EFFECTS OF EXPOSURE TO LOW-LEVEL CARBON MONOXIDE AT HIGH ALTITUDE IN SENSITIVE SUBJECTS

Final Report
Contract No. A833-159

Prepared for:

California Air Resources Board
Research Division
2020 L Street
Sacramento, CA 95814

Submitted by:

Principal Investigator
Michael T. Kleinman, Ph.D.

Co-Principal Investigator
David Leaf, M.D., M.P. H.

University of California, Irvine
Department of Community and Environmental Medicine
Irvine, CA 92717

DECEMBER 1991

Acknowledgements

We wish to thank Richard Vandagrif, Steve Oddo, Charles Bufalino, Sean Stuckley, Rhoda Peng and Amy Kleinman for their help in conducting many of the technical aspects of this study. We also wish to acknowledge the help of Dr. Katherine Ossan, biostatistician, of the U.C. Irvine Cancer Research Center, who reviewed the experimental design and statistical analysis portions of this program. This research project was part of programs supported by the Irvine Occupational Health Center, and we wish to especially acknowledge the backing and encouragement of Dr. Dan Menzel, Department of Community and Environmental Medicine, and Dr. Thomas Cesario, Department of Medicine, who made facilities and equipment available to this project. We also wish to acknowledge the help of the cardiology divisions of the Long Beach and Loma Linda Veterans Administration Hospitals for their help in identifying candidate subjects for this study. Finally we wish to thank the California Air Resources Board and members of the staff of the Research Division who actively followed the progress of this study and offered a great deal of valuable and insightful advice.

This report was submitted in fulfillment of ARB Contract No. A833-159, "Effects of Exposure to Low-level Carbon Monoxide at High Altitude in Sensitive Subjects" under the sponsorship of the California Air Resources Board. Work was completed as of June 30, 1991.

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as an actual or implied endorsement of such products.

Table of Contents

List of Tables

List of Figures

Summary and Conclusions

The primary objective of this study was to determine whether carbon monoxide (CO) exposure at simulated high altitude in human volunteers known to be at risk to CO-induced health effects caused greater changes in respiratory physiological, cardiological or hemodynamic parameters than CO exposures at sea level. A secondary objective was to measure the rate of endogenous CO production in order to refine physiologic models for computing COHb values from CO exposure data. The secondary objective was not fully realized because the available methods for COHb determination were able to accurately measure the washout of CO from selected exposed subjects, however the small incremental change in the washout slope which would have been attributed to endogenous production was not large enough to reliably detect. On the other hand the physiologic models did accurately predict the COHb concentrations in the subjects exposed under the condtions of this study, suggesting that they could be useful for estimating effects of environmental CO exposures.

The National Ambient Air Quality Standard for CO is equivalent to 9 ppm averaged over an 8 hr period. The State of California has established the same standard for sea level areas as the NAAQS, but in addition has established a more stringent 6 ppm standard (averaged over 8 hr) for areas at high altitude, such as Lake Tahoe. Individuals with coronary artery disease were exposed to carbon monoxide (CO) at sea level and at simulated high altitude (2.1 km or 7000 ft). Control exposures were performed with subjects breathing clean air at sea level and simulated high altitude. At conditions which were designed to reduce oxygen delivery to metabolizing tissues by about the same degree (reducing percent of oxygen saturation of arterial blood by 4%) both CO and simulated high altitude reduced time to onset of angina during exercise and increased the amount of cardiac effort to accomplish the same amount of work, compared to sea level, clean air conditions. The effects of CO and increased altitude were additive; statistical analyses using repeated measures analysis of variance did not demonstrate any significant interactions between carbon monoxide and altitude, although there were significant main effects of both factors. The lack of statistical interactions for any of the endpoints examined is consistent with an

