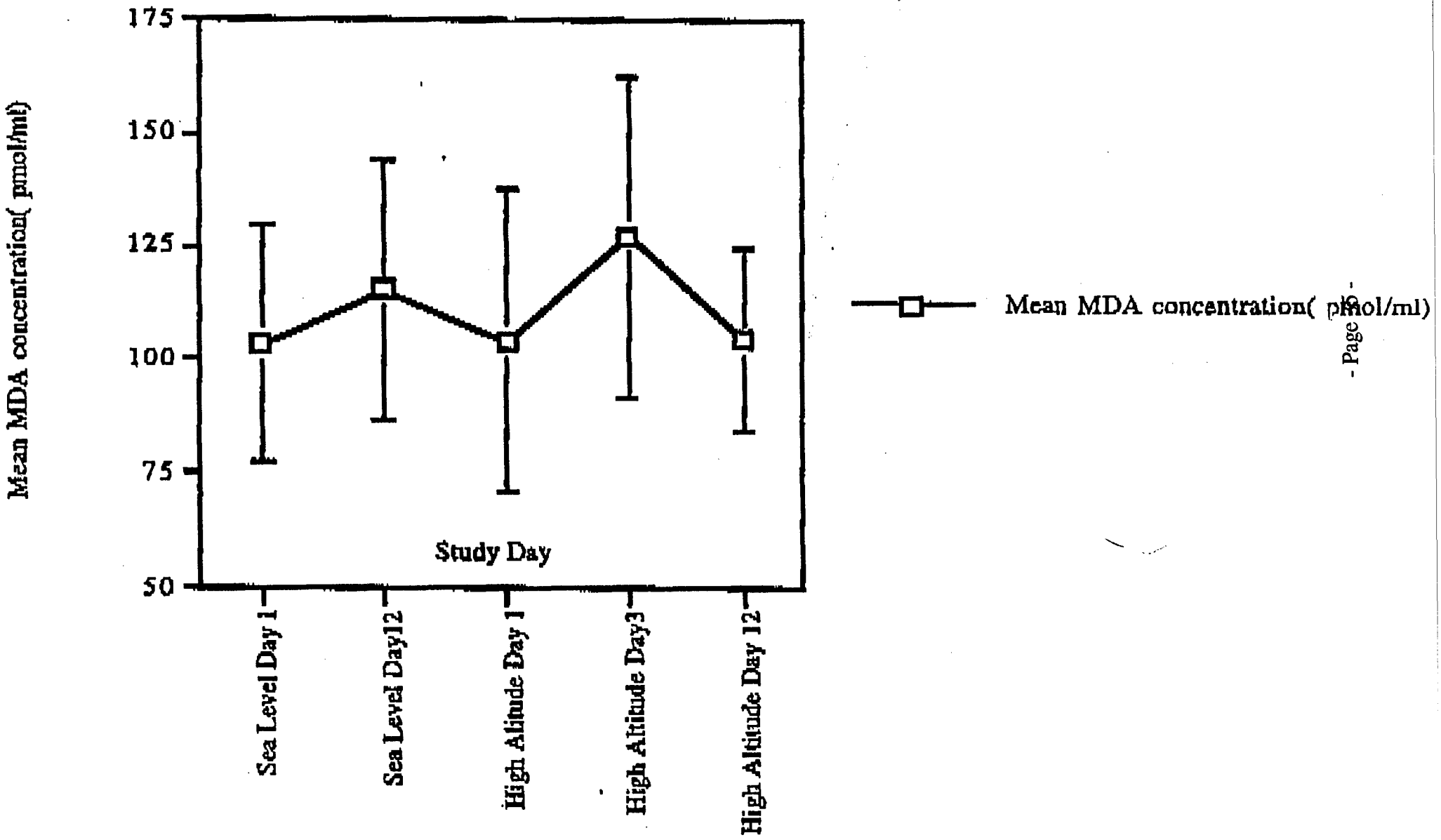

Mean MDA concentration(pmol/ml)
175
150
125
100
75
50
Study Day
Sea Level Day 1
Sea Level Day12
High Altitude Day 1
High Altitude Day3
High Altitude Day 12
Mean MDA concentration(pmol/ml)

Figure 9

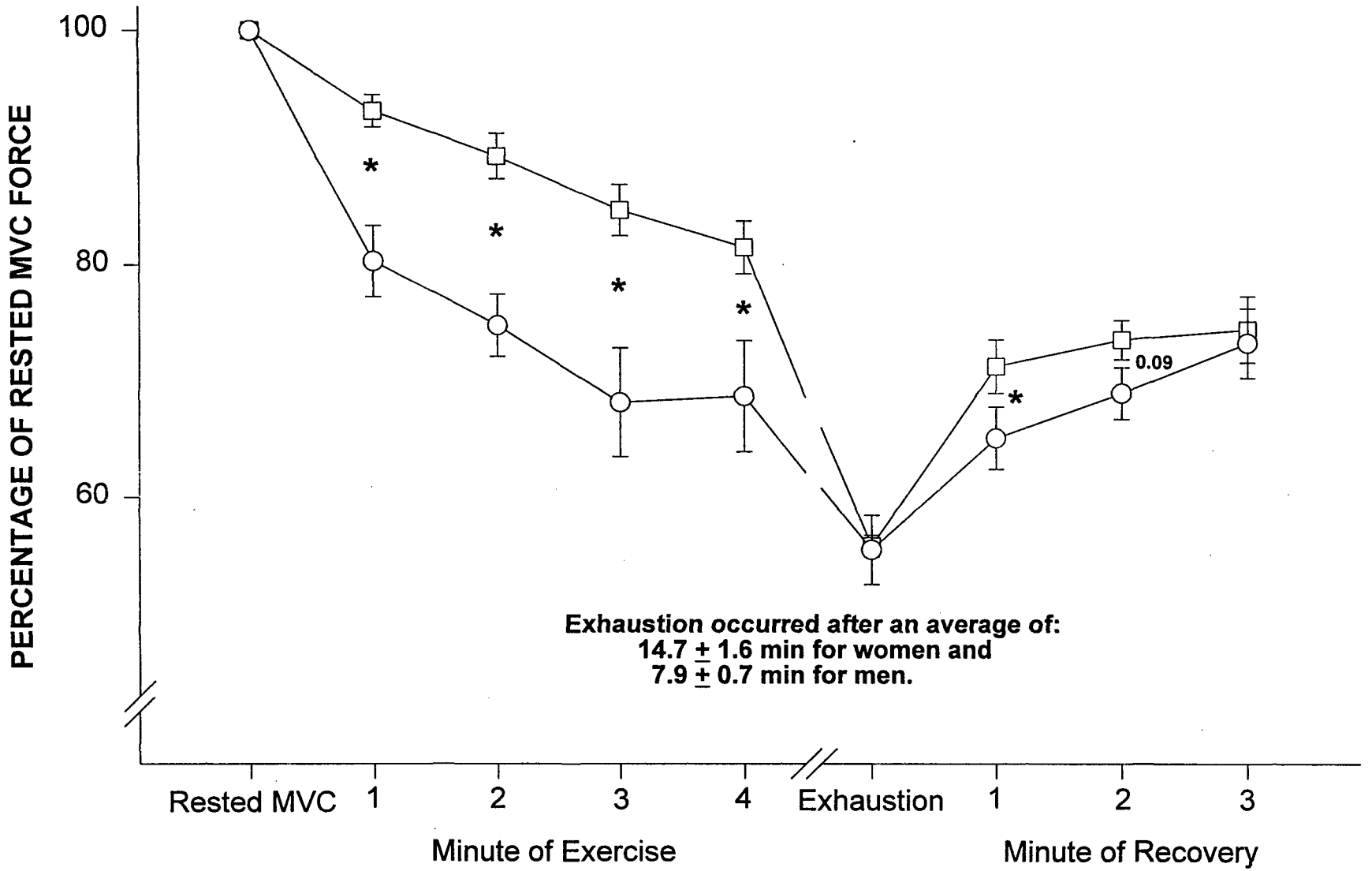
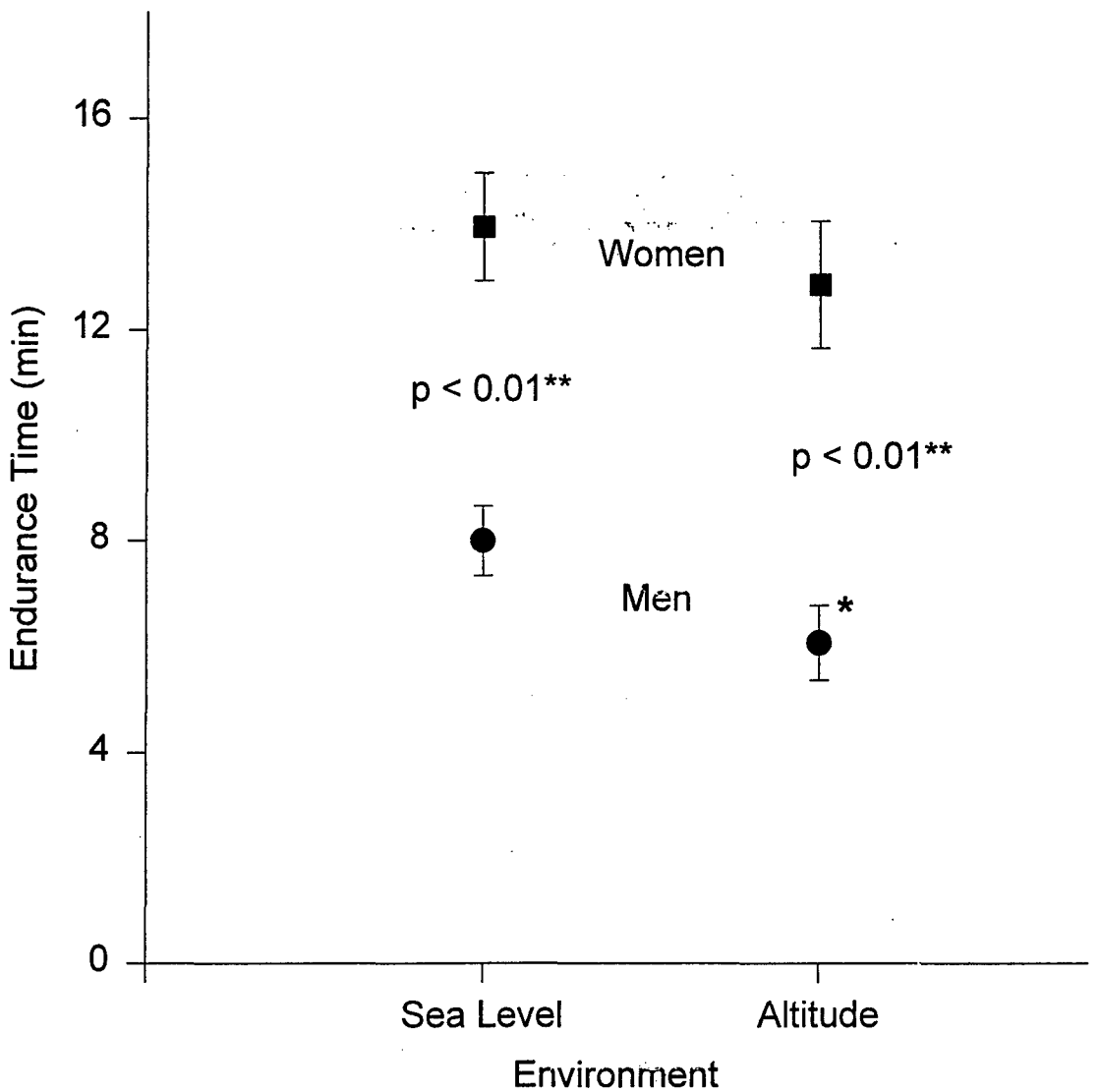

PERCENTAGE OF RESTED MVC FORCE
100
80
60
*
*
*
*
*
0.09
Exhaustion occurred after an average of:
14.7 ± 1.6 min for women and
7.9 ± 0.7 min for men.
Rested MVC
1
2
3
4
Exhaustion
1
2
3
Minute of Exercise
Minute of Recovery

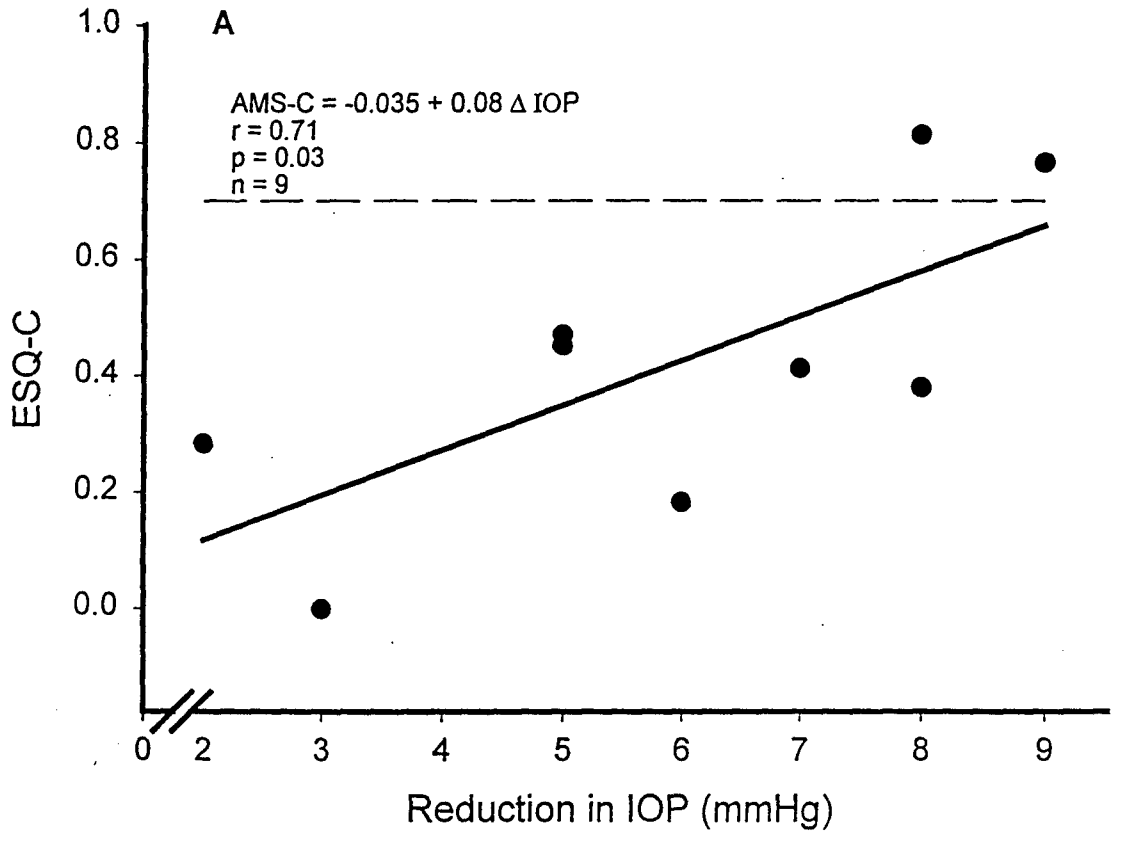
Figure 10

Adductor Pollicis Muscle Endurance Time
Women Compared to Men



*(p = .01, SL vs HA)
** (p < 0.01, Men vs Women)

Figure 11


A
AMS-C = -0.035 + 0.08 Δ IOP
r = 0.71
p = 0.03
n = 9
ESQ-C
1.0
0.8
0.6
0.4
0.2
0.0
0
2
3
4
5
6
7
8
9
Reduction in IOP (mmHg)