additive but not a synergistic effect of CO exposure at high altitude. This study verified that, for the reduction in duration of symptom-limited exercise, the magnitude of response was a function of the reduction in oxygen-carrying capacity of arterial blood, expressed as the percent of saturation ($\%SaO_2$). The experiments were well controlled and experimental conditions were successfully matched between exposure tests so that experiment-related variations were minimized. Using data obtained in this study, and models for determining the effects of carbon monoxide and altitude on arterial blood oxygen carrying capacity, we estimated that the effect of breathing air with CO concentrations at the NAAQS (9 ppm) at the altitude of Denver, CO was approximately equivalent to breathing 6 ppm CO-contaminated air at Lake Tahoe, CA, when the joint effects of both altitude and CO are taken into account. The additive nature of the effects of CO and altitude, and the fact that the effects were proportional to the reduction in oxygen carrying capacity of arterial blood, permitted us to express responses as a linear function of $\%SaO_2$. We performed a linear regression analysis, incorporating the data from this study with data reported on CO-induced changes in time to onset of angina (TTA), and esimated that a 1% reduction in SaO_2 (or a 1% increase in carboxyhemoglobin) would cause a 2.1 ± 0.8% reduction in TTA. Using this relationship, we calculate that CO-related time to onset of angina, for individuals with coronary artery disease exposed at the altitude of Lake Tahoe, would be decreased by about 35% if ambient CO exposures were increased from the level of the California State Air Quality High Altitude Standard (6 ppm for 8 hr) to an ambient concentration of 9 ppm for 8 hr (the National Ambient Air Quality Standard and California State Sea Level Air Quality Standard).

Recommendations

Several recommendations can be made as a result of this research.

1. Because individuals with coronary artery disease tended to exhibit increased numbers of abnormal heart beats following acute CO and high altitude exposures, we recommend that studies focussing on this subgroup should be considered. The protocol and subject selection criteria used in this study could be modified to perform a study of that nature.

2. This study involved only acute exposures of non-acclimatized subjects and the results might therefore not fully address acclimatized high altitude residents. Additional studies could be considered to determine whether or not the effects of CO exposure on acclimatized individuals are different from those seen in non-acclimatized subjects.

3. Other potential effects on subgroups at risk of high altitude CO exposures should be examined. For example, pregnant women and fetuses might be more susceptable to the effects of CO at high altitude because of low hemoglobin concentrations in the former group and more rapid metabolic rates in the latter group. Cost-effective studies could be performed using laboratory animal models which could be exposed with or without exercise.

Introduction

Carbon monoxide (CO) is emitted from virtually all sources of incomplete combustion, including: internal combustion engines (e.g. automobiles, trucks and gasoline fueled small engines); fires, both natural and manmade; improperly adjusted gas and oil appliances (e.g. space heaters, water heaters, stoves and ovens); and tobacco smoking. Because of the large number and the ubiquity of CO sources with significant source strengths (e.g. tobacco smoke contains about 1% CO by volume, or 10,000 ppm CO), ambient CO concentrations show large temporal and spatial variations. The exposure of individuals to CO is, therefore, also quite variable, depending upon the types of activities in which a person is engaged and how long they are engaged in those activities, where the activity takes place (e.g. indoors, at a shopping mall, outdoors, in a vehicle, at work or school, in a parking garage), and the proximity to CO sources. Urban ambient levels range from about 1 ppm in clean residential neighborhoods to 150 ppm (maximum eight-hour average concentration) (Larsen and Burke, 1969) under "worst-case" conditions, such as might be measured in heavy urban traffic.

Exposure to high levels of CO (>500 ppm) for even a few hours can fatally deprive the brain of oxygen. Carbon monoxide reversibly reacts with proteins and competes with oxygen for binding sites on hemoglobin and myoglobin molecules, thus compromising the ability of these proteins to transport oxygen. Hemoglobin is an iron-containing molecule with binding sites for 4 O_2 molecules; the affinity of these hemoglobin binding sites for CO is approximately 240 to 250 times greater than that for oxygen (Roughton, 1970). The resulting carboxyhemoglobin (COHb) is relatively stable, and in normal individuals, the average COHb molecule has a lifetime of about four hours. In the body, a small amount of CO (0.4 ml/hr) is naturally generated by metabolism of heme-containing proteins. The resulting carboxyhemoglobin level, reported as percent of saturation, due to endogenous CO production in the absence of exogenous sources of CO is on the order of 0.4% to 0.6% COHb.

Although there is some information which indicates a direct toxic effect of CO, most of the observed health effects in environmentally exposed humans may be attributable to the reduction in the oxygen carrying capacity of the hemoglobin in blood (CO-induced hypoxemia) and the reduced delivery of O_2 to metabolizing tissues such as the brain, heart and other muscles (CO-induced hypoxia). In addition, the binding of CO at one of the hemoglobin binding sites increases the O_2 affinity of the remaining hemoglobin binding sites, thus interfering with the release of O_2 at the tissue level (When O_2 content of blood [mL O_2 / mL blood] is plotted vs. O_2 partial pressure [mm Hg] the increased O_2 affinity is seen as the so-called leftward shift in the curve for blood partially loaded with CO) (Longo, 1976). CO-induced tissue hypoxia is therefore a joint effect of the reduction in O_2 carrying capacity and the reduction of O_2 release at the tissue level. Organs such as the brain and heart, which under normal conditions, utilize about 75% of the arterially delivered O_2 (Ayers et al., 1970) as compared to peripheral tissues and other organs (which normally extract about 25% of the O_2 present in arterial blood), are therefore likely to be the most sensitive targets for hypoxic effects following low-level CO exposures in the absence of any known compensating mechanisms. The potential for adverse health effects is increased under conditions of stress, such as exercise, which increases O_2 demand at the tissue level to sustain metabolism. Individuals without underlying cardiopulmonary disease can cope with exercise induced stress by increasing ventilation and cardiac output to increase the delivery of oxygenated blood to metabolizing tissues. Individuals with coronary artery disease, however, have limited capacity for making such adjustments, hence they are among the groups most at risk of experiencing potential adverse effects due to CO exposure.

Other factors which reduce the delivery of O_2 to metabolizing tissues can potentially interact with the effects of CO exposures. For example, lung diseases which obstruct ventilation or impede diffusion of O_2 from the lung into the bloodstream, anemia which reduces the amount of available hemoglobin, or inhalation of solvents or chemicals that increase COHb (e.g. methylene chloride) or methemoglobin concentrations (e.g. nitric oxide) can all reduce the efficiency of O_2 delivery. The reduction of the amount of O_2 inspired, for example at high altitudes, can also reduce the amount of O_2 carried by the blood.

Regardless of the agent involved, reduction in the amount of O_2 in the blood relative to the normal O_2 content (hypoxemia) can result in reduced O_2 concentrations in tissues (hypoxia). In extreme cases hypoxia can cause tissue ischemia, resulting in the death or injury of metabolizing cells.

The biological effects of hypoxia induced by CO and hypoxia induced by high altitude may not be identical. Decreased oxyhemoglobin and increased carboxyhemoglobin produce different physiological responses with respect to the partial pressure of O_2 in the blood, the affinity of O_2 for Hb, the direction of shifts in the oxyhemoglobin dissociation curve, and ventilation drive (NAS, 1977). Studies have been performed in which physiological responses to high altitude and CO were measured. Astrup and Pauli (1968) reported on individuals with mean COHb concentrations of 12% (range = 5% to 20%) at an altitude of 3.4 km (11,300 ft), and Vogel and Gleser (1972) compared oxygen transport during exercise at an altitude of 4 km (13,125 ft) and a COHb concentration of 20%. In both of these studies the effects of CO and altitude were equivalent. Pitts and Pace (1947) reported that with respect to heart-rate response to work, a 1% increase in COHb (up to 13%) was equivalent to a 108.2 m (355 ft) rise in altitude, for individuals exposed at altitudes between 2.1 and 3.0 km (7000 to 10,000 ft). These studies contained some methodological uncertainties and measurements have not been made which precisely address the possible additive (or more than additive) effects of CO exposures and altitudes at concentrations of COHb more reasonable for ambient exposures and matched with respect to hypoxemia due to altitude and hypoxemia due to CO exposure. There were few studies of CO health effects at altitudes in the range frequented by most high altitude residents and visitors, and no high altitude studies were performed with individuals considered to be in a group of high potential risk of adverse effects from CO exposure (people with coronary artery disease). Approximately 95% of the 2.2 million high altitude residents live at 1.5 to 2.1 km [5000 to 7000 ft]; these areas also attract tourists both in summer and winter seasons. The city of Leadville, CO (3.1 km) is reputed to be the highest altitude city in the U.S. with a permanent residential population which is in excess of 4000 people. The present study was therefore designed to:

a) identify and recruit sensitive volunteer subjects (with ischemic heart disease);

b) expose these subjects to CO at sea level and high altitudes under carefully controlled, reproducible and matched conditions, such that the decrease in oxyhemoglobin would be 4% (equivalent to 2.1 km altitude) and the COHb concentration would be 4%;

c) carefully and precisely measure cardiopulmonary and exercise physiological parameters during graded exercise tests at sea level and high altitude conditions;

d) statistically analyze the resulting data to determine the possible additive (or more than additive) effects of CO and high altitude exposure.

Methods

Design

This study was a randomized prospective intervention trial with each subject serving as his own control. Eighteen male non-smokers with physician-diagnosed ischemic heart disease and stable angina were exposed to 100 ppm CO or to clean air under sea level and simulated high altitude (2.1 km). The subjects then performed a cardiopulmonary monitored graded exercise test. The study was blinded to both subjects and investigators; the subjects, the exercise technician, the physician and the nurse participating in the exercise testing were unaware of the exposure atmosphere during the course of the exercise and effects evaluations portions of the studies. A total of 24 subjects entered the study, but six were discontinued, during or after the study, for medical or other reasons.