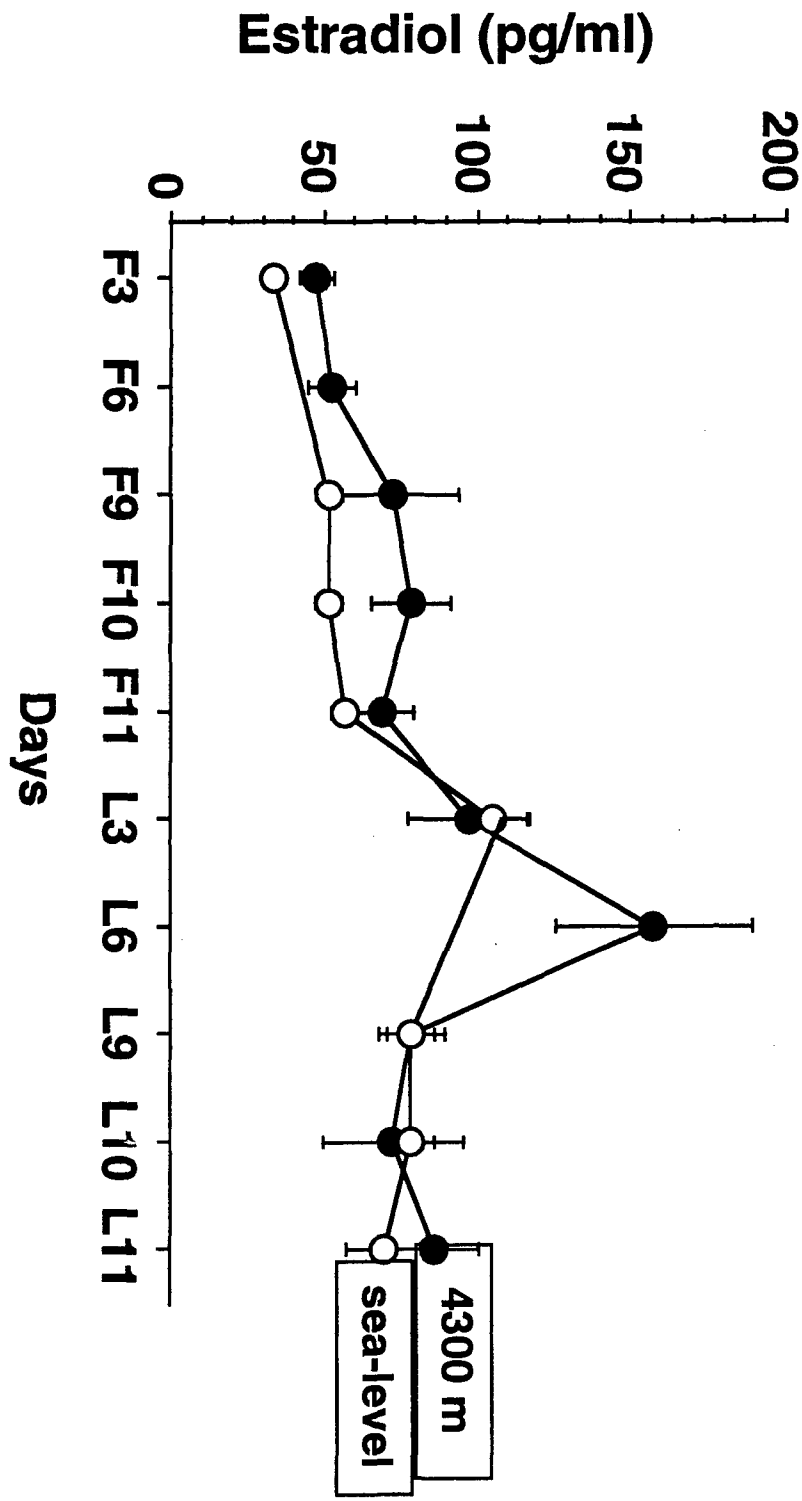

Estradiol (pg/ml)
0
50
100
150
200
F3
F6
F9
F10
F11
L3
L6
L9
L10
L11
Days
4300 m
sea-level

Figure 12

Figure 13

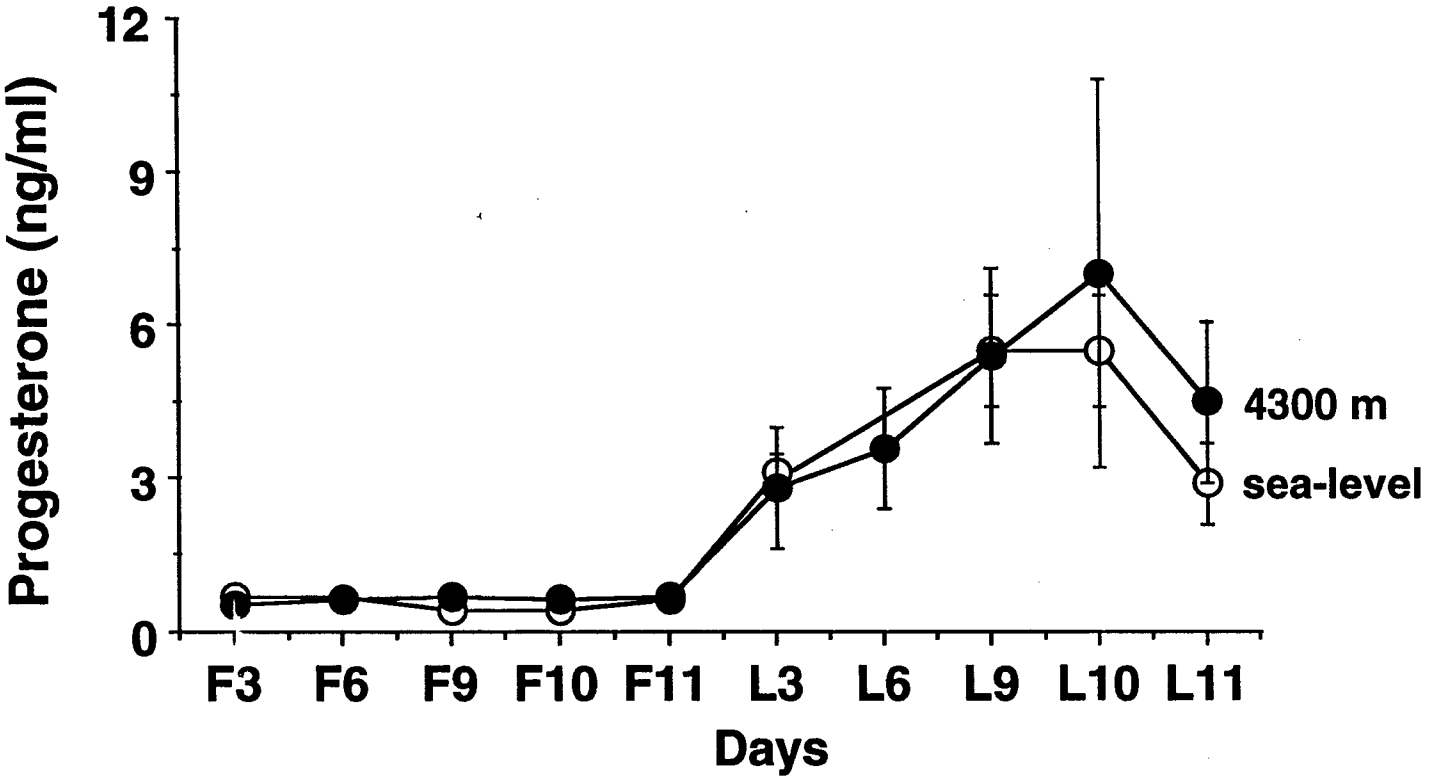

Progesterone (ng/ml)
12
9
6
3
0
F3
F6
F9
F10
F11
L3
L6
L9
L10
L11
Days
4300 m
sea-level

Figure 14

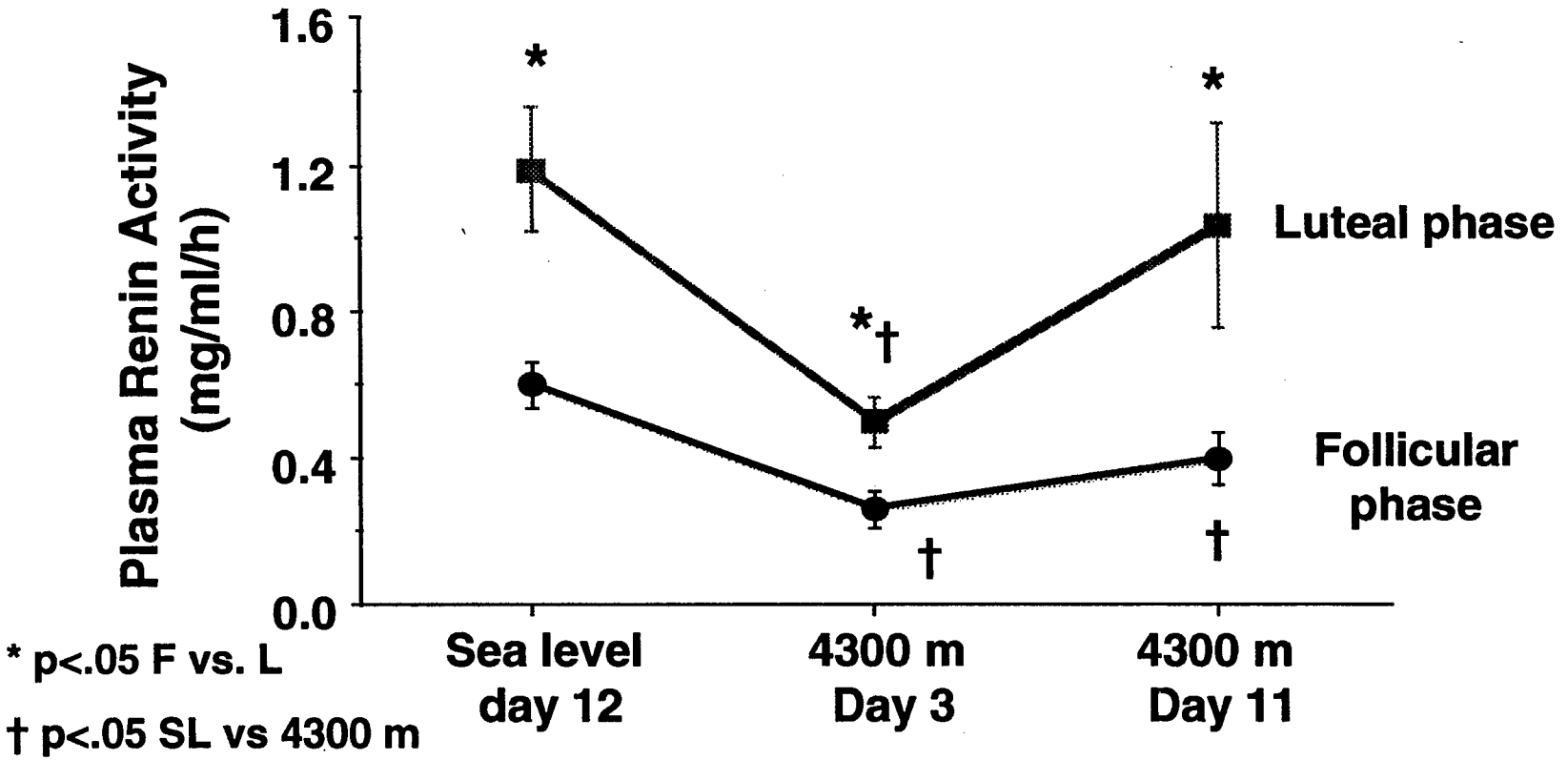
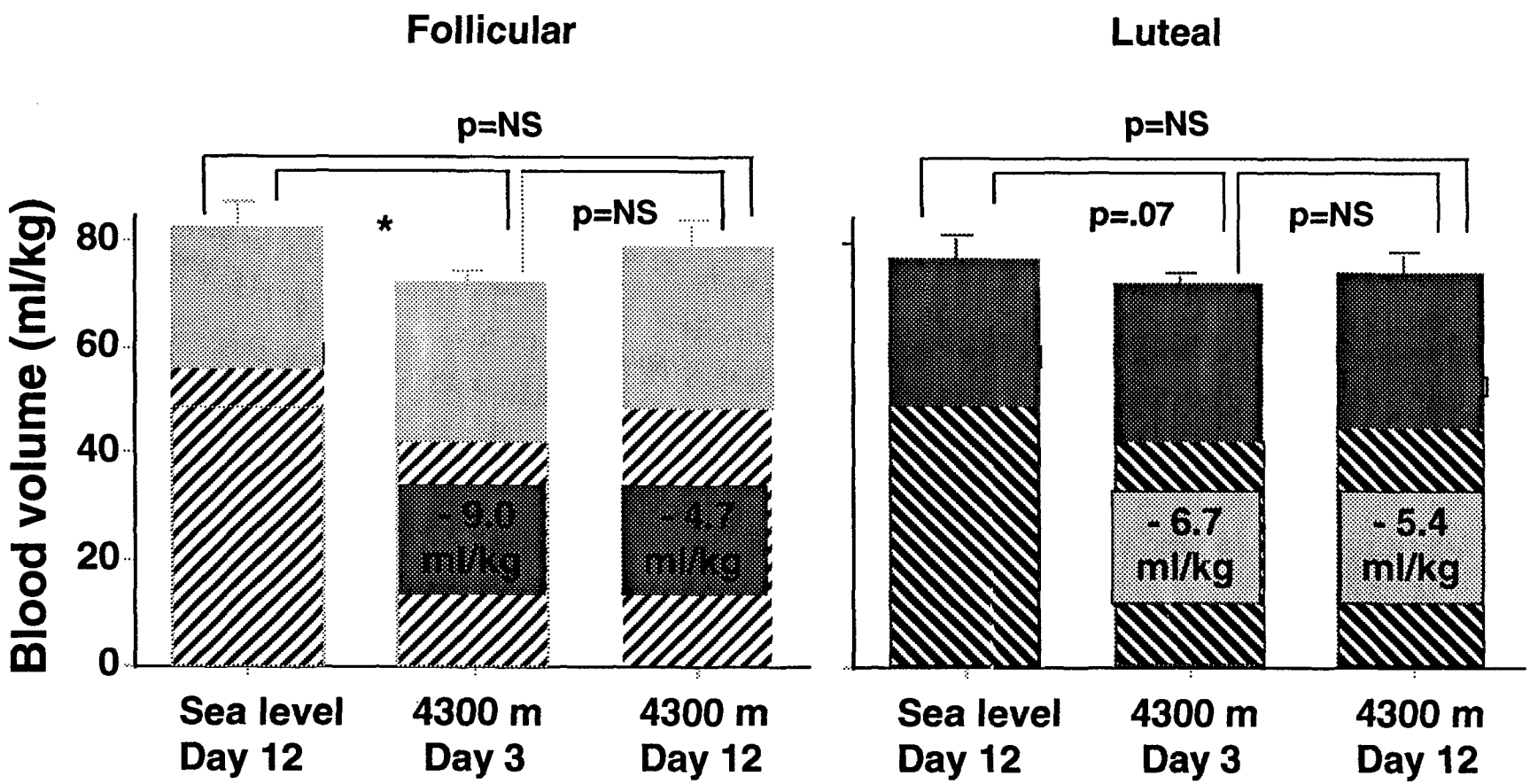
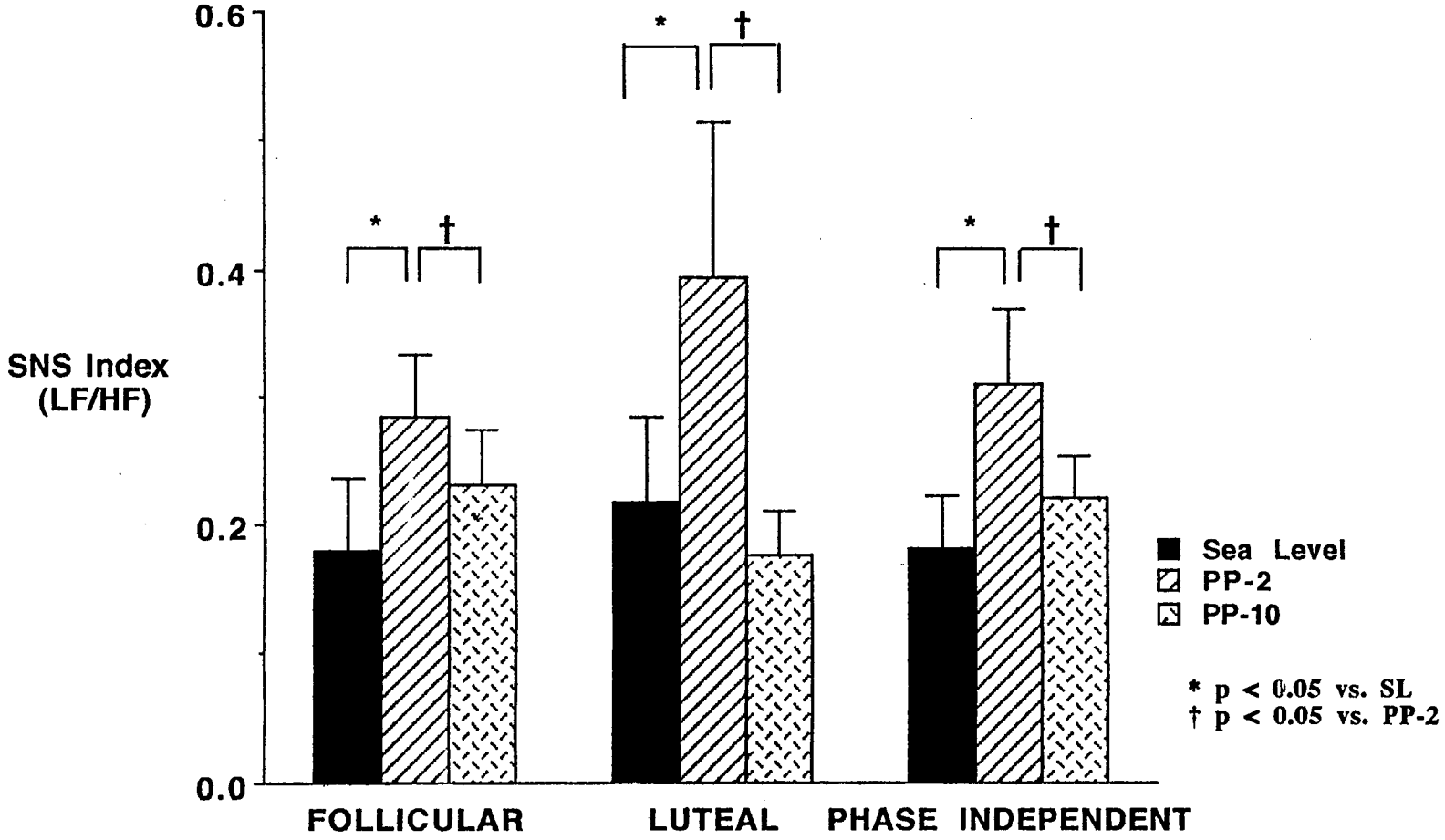