Informed consent was obtained from each eligible subject on entry to the study. Eligibility requirements included no tobacco smoking for at least 6 months prior to the study and no changes in medications for months prior to entry. The study protocol was approved by an Institutional Review Board at the University of California, Irvine. At entry, each subject completed a questionnaire reporting their medical history and health habits. The information included the number of vessels involved in disease, previous surgery and myocardial infarctions, previous history of other diseases, and historical information

regarding potential environmental and occupational exposures to CO. A physician reviewed each subject's medical history and performed a cardiovascular and respiratory system examination prior to the subject's entry into the study. Stable angina was defined as pain or discomfort in the area of the chest (with or without radiation to other areas), reproducibly precipitated by exertion and relieved by rest or sublingual nitroglycerine, with no recent changes in the frequency, duration, time of appearance, or precipitating factors of pain (Silverman and Grossman, 1984). ST segment depression was measured at 0.06 sec after the J-point, consistent with measurements made in previously reported studies (Allred et al., 1989; Kleinman et al., 1989). Pulmonary function testing was performed, then subjects completed a graded cardiopulmonary exercise stress test up to the point of onset of anginal pain, to document the presence of exertional angina.

Twenty-four subjects were entered into this study and 18 were able to complete the full series of exposure-cardiopulmonary exercise stress test sessions. Reasons for dropout included subjects unwilling to continue (2 cases), a subject exhibiting symptoms of unstable angina (1 case), subjects developing cardiopulmonary complications unrelated to their participation in the study (2 cases) and a subject that failed to exhibit evidence of exercise-induced ischemia during testing. The 18 subjects were tested on four occasions separated by an average interval of about one week. Each test consisted of an exposure to one of four exposure atmospheres (clean air at sea level [CA/SL]; clean air at 2.1 km simulated altitude [CA/HA]; 100 ppm CO at sea level [CO/SL]; 100 ppm CO at simulated 2.1 km altitude [CO/HA]). The order of atmosphere exposures was randomized. Each exposure was followed by a graded cardiopulmonary stress test; the initial workload was 30 watts and workload was increased 10 watts at the end of each minute of exercise.

The protocol minimized several potentially confounding factors. For example, the investigators controlled the times subjects took medication prior to testing and the subjects' carbohydrate consumption before exercise tests. Subjects also refrained from drinking caffeinated beverages prior to testing. On the morning of a test, subjects were transported to the laboratory in a van equipped with a clean air "tent," reducing the effects of

extraneous CO exposure even when the subject was driven in heavy traffic conditions.

Prior to each subject's exposure, a physician performed a limited physical examination and evaluated a resting 12-lead electrocardiographic tracing (EKG). A catheter was emplaced for venous blood sampling and a sample was obtained to determine the subject's baseline COHb level. Blood samples were taken at initiation and then at 30 min intervals during exposure. The exposure duration required to titrate the subject to 4% COHb was computed. At the end of the exposure period, the altitude exposure was continued but carbon monoxide exposure was discontinued, and the subject performed an exercise test. The subjects were brought to the laboratory following an overnight (10 hr) fast and were provided with 8 oz of fruit juice and were at rest before undergoing the 2 hr atmosphere exposures. Red blood cell (RBC) fatty acid profiles were obtained at the first and last exposure days for 6 of the subjects in order to document the stability of dietary fat intake during the study.

Laboratory Measurements

Carbon monoxide (1% in air) was metered from a cylinder of compressed gas into a flow of clean, HEPA filtered air. High altitude was simulated by metering purified nitrogen (99.9995%; Matheson Gas) into clean air to reduce the O_2 to 16.1% (equivalent to the reduced partial pressure of O_2 at an altitude of 2.1 km or 7000 ft). The gases were mixed at atmospheric pressure in a chamber and delivered to a low-deadspace respiratory mask (Vac-U-Metrics) which was fitted and carefully sealed over the subject's nose and mouth to eliminate leaks. The mask was fitted with one-way valves to separate inspiratory and expiratory flows. Excess exposure atmosphere and expired gases were vented to a fume hood and exhausted from the room. Carbon monoxide concentrations in the exposure atmospheres were monitored using a non-dispersive infrared absorption unit (Dasibi Model 3003) which was calibrated against certified CO gas mixtures of known concentrations. External quality assurance audits on the CO monitors were performed by California Air Resources Board personnel, using standards traceable to the National Institute of Standards and Technology. Exercise was performed on an electromagnetically-braked cycle ergometer

(Gould-Goddart). No more than 15 min were allowed from end-exposure to the start of the test, and the time of day was consistent between tests ± one hr.

The exercise test consisted of three segments; a pre-exercise (resting) period of 5 min, a graded exercise period to the time of onset of angina (unless stopped by the physician or voluntarily by the subject), and a recovery period of eight min. Continuous single-lead EKG monitoring was maintained during rest, exercise, and recovery periods. Respiratory gas exchange measurements were obtained continuously during each of the three exercise test segments. The five-minute pre-exercise period was begun after allowing sufficient time for the subject to achieve a regular breathing pattern and for stabilization of respiratory gas exchange measurements. At the end of five min, a 12-lead EKG was obtained and the graded cardiopulmonary exercise stress test was begun. The subject was instructed to pedal the ergometer to 60 rpm and to continue until he either felt the onset of anginal pain, could not continue the test for any other reason, or was told to stop by the physician. The initial work load was 30 watts and was increased 10 watts at the end of each min of exercise. EKG's were obtained at the end of each min of exercise, and blood pressure readings were taken after every second min. At the end of exercise, the subject's time to angina, blood pressure, EKG, and duration of angina were recorded. The subject remained seated on the ergometer for the eight minute recovery period during which EKG, blood pressure, and respiratory gas exchange data continued to be recorded at regular intervals.

Respiratory gases were monitored continuously; fractions of inspired and expired O_2 by an electrochemical detector (Amtek S-3A) and fractions of expired CO_2 by an infrared analyzer (Beckman LB-2). These gas monitors were calibrated before each test against certified, NIST traceable gas mixtures. Expiratory gas flow was monitored on a breath-by-breath basis using a turbine pneumotachometer. The gas analyzers and the pneumotachometer were interfaced to a computer which calculated and recorded minute ventilation (Ve), fractions of inspired and expired O_2, fractions of expired CO_2, oxygen uptake (VO_2), carbon dioxide output (VCO_2), and ventilation equivalents for O_2 and CO_2 (Ve/VO_2 and Ve/VCO_2, respectively) at 15 sec intervals. A 12-lead EKG tracing

(Marquette MAC-II) was taken during the last 20 seconds of each minute during the exercise test. The EKG was calibrated such that 1.0 mm = 0.1 mv. The numbers of abnormal heart beats, changes in the levels of ST segments and T-waves, and the heart rate were determined.

Venous blood samples were collected six times on each exposure day; on arrival at the laboratory before exposure (to obtain baseline data, and to verify that the subject had not been smoking recently), four times during the exposure, and about 10 min after exercise. Blood samples were analyzed for concentrations of total hemoglobin (THb), oxyhemoglobin (O_2Hb), carboxyhemoglobin (COHb), and methemoglobin (MetHb) using a cooximeter (Instrumentation Laboratories Model IL282 CO-Oximeter) which was periodically checked against a gas chromatograph with a reducing gas detector (Trace Analytical). The CO-Oximeter was calibrated daily against standards (Fisher Scientific) which provided a range of COHb concentrations from 2% to 95%. RBC fatty acids were measured by gas chromatography after preparation according to the method of LePage (1986). Fatty acid profiles included major saturated, mono-, and polyunsaturated acids.

The Coburn-Foster-Kane equation (Coburn et al., 1965) has been important for modeling COHb concentrations in CO-exposed individuals. One of the parameters used in the equation for which there are few experimental data is the rate of endogenous CO production. As a sub-study within this project, we evaluated the use of exogenously administered non-radioactive, isotopically labeled $C^{18}O$ to differentiate between the rates of elimination of endogenous and exogenous CO and thus allow us to better estimate endogenous CO production rates. The experimental design was to administer a bolus of $C^{18}O$ to volunteers via inhalation and then to collect serial samples over a 2 hr period which were analyzed using a gas chromatograph and a mass spectromenter to determine the rates of removal of $C^{18}O$ and $C^{16}O$.

Authenticity of the altitude simulation was tested by using a pulse oximeter (Nellcor Model N100 Oximeter with a D25 Oxysensor) to determine that the saturated oxygen

content of arterial blood was reduced from the normal sea level value of about 98% to an expected 2.1 km altitude value of about 94% during simulated altitude exposures. Arterial blood gases were measured (Radiometer Blood Gas Analyzer) in one subject to demonstrate that effects of CO and altitude were additive in reducing the percent O_2 saturation of arterial blood.