Plasma Renin Activity (mg/ml/h)
1.6
1.2
0.8
0.4
0.0
*
*
*†
†
†
Luteal phase
Follicular phase
Sea level day 12
4300 m Day 3
4300 m Day 11
* p<.05 F vs. L
† p<.05 SL vs 4300 m

Figure 15


Follicular
Luteal
p=NS
p=NS
*
p=NS
p=.07
p=NS
Blood volume (ml/kg)
80
60
40
20
0
- 9.0 ml/kg
- 4.7 ml/kg
- 6.7 ml/kg
- 5.4 ml/kg
Sea level Day 12
4300 m Day 3
4300 m Day 12
Sea level Day 12
4300 m Day 3
4300 m Day 12

SNS Index Increases with Early Hypoxic Exposure and Returns to Near Sea Level Values after 10 days in Women at 4,300 m Altitude



PNS Index Falls with Early Exposure to Hypoxia but Returns To Sea Level Values after 10 days at 4,300 m

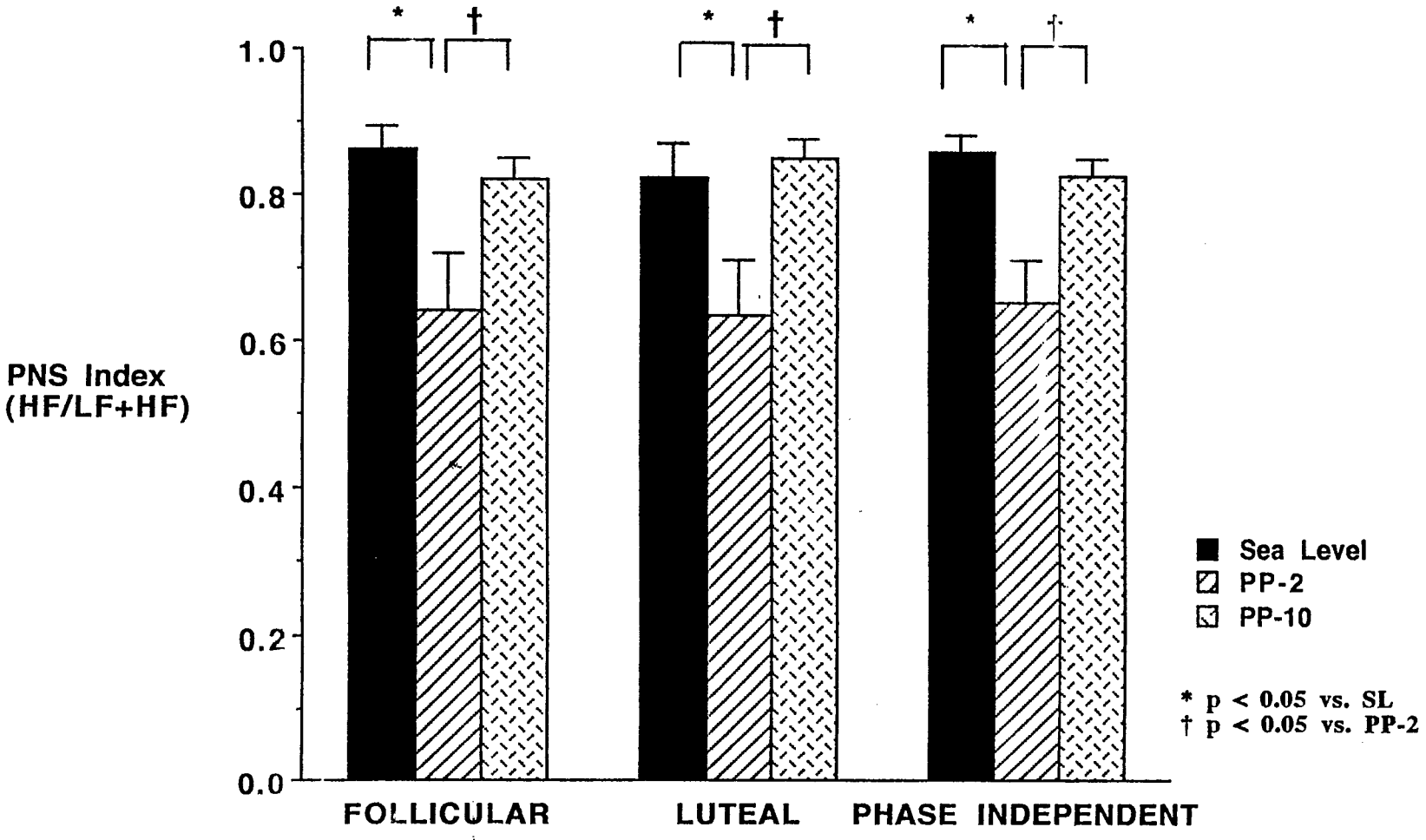
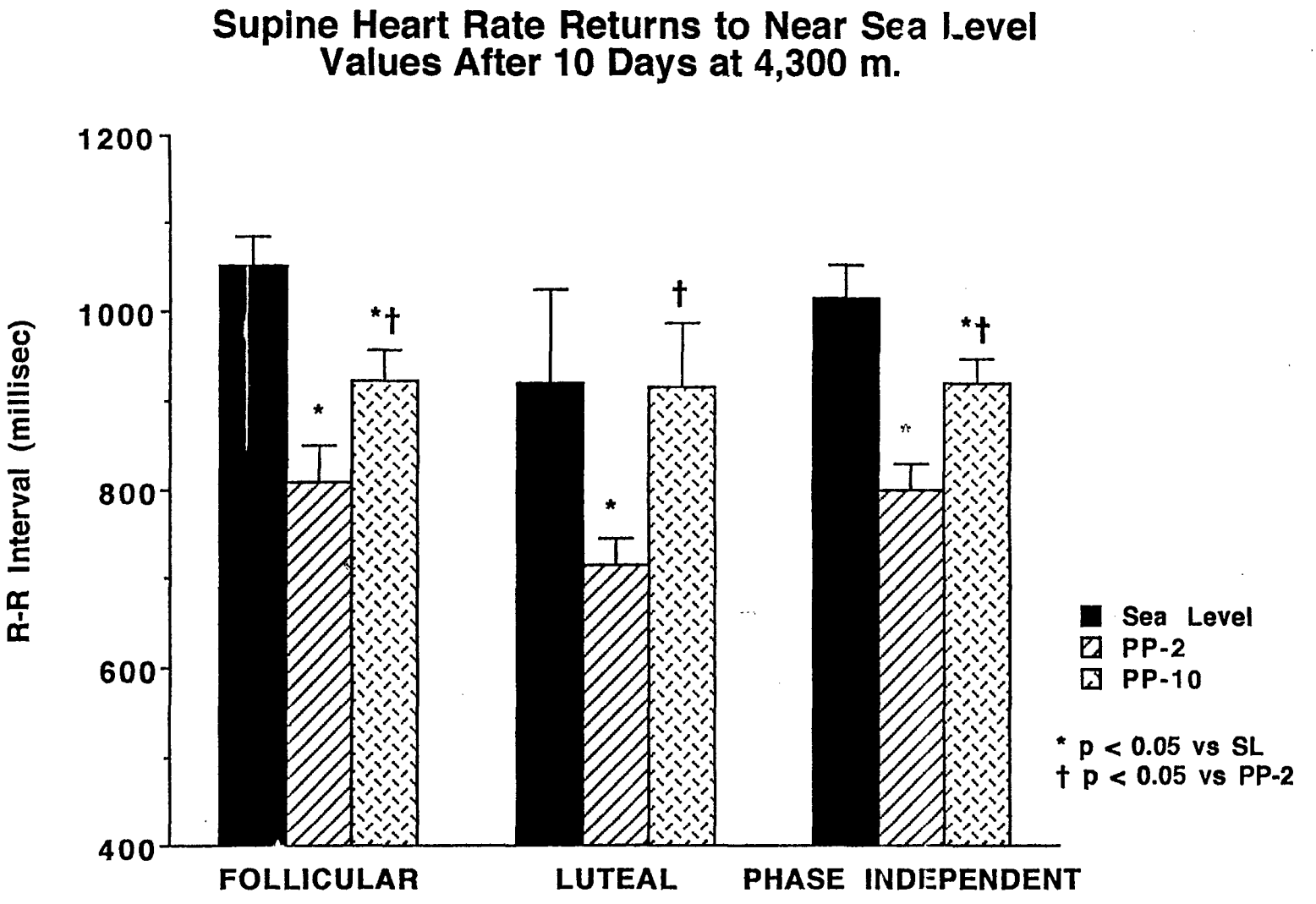
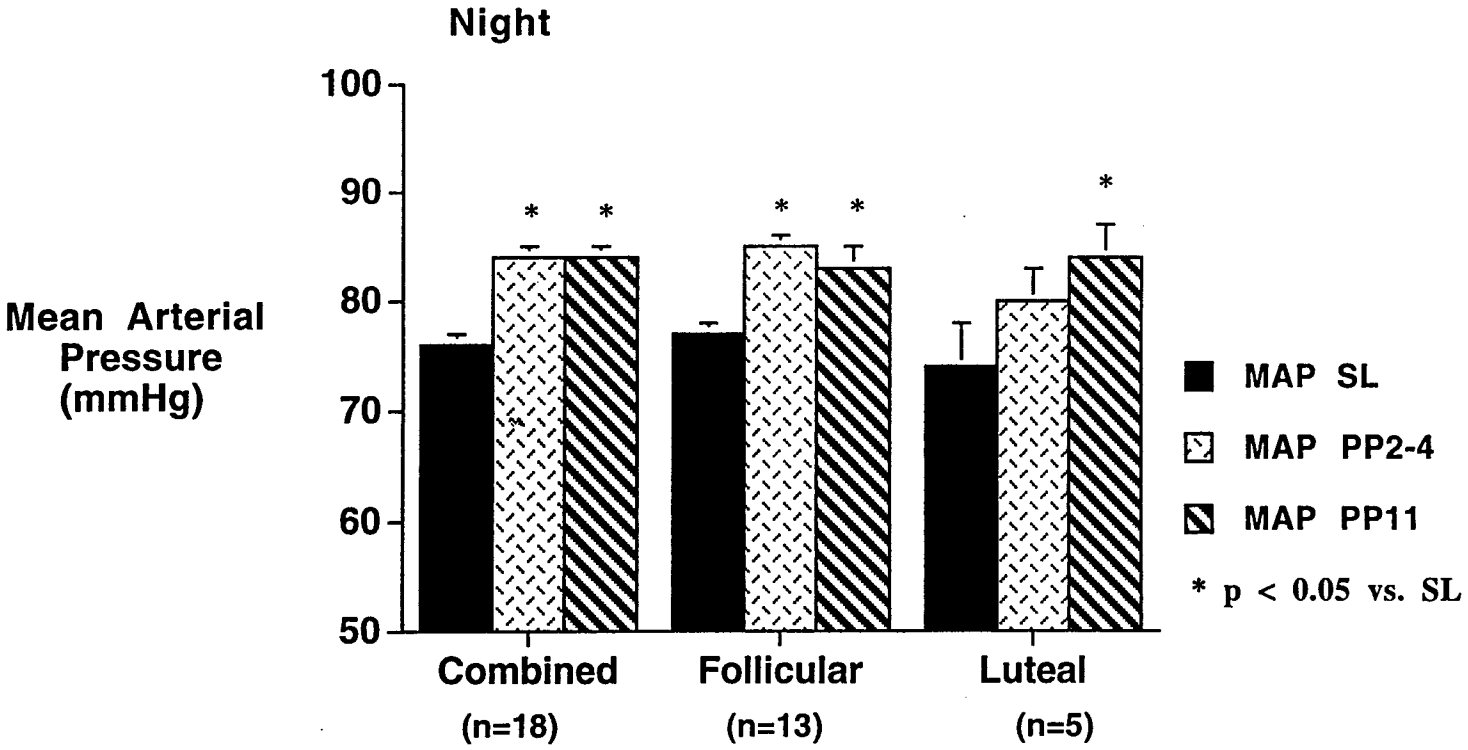


Figure 18


Supine Heart Rate Returns to Near Sea Level Values After 10 Days at 4,300 m.
R-R Interval (millisec)
1200
1000
800
600
400
*†
*
†
*
*†
*
FOLLICULAR
LUTEAL
PHASE INDEPENDENT
Sea Level
PP-2
PP-10
* p < 0.05 vs SL
† p < 0.05 vs PP-2

Cycle Independent Increase in Nocturnal Mean Arterial Pressure at High Altitude

(Combined Data - 1996 and 1998)