Statistical Analysis

The respiratory gas exchange and cardiovascular data were analyzed using two-factor analyses of variance (ANOVA) with repeated measures. The two factors analyzed were exposure ATMOSPHERE (clean air or CO) and ALTITUDE (sea level or simulated 2.1 km altitude). Each subject acted as his own control. Individual group mean values were compared using the Tukey multiple comparison test (Huntsberger and Leaverton, 1970). Because all subjects did not experience angina on all 4 exposures, the data are analyzed for the entire group, and separately for those experiencing angina. There was some unrecoverable loss of data during the course of the experiment due, in general, to technical problems. The affected parameters were systolic and diastolic blood pressures (one missing value in each of 6 cases), heart rate (one missing value in one case), and the exercise physiology data set (one missing value for each of the parameters in each of 4 cases). There were no missing values for time to onset of angina, ST segment depression or numbers of PVC's. Repeated measures ANOVA provides a powerful statistical method for evaluating data from the type of experimental design used in this study, however cases with incomplete data for the dependent variable in the test cannot be included. To maximize the number of acceptable cases a least squares method was used to estimate values for the missing data (Snedecor and Cochran, 1980) employing the UCLA Biomedical Data Analysis Program AM (BMDP, 1988). Data sets were evaluated with and without substitution of missing data. This estimation procedure yielded group mean values for the complete data set which differed from the true means of the valid data by less than 4% for the variable with the largest number of missing values. ANOVA results for the affected parameters with missing data substitution are discussed below. Selected measurements which were functions of the amount of work performed, or the duration of exercise, were compared using least squares

linear regression of the parameter values vs. exercise duration. Slopes and intercepts were compared using two-sided t-tests.

Paired t-tests of levels of major fatty acids in plasma, including saturates, mono-, and polyunsaturates, revealed no significant differences between from time of entry to the last day of cardiopulmonary exercise testing.

Results

Subject Characteristics

All of the subjects in the study were males with stable angina pectoris. The subjects' ages, weights and indications of cardiovascular abnormalities are summarized in Table 1. The subjects ranged in age from 58 to 83 years, with a mean ± standard error (s.e.) age of 67 ± 8 years. The subjects ranged in weight from 69.5 kg to 124.5 kg with a mean ± s.e. of 81.3 ± 15 kg. All of the subjects had a positive exercise stress test, and all but one of the subjects (# 7) had objective clinical findings (in addition to the positive exercise test) indicative of ischemic heart or coronary artery disease. About half of the subjects reported experiencing an episode of angina within 1 wk prior to their initial screening study. The remaining subjects reported a range of from 2 wk to six months since their last attack of angina. Thirteen of the subjects had reported one, or more, myocardial infarctions, ranging in time from 9 to 216 months prior to this study. Nine of the subjects had previous coronary bypass surgery. Twelve of the subjects were under treatment for hypertension and 7 subjects had diabetes. None of the subjects had a history of emphysema or chronic bronchitis and one subject reported a history of asthma.

Most of the subjects (16 out of 18) were ex-cigarette smokers, 3 were ex-pipe smokers, and 2 had never smoked. The average ex-cigarette-smoking subject had smoked 32 cigarettes per day; for the group, the range was from 2 to 50 cigarettes per day. The subjects reported having smoked in a range from four to 40 years, and all had quit smoking at least eight months prior to entering our study.