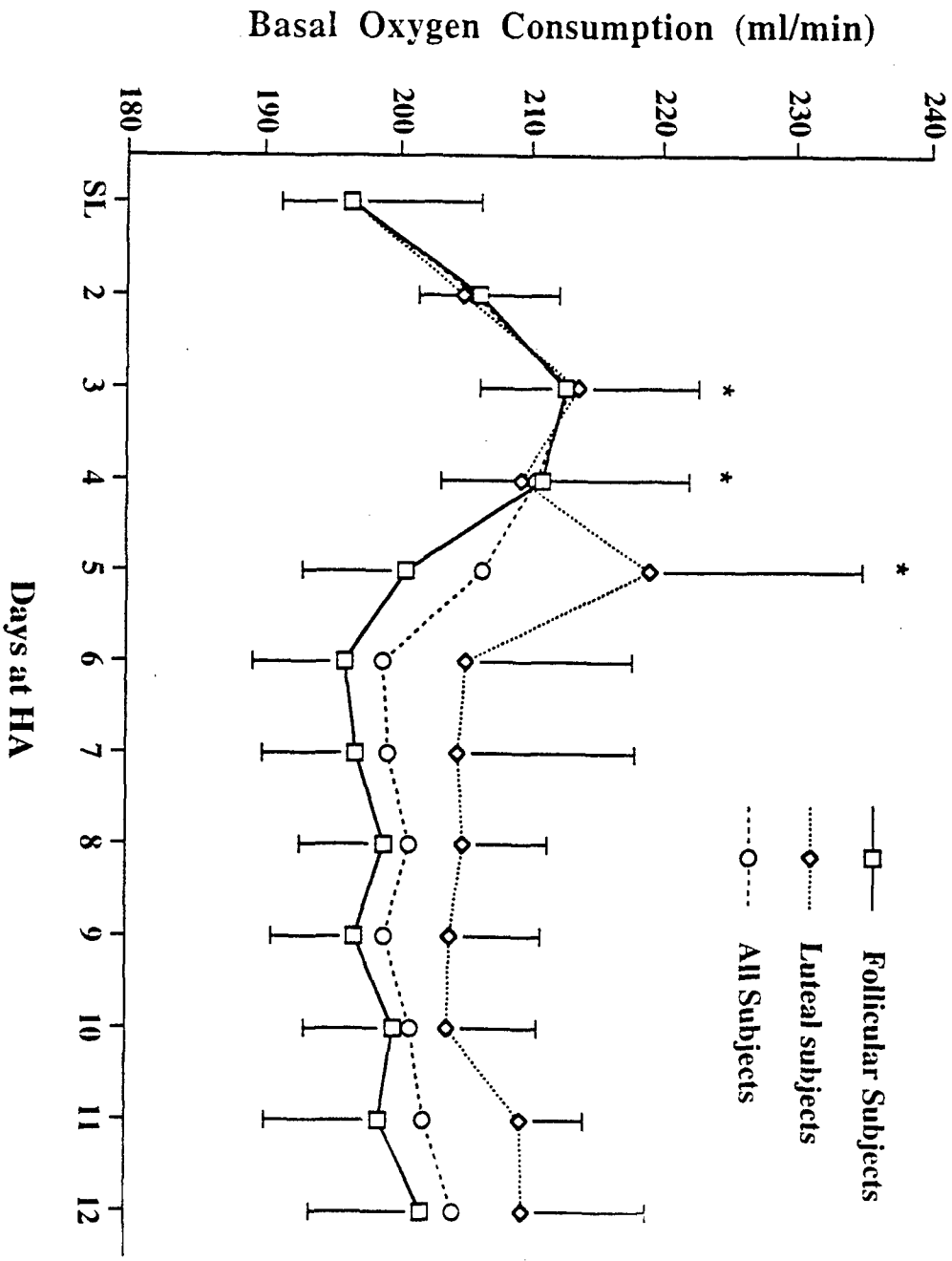

Basal Oxygen Consumption (ml/min)
180
190
200
210
220
230
240
SL
2
3
4
5
6
7
8
9
10
11
12
Days at HA
*
*
*
Follicular Subjects
Luteal subjects
All Subjects

Figure 20

Figure 21

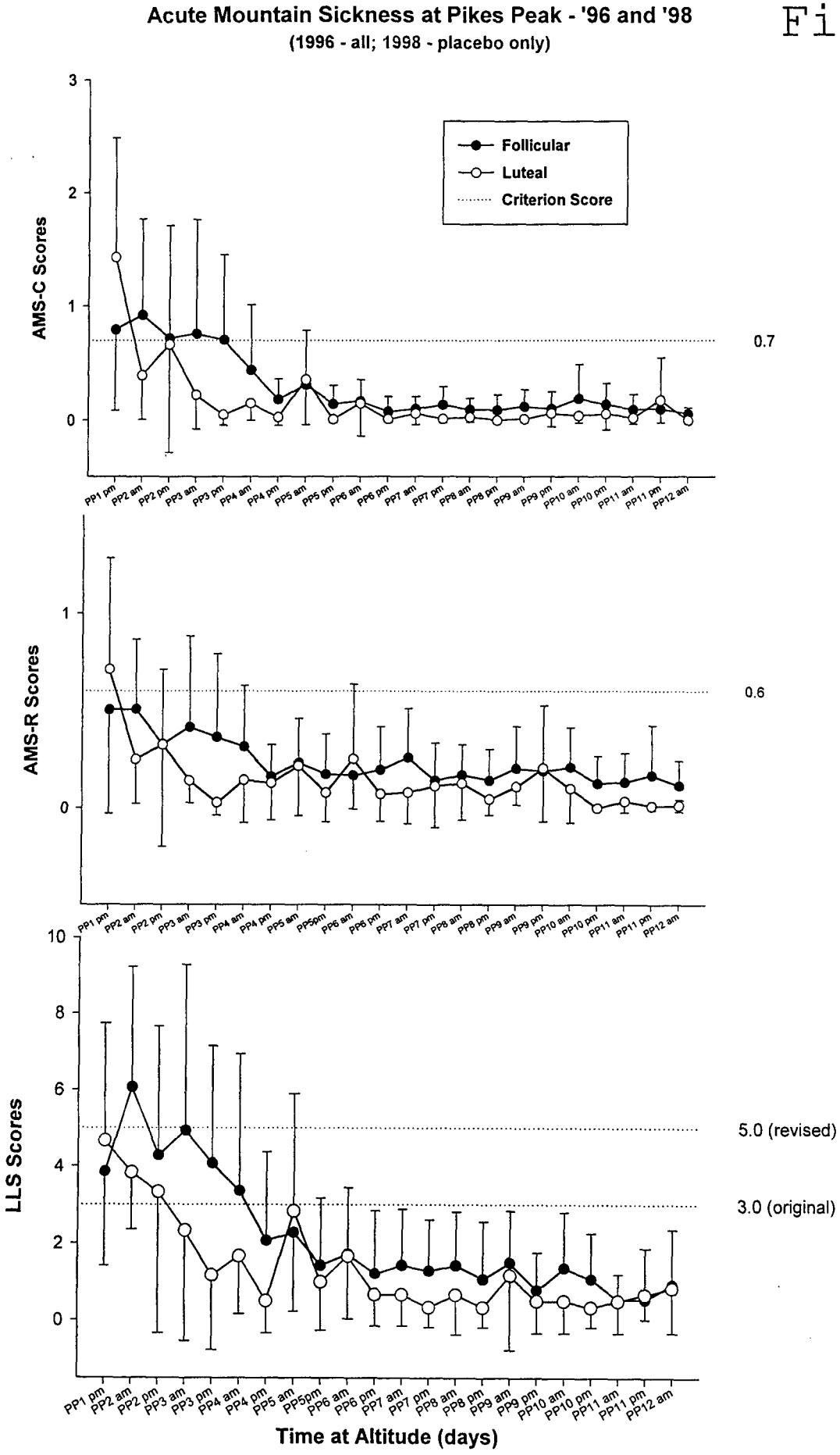

Acute Mountain Sickness at Pikes Peak - '96 and '98
(1996 - all; 1998 - placebo only)
Follicular
Luteal
Criterion Score
AMS-C Scores
0.7
AMS-R Scores
0.6
LLS Scores
5.0 (revised)
3.0 (original)
Time at Altitude (days)

Figure 22

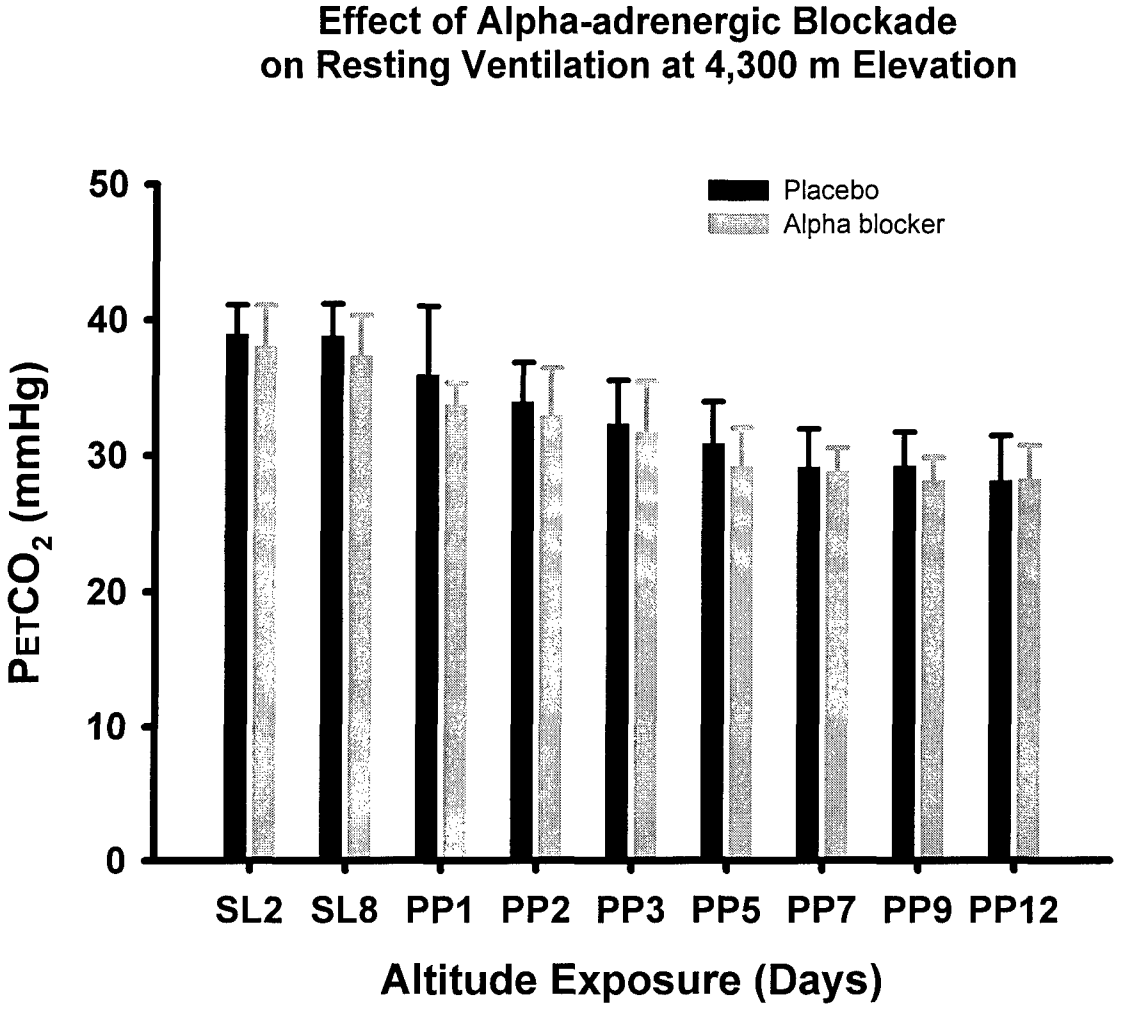

Effect of Alpha-adrenergic Blockade
on Resting Ventilation at 4,300 m Elevation
Placebo
Alpha blocker
50
40
30
20
10
0
PETCO2 (mmHg)
SL2
SL8
PP1
PP2
PP3
PP5
PP7
PP9
PP12
Altitude Exposure (Days)

Figure 23

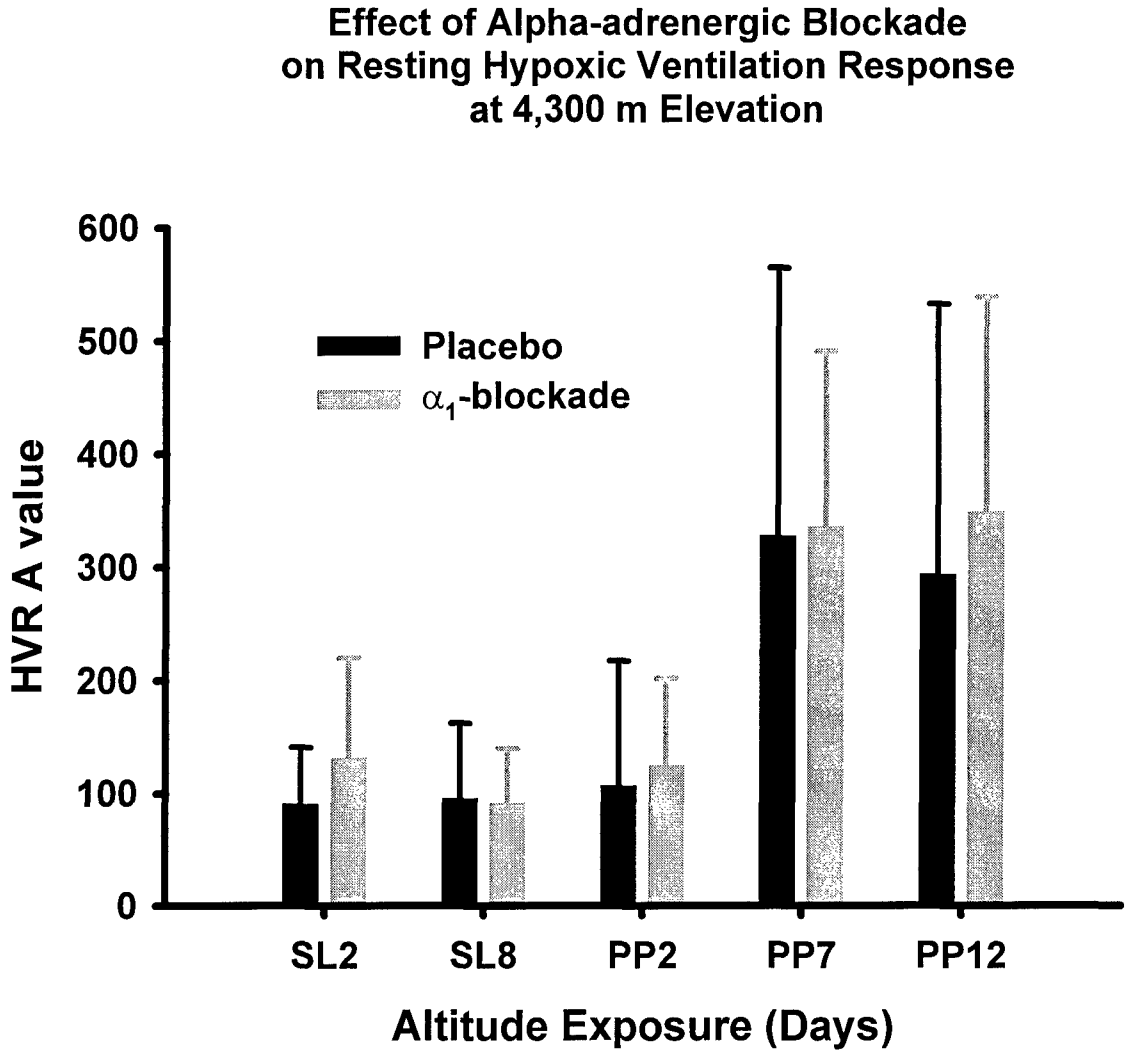

Effect of Alpha-adrenergic Blockade
on Resting Hypoxic Ventilation Response
at 4,300 m Elevation
HVR A value
600
500
400
300
200
100
0
Placebo
α1-blockade
SL2
SL8
PP2
PP7
PP12
Altitude Exposure (Days)

Figure 24

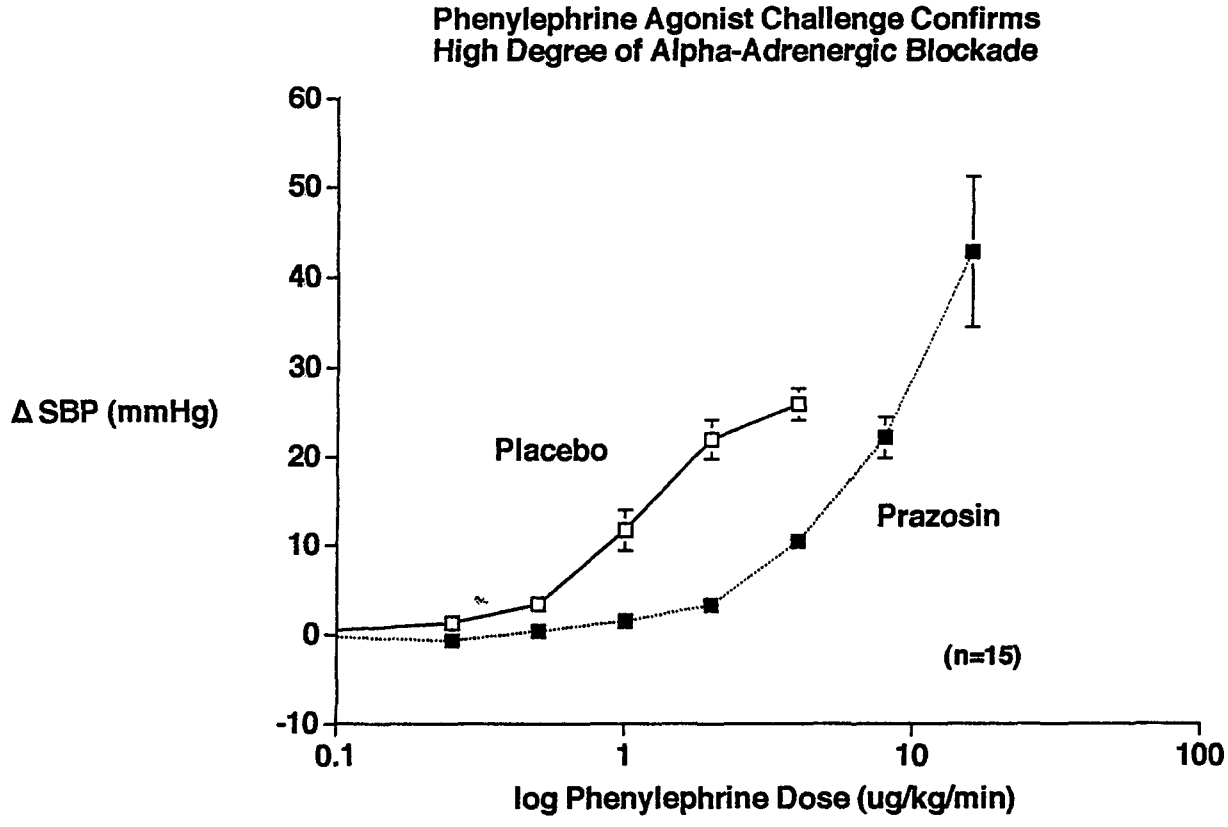

Phenylephrine Agonist Challenge Confirms
High Degree of Alpha-Adrenergic Blockade
Δ SBP (mmHg)
60
50
40
30
20
10
0
-10
Placebo
Prazosin
(n=15)
0.1
1
10
100
log Phenylephrine Dose (ug/kg/min)

Figure 25

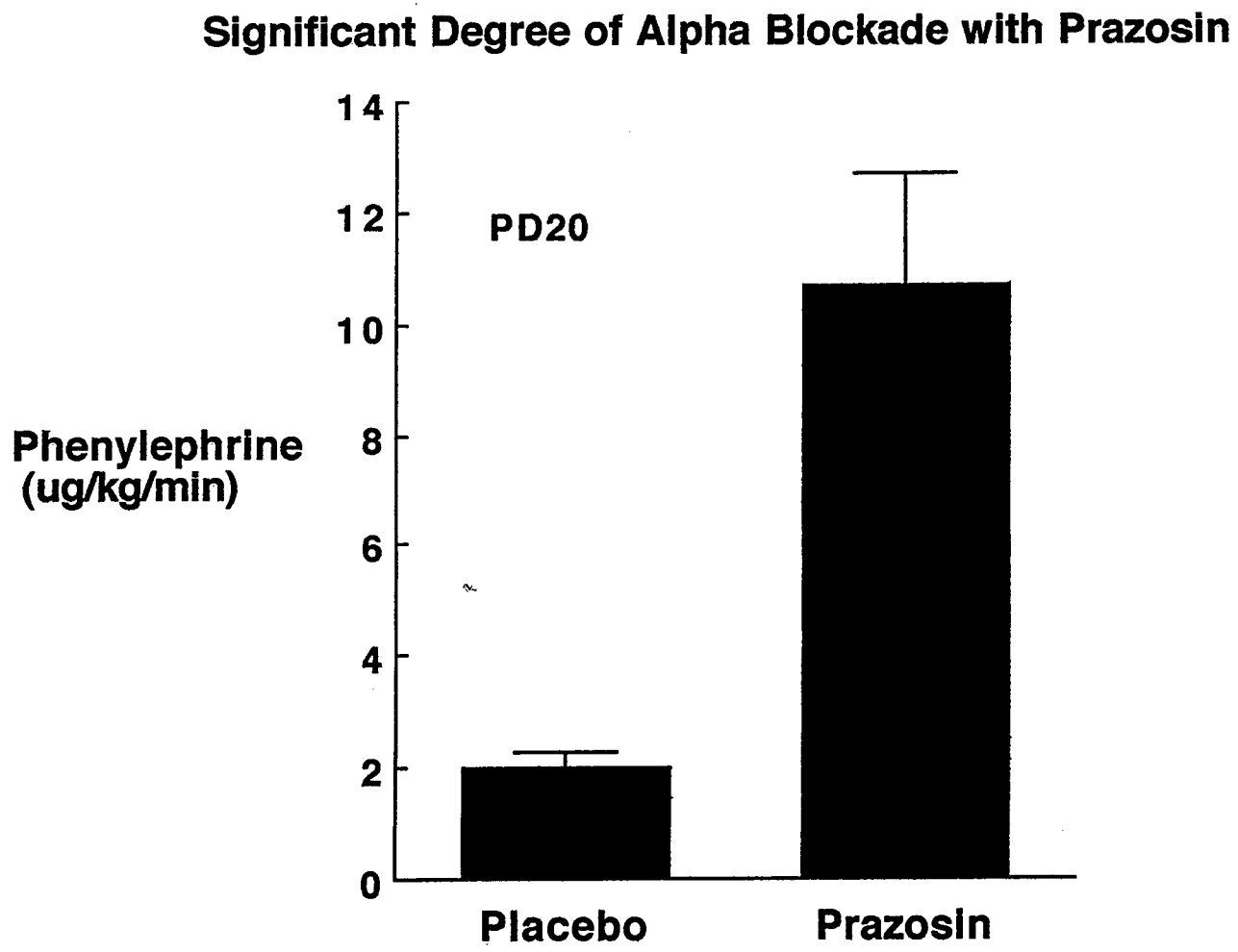
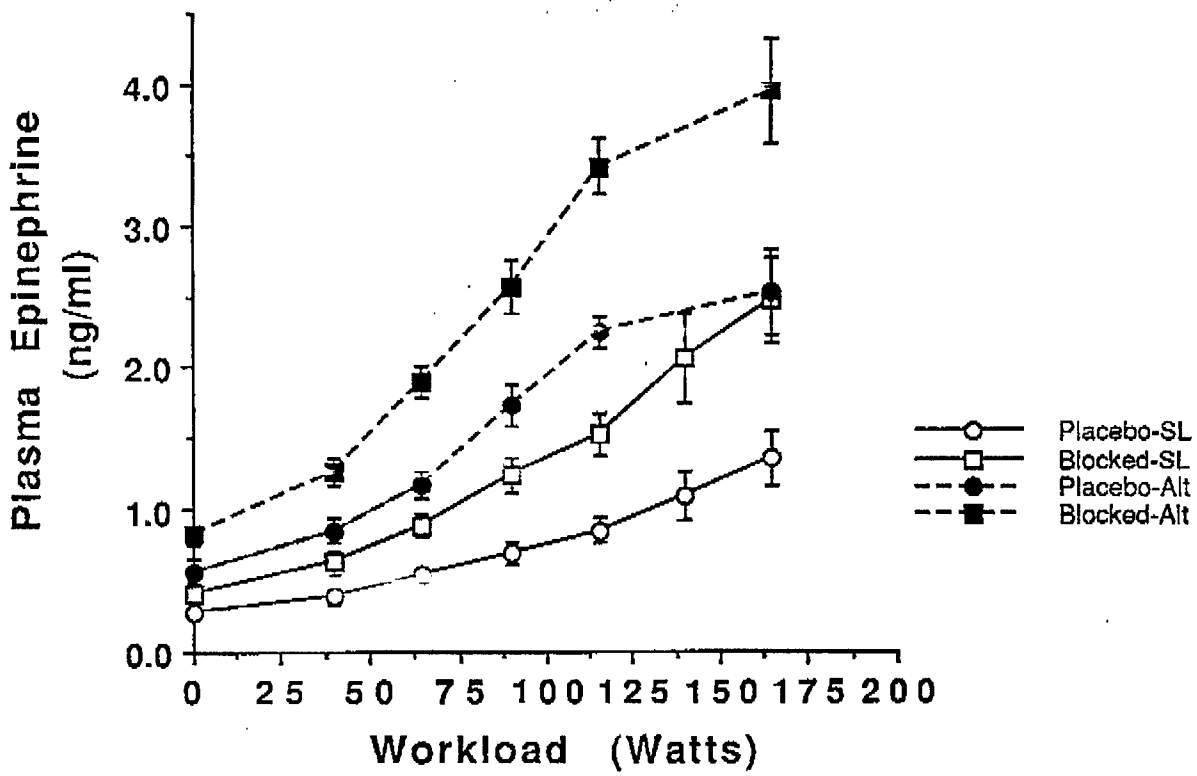

Significant Degree of Alpha Blockade with Prazosin
PD20
Phenylephrine (ug/kg/min)
14
12
10
8
6
4
2
0
Placebo
Prazosin

Figure 26


Plasma Epinephrine (ng/ml)
4.0
3.0
2.0
1.0
0.0
0 25 50 75 100 125 150 175 200
Workload (Watts)
Placebo-SL
Blocked-SL
Placebo-Alt
Blocked-Alt

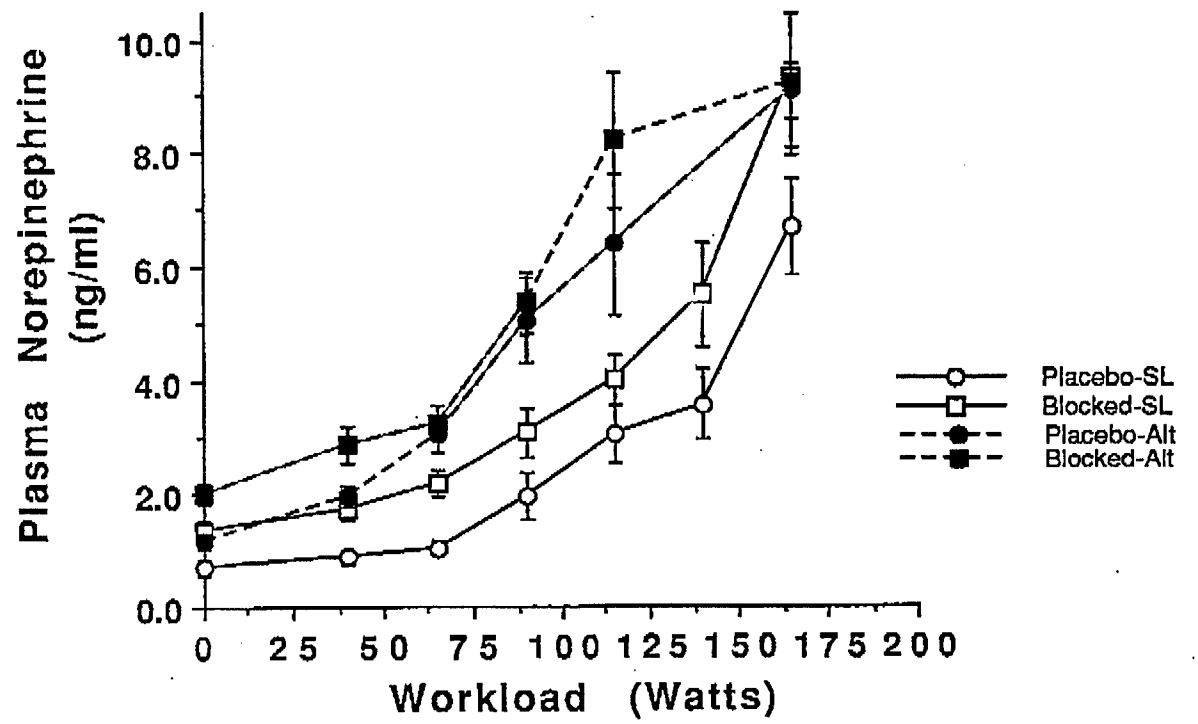

Plasma Norepinephrine (ng/ml)
10.0
8.0
6.0
4.0
2.0
0.0
0 25 50 75 100 125 150 175 200
Workload (Watts)
Placebo-SL
Blocked-SL
Placebo-Alt
Blocked-Alt