Table 1. Characteristics of Subjects

Subject*	Age	Weight (kg)	Blood COHb levels (% saturation)							
			CA/SL		CO/SL		CA/HA		CO/HA	
			End Expos.	End Exer.	End Expos.	End Exer.	End Expos.	End Exer.	End Expos.	End Exer.
1 b,d	79	71.8	0.8	0.6	4.0	3.9	0.6	0.5	3.8	3.5
2 d	58	76.8	0.2	0.1	4.8	4.0	1.0	0.8	4.9	4.5
3 a,b,d	62	73.2	0.7	0.5	4.6	3.8	1.1	0.3	3.8	3.6
4 a	62	76.8	0.8	0.8	4.0	3.4	0.9	0.5	4.0	4.0
5 a,d	61	90.0	0.7	0.6	4.4	4.0	0.6	0.5	4.5	3.9
6 a,b	64	69.5	0.5	0.3	4.5	3.8	0.8	0.4	5.1	4.7
7	83	70.4	0.4	0.4	3.7	3.1	0.2	0.2	3.7	3.0
8 a,c,d	61	124.5	0.8	0.4	4.6	4.2	0.5	0.4	5.1	4.4
9 d	63	80.9	1.0	0.9	4.3	4.3	0.6	0.4	4.4	3.7
10 a,b,c	63	73.2	0.8	0.8	3.7	3.4	0.2	0.2	4.1	3.6
11 a,b,d	66	74.1	0.5	0.5	4.2	3.6	0.5	0.5	4.1	3.9
12 b	83	77.7	0.5	0.3	3.9	3.3	0.5	0.4	3.9	3.3
13 a,b,d	63	70.9	0.8	0.6	4.5	4.3	0.5	0.2	3.9	4.1
14 a,d	68	80.9	0.5	0.6	4.3	3.1	1.3	1.1	3.1	2.8
15 a,b	75	75.0	0.6	0.5	3.9	3.4	0.6	0.5	4.9	4.5
16 a,b	62	115.9	0.5	0.3	3.9	3.4	0.8	0.8	4.2	3.8
17 a	64	75.0	0.8	0.7	3.8	2.8	0.6	0.8	3.9	3.3
18 a	70	88.2	1.8	1.4	3.7	2.9	1.0	0.5	3.6	3.1
Mean ± SD§	67±8	81.3±15	0.64 ± 0.32		3.86 ± 0.51		0.59 ± 0.28		3.96 ± 0.59	

*a = previous myocardial infarction, b = previous coronary artery bypass surgery, positive Thallium exercise test, d = positive angiogram, cardiac catheterization or angioplasty.

§Mean COHb values are the average of the end exposure and end exercise values.

Pre-exposure COHb levels (percent of saturation of hemoglobin by CO) in the subjects ranged from 0.2% to 2.1%, with a mean of 0.7 %. These values are consistent with a history of non-smoking residence in urban areas. The COHb levels measured in subject's blood after CO exposures and 10 min after completion of the exercise test are summarized in Table 1. Subjects were exposed for approximately 2 hr, at rest, to carbon monoxide (CO; 100 ± 2 ppm) or clean, filtered air (CA; 2 ± 1.5 ppm), under sea level (SL) and simulated high altitude (HA) conditions, via a respiratory mask. Group mean COHb levels during exercise (estimated as the average of the end exposure and end exercise values) were in excellent agreement between the SL and HA conditions (3.86 ± 0.51 and 3.96 ± 0.59, respectively; mean ± s.d.). COHb levels on clean air exposure days were also comparable under SL and HA conditions (0.64 ± 0.32 and 0.59 ± 0.28, respectively; mean ± s.d.).

Prediction of COHb and measurements of endogenous CO production

Two analytical methods were attempted for measuring $C^{18}O$ in the presence of $C^{16}O$. The first method used chromatographic separation of CO liberated from small samples of serially collected blood followed by quantitation using a sensitive reducing gas detector. Unfortunately, technical problems with the instrument caused the data to be unreliable. The second method used a medical gas mass spectrometer which could detect $C^{18}O$ but not $C^{16}O$ and an infrared absorption analyzer to measure the combined $C^{18}O$ and $C^{16}O$ in serially-collected breath samples. The net differences between these measurements should reflect the contribution of endogenously produced CO plus a small contribution from exogenous CO in inhaled breathing air. The measured differences were, however, were within the limits of experimental error for the determinations. A removal halftime of about 4 hr was observed, but reliable estimates of CO production could not be obtained. Additional development is continuing to improve the measurement precision and allow these estimates to be made. On the other hand, we did use the Corburn-Foster-Kane equation as part of a model to calculate COHb concentrations for subjects at sea level and 2 km altitude, assuming an ambient CO 8 hr average concentration of 1.5 ppm, and compared those estimates with values measured for our subjects (summarized in Table 1). The measured COHb concentrations (0.6 ± 0.3%) agreed extremely well with the modeled

estimates (0.60% [sea level] and 0.67% [2 km]), suggesting that current estimates of parameters used in the model may be adequate for predictions of COHb for individuals with coronary artery disease.

Exercise test results

The exercise tests were performed with the subjects breathing clean, filtered air with the inspired O_2 percentage appropriate for the simulated altitude (sea level or 2.1 km). Carbon monoxide was not supplemented during exercise on the CO exposure days. COHb concentrations on CO exposure test days dropped 14.6% after exercise at sea level and 10.2% after exercise at simulated 2.1 km altitude; the differences in CO removal rates were not significant between altitude conditions (2 sided paired t-test).