Figure 27

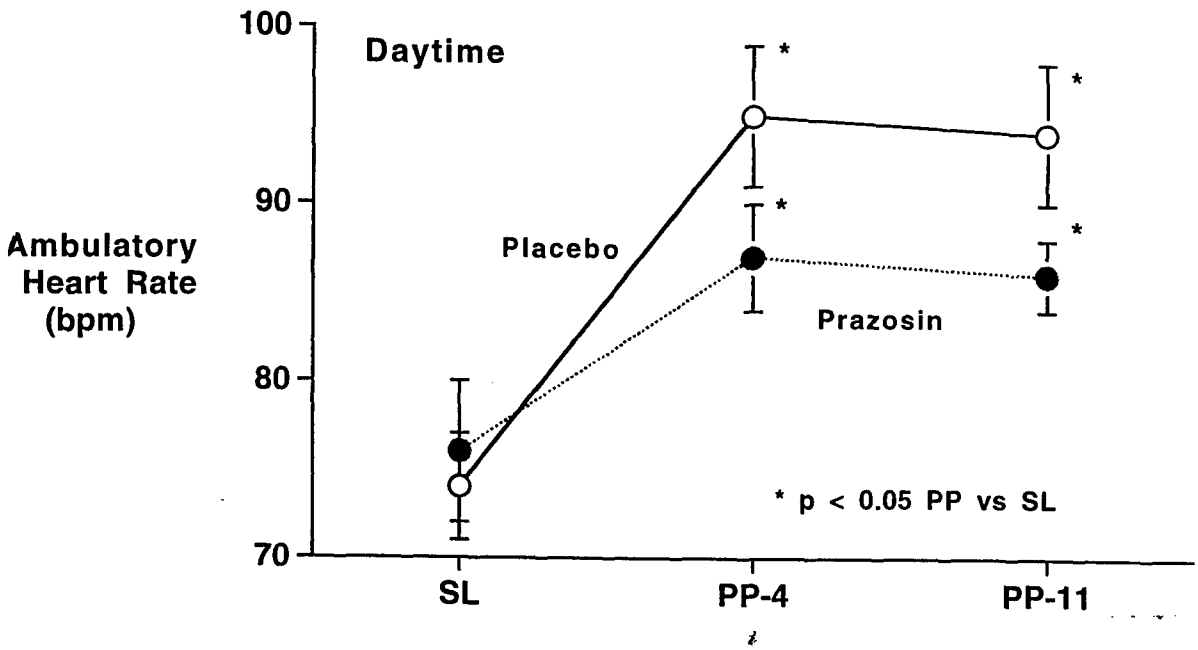
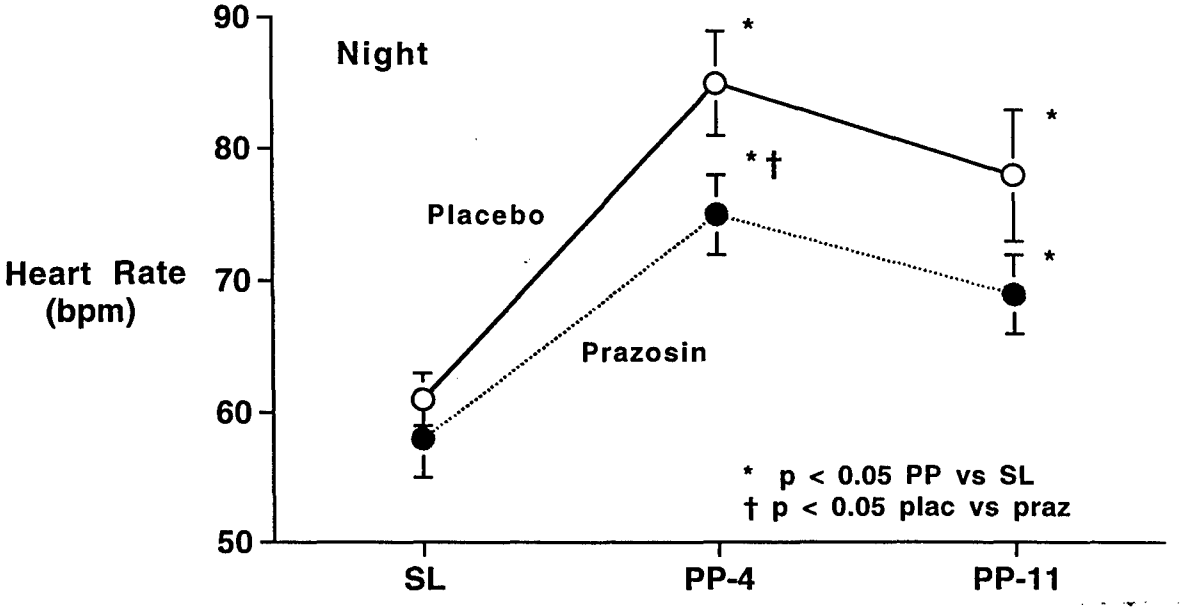
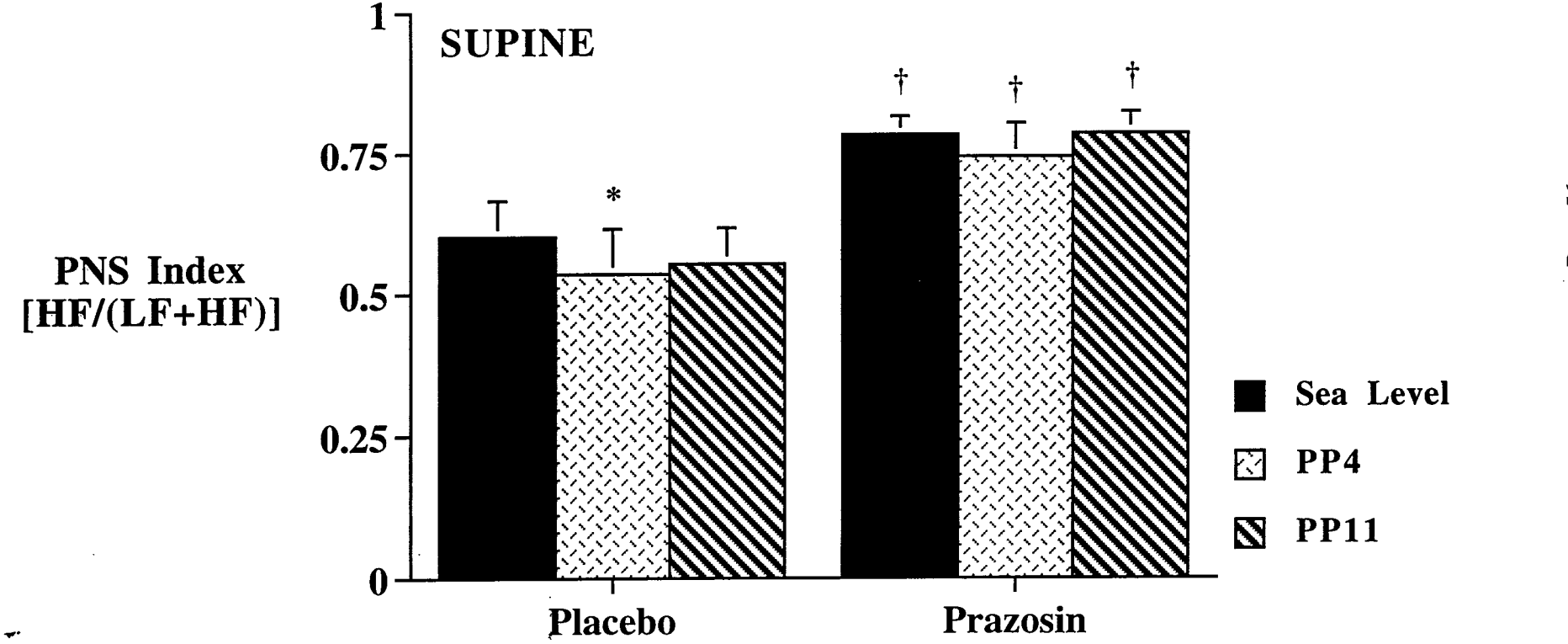

Ambulatory Heart Rate (bpm)
100
90
80
70
Daytime
Placebo
Prazosin
*
*
*
*
* p < 0.05 PP vs SL
SL
PP-4
PP-11

Figure 28


Night
Placebo
Prazosin
Heart Rate (bpm)
90
80
70
60
50
SL
PP-4
PP-11
* p < 0.05 PP vs SL
† p < 0.05 plac vs praz

Parasympathetic Index Increased With Alpha Receptor Blockade Both at Sea Level and 4,300 m Altitude



* p < 0.05 vs. Sea Level within group
† p < 0.05 vs. Placebo

Figure 30

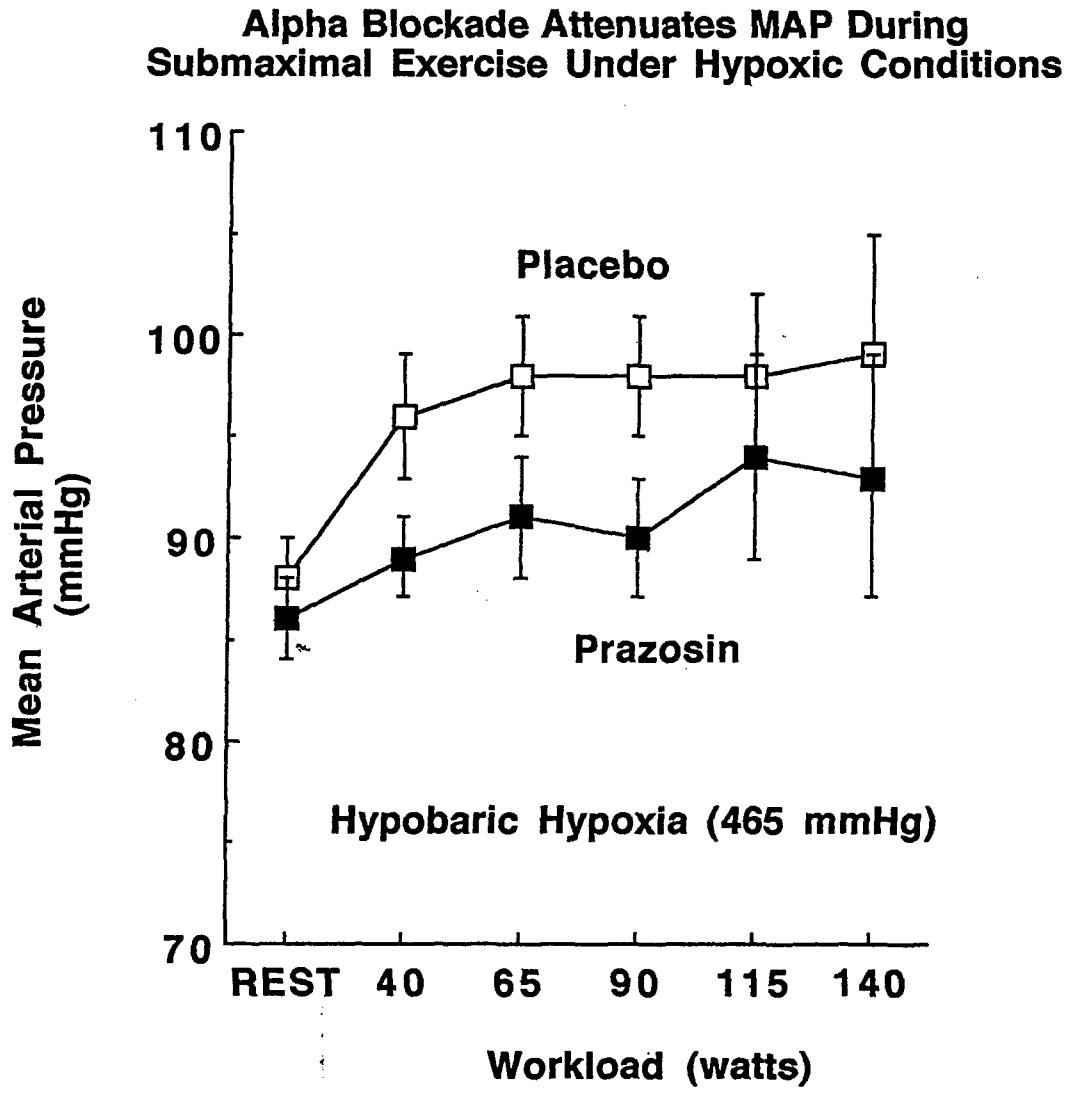
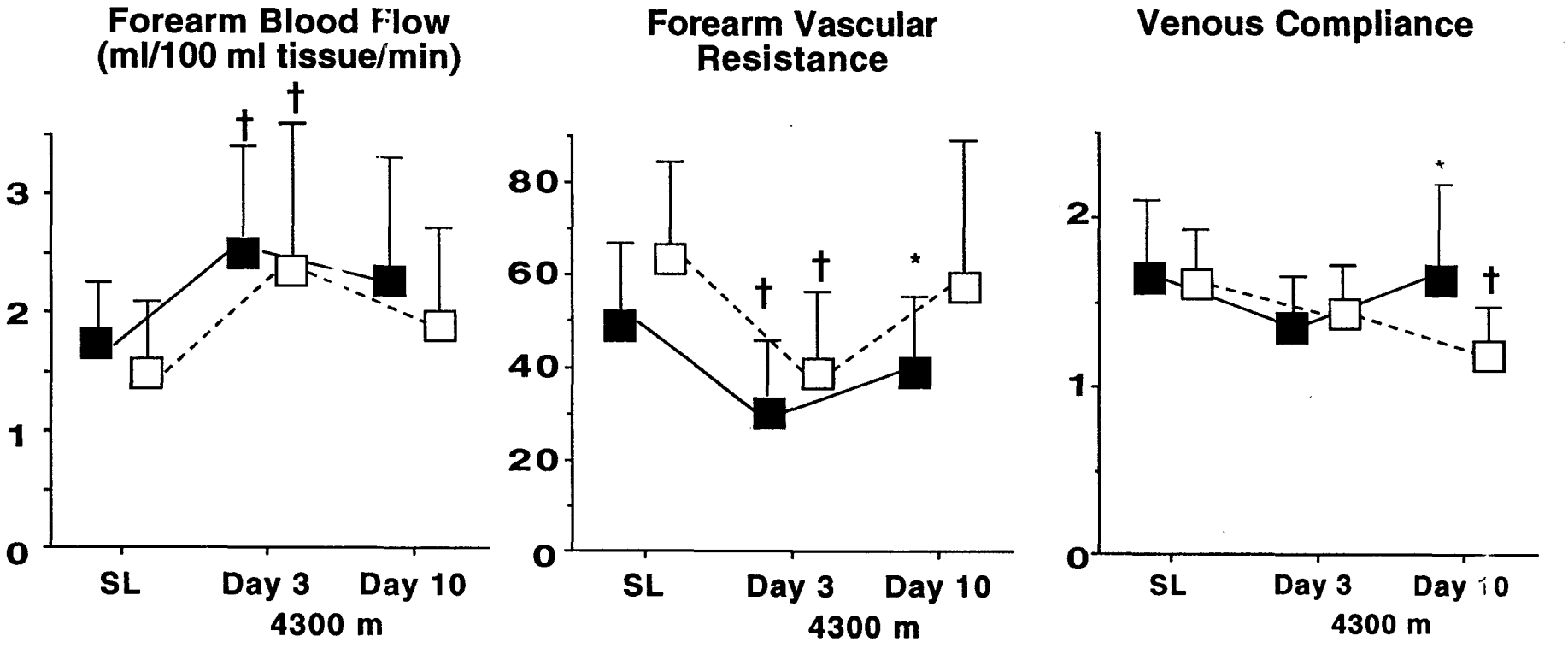

Alpha Blockade Attenuates MAP During
Submaximal Exercise Under Hypoxic Conditions
Mean Arterial Pressure (mmHg)
110
100
90
80
70
Placebo
Prazosin
Hypobaric Hypoxia (465 mmHg)
REST
40
65
90
115
140
Workload (watts)

Figure 31

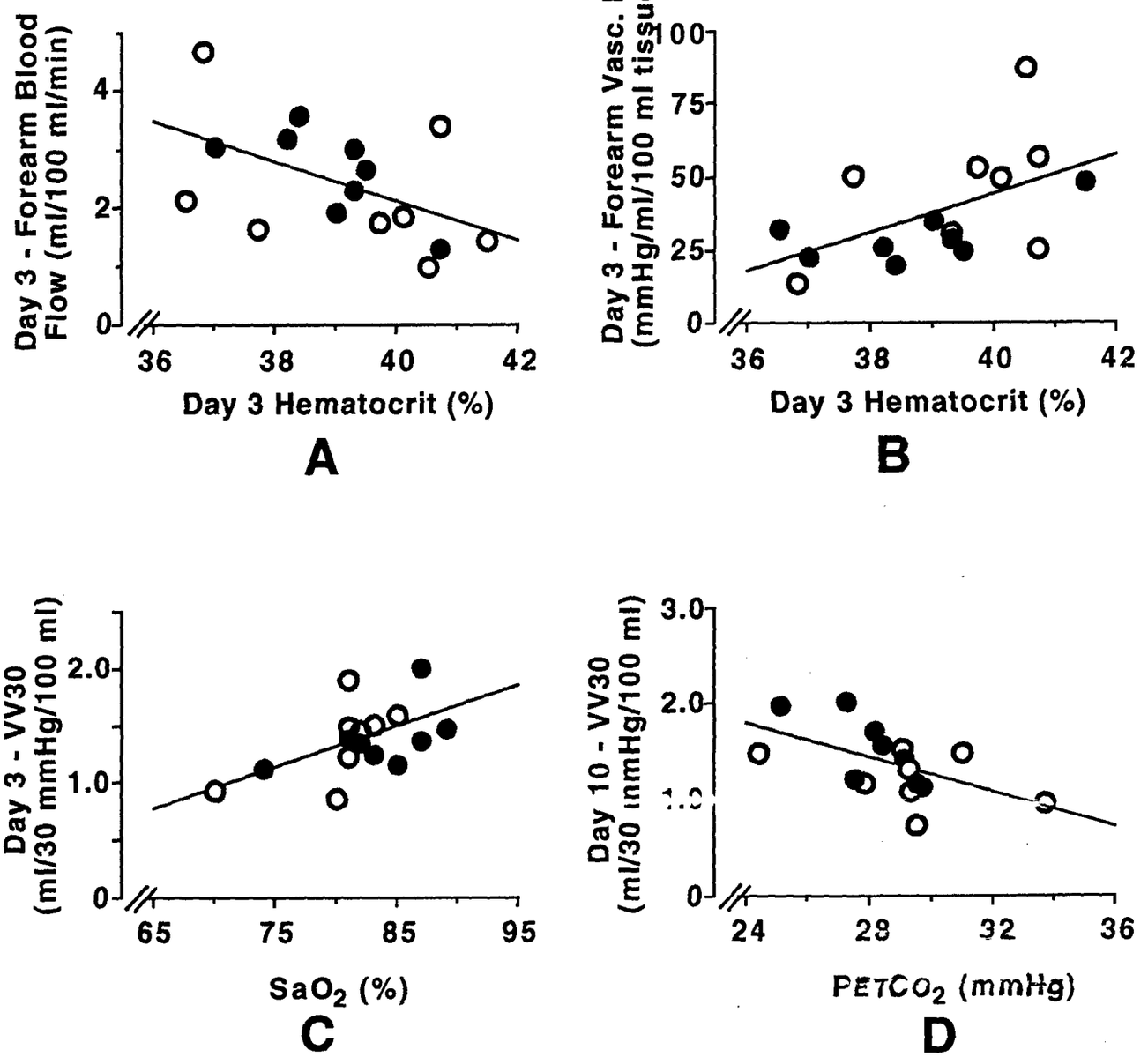


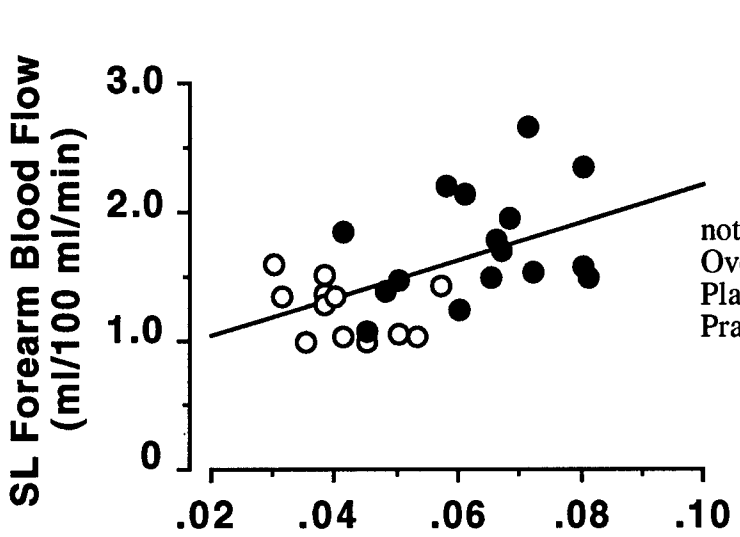
† p<.05 sea-level vs altitude within drug group on a particular day

* p<.05 prazosin vs placebo on a given day

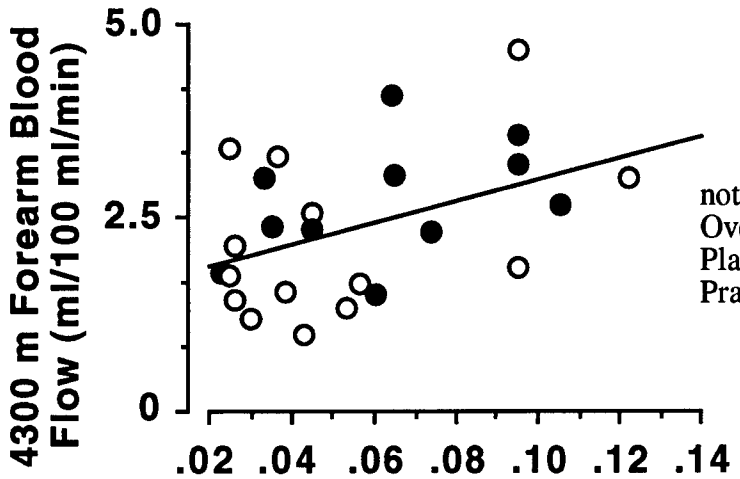
■ Prazosin □ Placebo

Figure 32

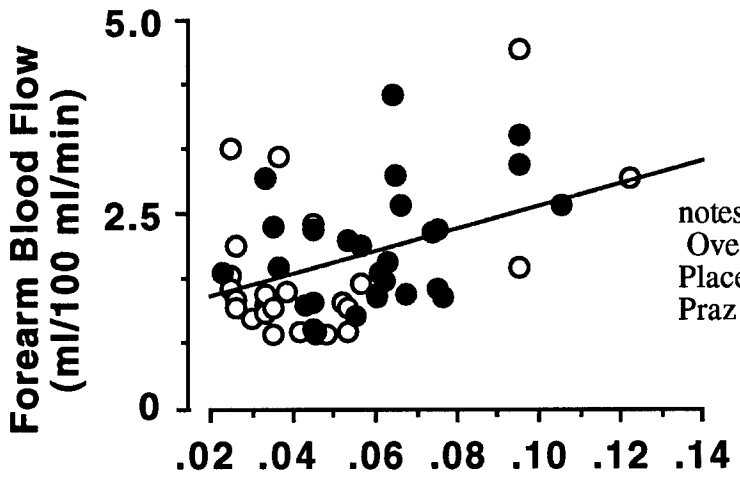

Day 3 - Forearm Blood Flow (ml/100 ml/min)
0
2
4
36
38
40
42
Day 3 Hematocrit (%)
A
Day 3 - Forearm Vasc. Resis. (mmHg/ml/100 ml tissue/min)
0
25
50
75
100
36
38
40
42
Day 3 Hematocrit (%)
B
Day 3 - VV30 (ml/30 mmHg/100 ml)
0
1.0
2.0
65
75
85
95
SaO2 (%)
C
Day 10 - VV30 (ml/30 mmHg/100 ml)
0
2.0
3.0
24
28
32
36
PETCO2 (mmHg)
D



notes for legend: SEA LEVEL data only
Overall correlation is r=.53, r2=.28, p<.005;
Placebo only r=.352, r2=.12, p=.26
Praz only r=.334, r2=.11, p=.21



notes for legend: 4300 M PP data only
Overall correlation is r=0.44, r2=.19, p<.05;
Placebo only r=0.40, r2=.16, p=.16
Praz only r=.42, r2=.17, p=.20



notes for legend: ALL data SL & PP FBF
Overall correlation is r=0.46, r2=.22, p<.001;
Placebo only r=0.46, r2=.21, p<0.05
Praz only r=0.37, r2=.14, p=0.05

Plasma Epineprhine (pg/ml)

Figure 34

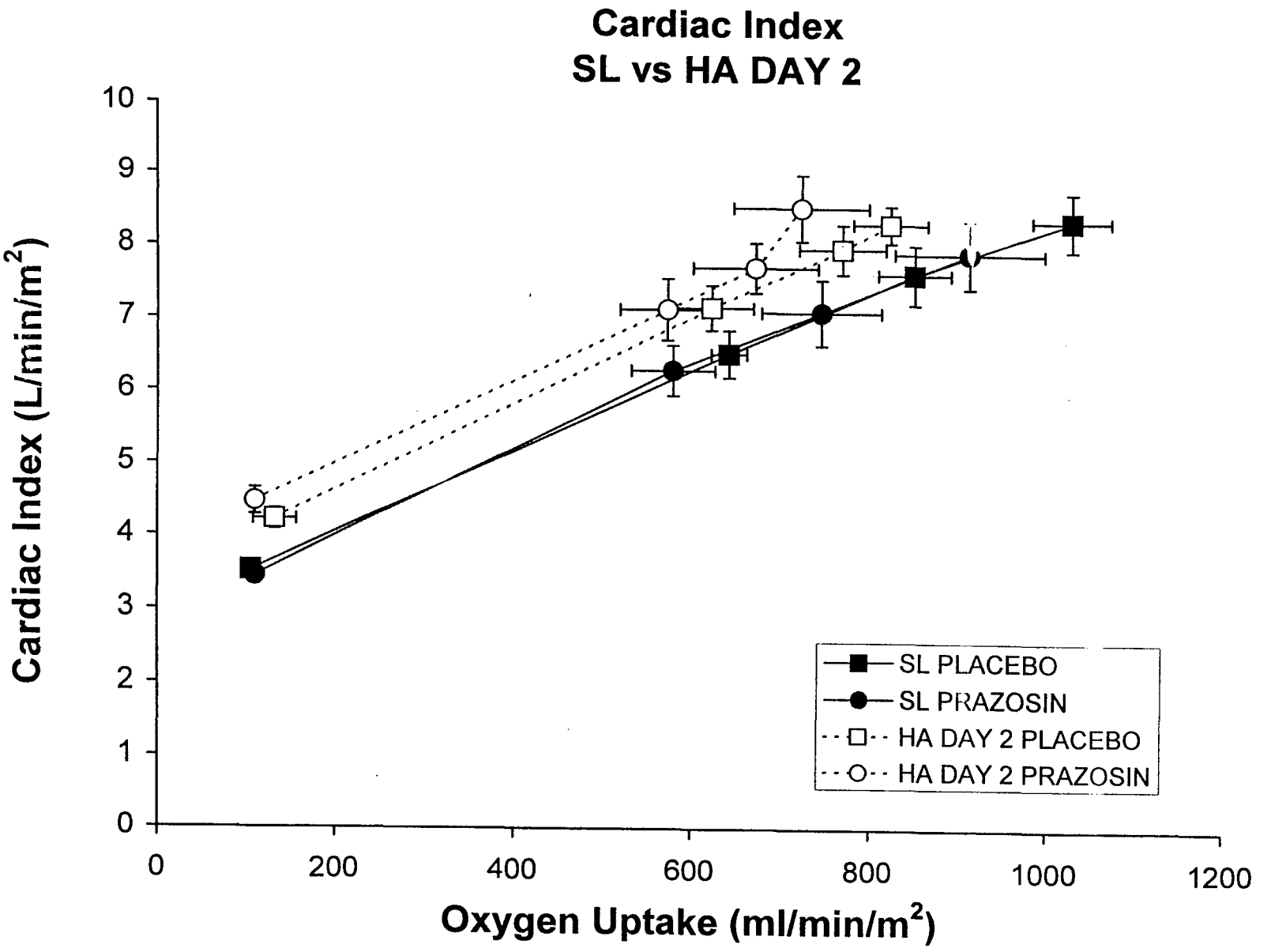

Cardiac Index
SL vs HA DAY 2
Cardiac Index (L/min/m2)
Oxygen Uptake (ml/min/m2)
0
1
2
3
4
5
6
7
8
9
10
0
200
400
600
800
1000
1200
SL PLACEBO
SL PRAZOSIN
HA DAY 2 PLACEBO
HA DAY 2 PRAZOSIN

Figure 35

Fig. 1: BMR SL to HA

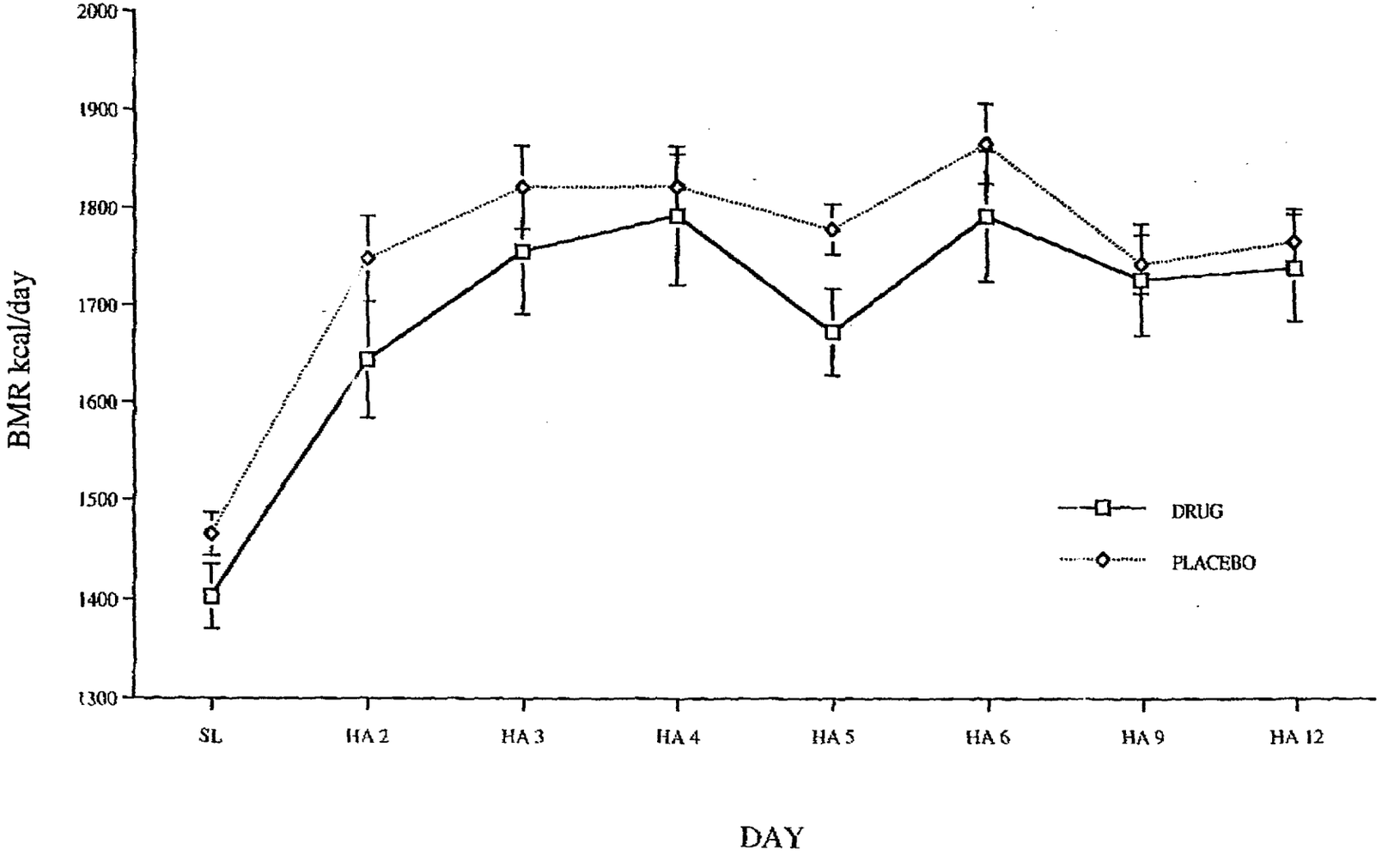


Figure 36

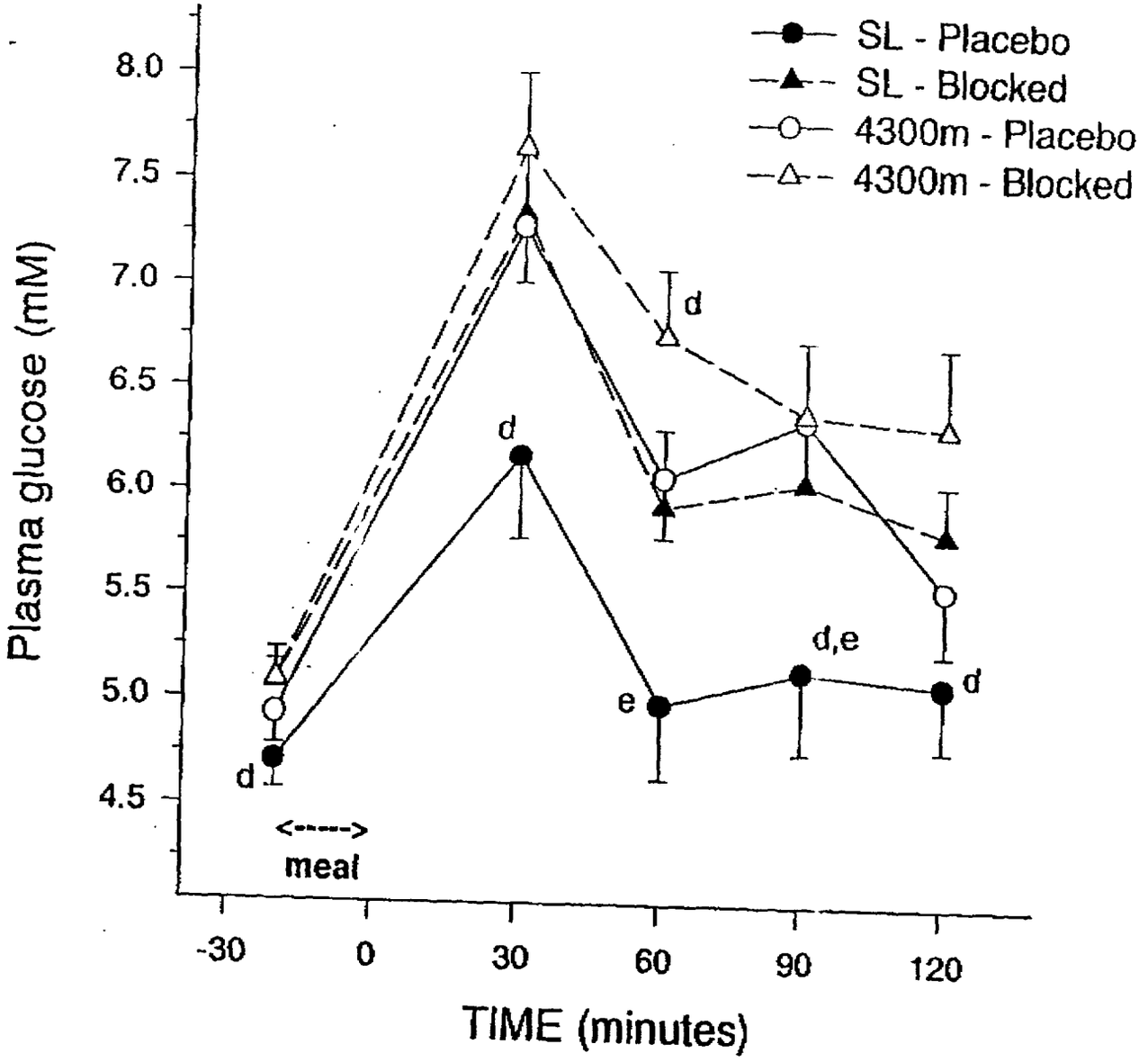
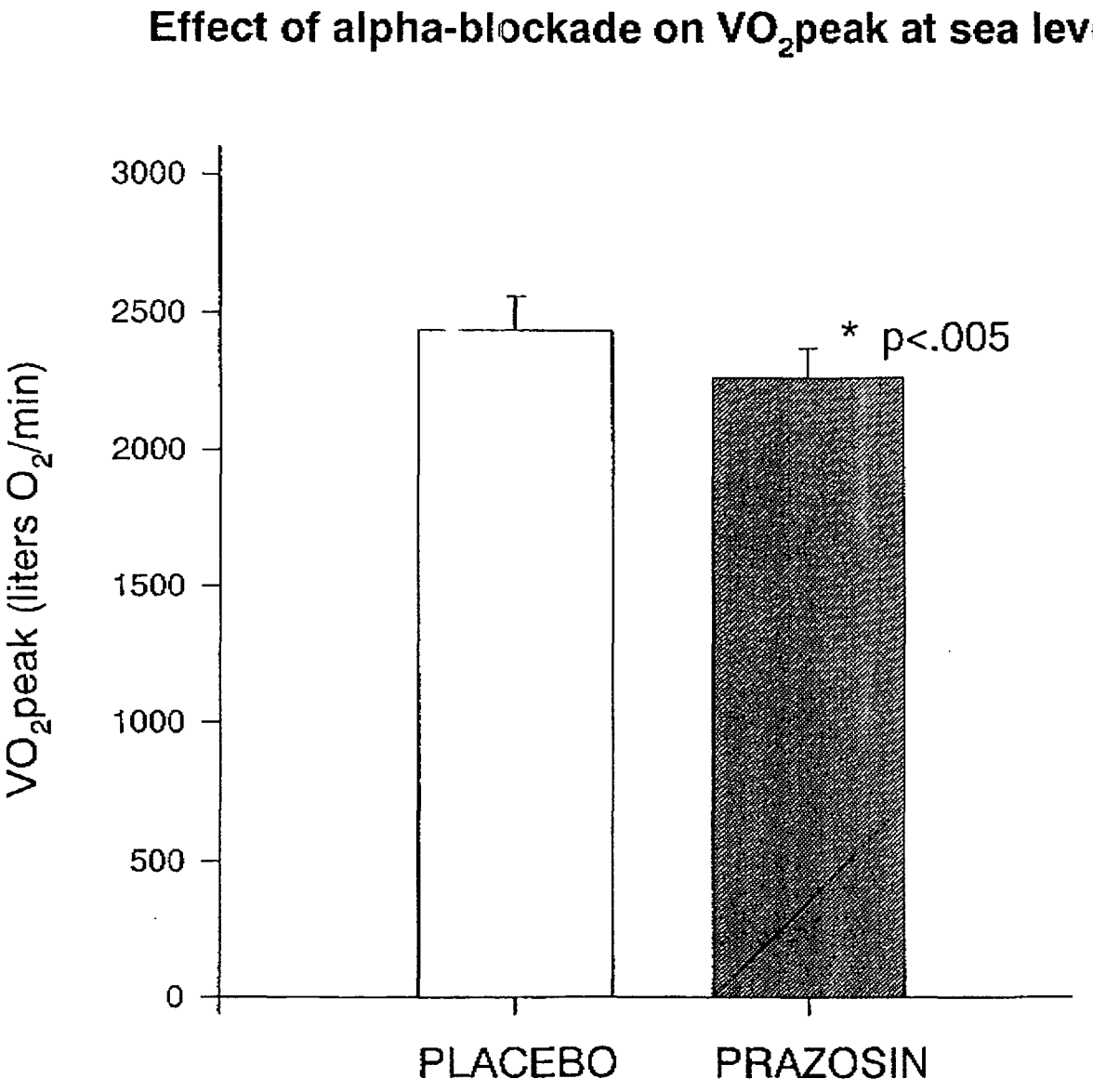

SL - Placebo
SL - Blocked
4300m - Placebo
4300m - Blocked
Plasma glucose (mM)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
d
d
d
e
d,e
d
<-----> meal
-30
0
30
60
90
120
TIME (minutes)

Figure 37


Effect of alpha-blockade on VO_2peak at sea level
VO_2peak (liters O_2/min)
3000
2500
2000
1500
1000
500
0
* p<.005
PLACEBO
PRAZOSIN

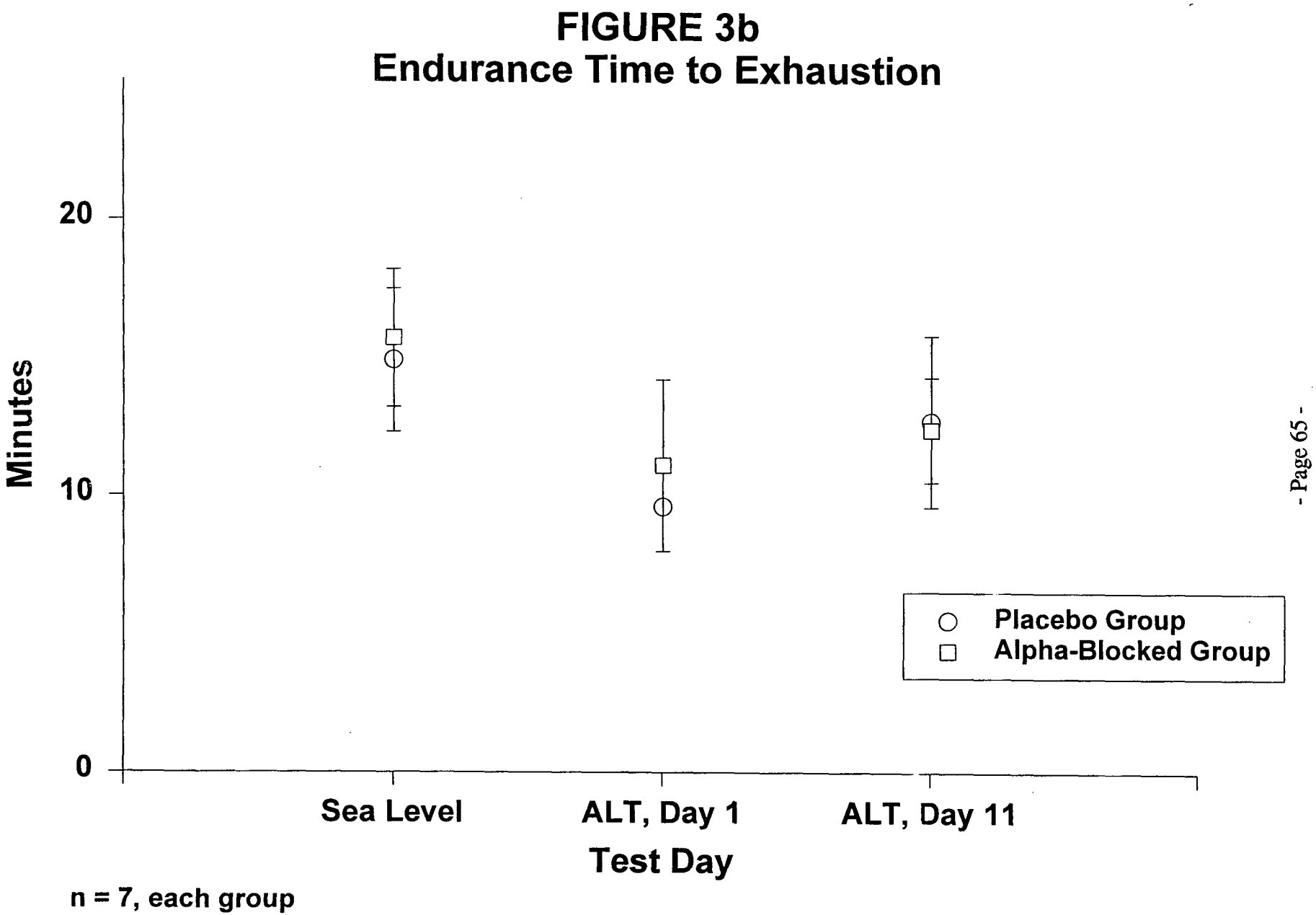


FIGURE 3b
Endurance Time to Exhaustion
Minutes
20
10
0
Sea Level
ALT, Day 1
ALT, Day 11
Test Day
Placebo Group
Alpha-Blocked Group
n = 7, each group

Table 1. Resting ventilatory responses to 4,300 m altitude sojourn. Subjects in follicular and luteal phases grouped together.

	N		$\dot{V}E$ ($l \cdot min^{-1}$)	$P_{ET}CO_2$ (mmHg)	SpO_2 (%)	HVRs ($\Delta\dot{V}E/\Delta SpO_2$) ($l \cdot min^{-1} \cdot \%^{-1}$)	HCVRs ($\Delta\dot{V}E/\Delta PCO_2$) ($l \cdot min^{-1} \cdot mmHg^{-1}$)	HCVRx (mmHg PCO_2)
	F	L						
SL	15	7	8.1 ± 2.0	38.5 ± 2.5	98 ± 1	0.56 ± 0.33	1.91 ± 0.76	39.8 ± 3.3
Day 1*	14	7	9.1 ± 1.7†	34.8 ± 2.5†	80 ± 4†			
Day 2	14	7	9.8 ± 1.4	33.6 ± 2.5†	82 ± 5†	0.44 ± 0.29†	2.05 ± 0.93	36.5 ± 5.4†
Day 3	12	7	9.7 ± 1.7	31.7 ± 2.8†	82 ± 5†			
Day 5	13	7	11.1 ± 1.6	30.6 ± 2.9	86 ± 4			
Day 7	13	9	11.0 ± 1.6	29.7 ± 2.4	86 ± 2	1.19 ± 0.67	2.90 ± 1.48	34.0 ± 3.3
Day 12	16	6	10.9 ± 2.5	28.0 ± 2.3	88 ± 2	1.46 ± 0.93	2.80 ± 1.42	31.6 ± 2.6

F: follicular, L: luteal, $\bar{x}$ ± S.D. * Day 1 measurements made 2-3 h after arrival between 1400 -1600 h.

† indicates significant difference between that high altitude day and Day 12.

Table 2. Fibrinolytic-related Factors at Sea Level and Altitude

	SL F	SL L	HA F	HA L	ALL SL	ALL HA
ECLT	249±16	302±12*	220±21	265±4•	270±12	235±15†
Fib	269±11	292±22	286±15	309±14	278±11	293±11
TAT	8.0±1.9	8.2±1.3	20.1±6.4	7.7±2.9	8.1±1.3	15.3±4.3
VWF	.71±.08	.62±.08	.76±.12	.94±.22	.67±.06	.82±.11†
F1+2	.85±.06	.73±.04*	1.1±.16	.69±.05	.80±.04	.94±.11

TABLE 3 Adductor Pollicis Maximal Voluntary Contractile Force and Endurance Time to Exhaustion For Follicular and Luteal Groups at Sea Level and Altitude.

	Maximal Voluntary Contractile Force (kgs)					
	Follicular Group (n = 8)			Luteal Group (n = 5)		
	Days 1-2	Days 5-6	Days 9-10	Days 1-2	Days 5-6	Days 9-10
SL	11.6 ± 0.4	11.4 ± 0.4	11.5 ± 0.6	10.9 ± 0.6	12.0 ± 0.8	11.8 ± 0.6
ALT	12.6 ± 0.5	12.0 ± 0.7	12.2 ± 0.8	12.2 ± 1.0	12.2 ± 1.0	12.8 ± 1.2

	Endurance Time to Exhaustion (mins)					
	Follicular Group (n = 8)			Luteal Group (n = 5)		
	Days 1-2	Days 5-6	Days 9-10	Days 1-2	Days 5-6	Days 9-10
SL	13.4 ± 1.6	15.8 ± 1.4	18.3 ± 3.6	23.0 ± 6.6	14.4 ± 5.5	18.0 ± 5.2
ALT	14.6 ± 2.2	18.6 ± 3.3	21.1 ± 3.2	14.6 ± 2.5	17.4 ± 1.7	19.3 ± 2.5

SL = sea level, ALT = altitude. Values are means ± SE

Table 4 Double-Blind Phenylephrine Data

	Sea Level		Pikes Peak	
	Placebo (n=8)	Prazosin (n=8)	Placebo (n=8)	Prazosin (n=8)
Baseline:				
Systolic BP (mmHg)	106±2	100±2*	111±3	110±4†
Diastolic BP (mmHg)	65±1	64±2	73±4	71±2†
MAP (mmHg)	79±1	76±2	85±3†	84±2†
Heart Rate (bpm)	57±3	58±3	78±6†	70±4†
Peak Phenylephrine Dose:††				
Systolic BP (mmHg)	129±1	136±7	133±2	132±3
Diastolic BP (mmHg)	87±2	82±4	88±3	78±1*
MAP (mmHg)	101±2	100±5	103±2	96±2*
Heart Rate (bpm)	47±3	44±2	62±6†	54±3†
Peak Phenylephrine Dose (ug/kg/min)	1.57±0.43	9.50±1.50*	3.71±0.28†	17.00±2.36*†
PD_{20} (ug/kg/min)	1.23±0.28	6.87±0.90*	3.83±0.61†	15.05±2.78*†
SBP/R-R slope (msec/mmHg)	10.70±1.73	9.47±1.10	9.09±1.76	11.48±2.02

†† Higher dose of phenylephrine required with prazosin vs placebo both at sea level and Pikes Peak. PD_{20} = dose of phenylephrine required for 20 mmHg increase in systolic BP over baseline. MAP = mean arterial pressure. SBP/R-R slope is a measurement of baroreceptor function. * $p < 0.05$ placebo vs prazosin; † $p < 0.05$ sea level vs Pikes Peak. Mean ± S.E.

LIST OF PERSONNEL RECEIVING PAY FROM RESEARCH EFFORT

Persons paid, in part, by research contract to Univ of Colorado:
- Thomas E. Dahms, PhD
- Robert E. McCullough
- Rosann G. McCullough
- Maaike Meertens
- Lorna G. Moore, Ph.D.
- Deborah R. Van Pelt
- Eugene Wolfel, M.D.
- Stacy Zamudio, Ph.D.

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- Ryan De Lee
- Shannon Dominick
- Steven Eseji-Okoye, M.D.
- Jacinda T Mawson
- Sharon Moynihan

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- Randi Barken
- Susan Bradford
- Karrie Cesario
- Raj Rathod
- Jay Tokeshi
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- Anne Friedlander, Ph.D.
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- Robert Mazzeo, Ph.D.
- John T. Reeves, M.D.
- Douglas White

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