Measurement of Cardiac Function

Group means were computed for each response variable for data obtained on the 4 test days. Not every subject experienced angina, exhibited ST segment depression (ST↓), or exhibited preventricular contractions (PVC) on every test day. Since some of the endpoints to be discussed are symptom-specific, group mean values are presented in Table 2 for the entire group (all subjects; n=18), for the sub-group with angina on all 4 tests (angina group; n=13), and for the sub-groups with either ST↓ (n=12) or PVC (n=11), as appropriate. Repeated measures analyses of variance (ANOVA) on the time to onset of angina (TTA) for the angina subject sub-group demonstrated statistically significant main effects of both CO exposure (p=0.047) and altitude (p=0.026). The % changes in TTA from the clean air/sea level control exposure (CA/SL) for the CO exposure at sea level

Table 2. Results of Symptom Limited Exercise Tests - Cardiological Parameters

	Duration of Exercise (min)				Integrated ST Depression (mV • min)				Total PVC's (number observed)			
Subject	CA/SL	CO/SL	CA/HA	CO/HA	CA/SL	CO/SL	CA/HA	CO/HA	CA/SL	CO/SL	CA/HA	CO/HA
1 [a,b]	7.91	7.12	6.25	5.50	0.05	0.0	0.0	0.05	0.0	0.0	0.0	0.0
2 [a,b,c]	11.00	9.33	10.23	6.78	0.9	0.75	0.8	1.0	0.0	2.0	3.0	2.0
3 [a,b,c]	3.75	3.38	2.57	2.21	0.15	0.3	0.0	0.0	2.0	0.0	5.0	0.0
4 [a,b,c]	5.00	4.25	4.83	3.83	1.1	1.1	1.1	0.45	0.0	1.0	1.0	0.0
5 [a,b,c]	3.83	4.33	3.17	2.25	0.3	0.4	0.3	0.5	2.0	0.0	0.0	3.0
6 [a]	5.12	4.50	5.00	5.08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7 [b,c]	6.73	8.25	7.00	8.33	0.3	0.65	0.0	0.9	0.0	0.0	0.0	2.0
8 [a,b,c]	8.38	7.18	7.82	9.63	0.7	0.3	0.5	1.35	3.0	0.0	1.0	6.0
9 [a,b,c]	3.33	3.83	3.50	3.50	0.4	0.7	0.7	0.4	0.0	1.0	0.0	0.0
10 [c]	4.42	4.33	4.55	4.66	0.0	0.0	0.0	0.0	1.0	6.0	5.0	4.0
11 [a,c]	5.38	5.58	6.12	4.90	0.0	0.0	0.0	0.0	1.0	5.0	0.0	1.0
12 [b,c]	7.50	8.12	6.75	5.92	0.0	0.25	0.0	0.70	0.0	0.0	0.0	2.0
13 [b]	8.00	6.88	6.00	5.92	1.45	0.95	0.85	1.40	0.0	0.0	0.0	0.0
14 [a]	4.25	4.67	5.08	4.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15 [b,c]	2.75	3.75	3.75	4.25	0.0	0.0	0.15	0.0	0.0	3.0	0.0	0.0
16 [a,b]	6.07	3.65	4.17	4.40	0.25	0.05	0.1	0.0	0.0	0.0	0.0	0.0
17 [a]	7.25	6.15	4.67	5.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18 [a,c]	6.00	6.28	5.00	5.17	0.0	0.0	0.0	0.0	1.0	0.0	1.0	1.0
All Subjects Mean ± SD	5.93±2.14	5.64±1.83	5.36±1.85	5.13±1.85	0.3±0.4	0.3±0.3	0.3±0.4	0.4±0.5	0.6±0.9	1.0±1.8	0.9±1.7	1.2±1.7
Angina[a] Subjects Mean ± SD	5.94±2.20	5.40±1.74	5.26±2.03	4.87±1.93	0.3±0.4	0.3±0.4	0.3±0.4	0.3±0.4	0.7±1.0	0.7±1.4	0.9±1.5	1.0±1.8
ST↓[b] or PVC[c] Subjects Mean ± SD	6.04[b]±2.3	6.02[b]±1.9	5.71[b]±2.1	5.27[b]±2.2	0.5[b]±0.5	0.5[b]±0.4	0.4[b]±0.4	0.6[b]±0.5	0.8[c]±1.0	1.5[c]±2.2	1.3[c]±1.9	1.8[c]±1.9

Notes: a - subjects with angina on all 4 test days; b - subjects exhibiting ST segment depression (ST↓) on one or more test days; c - subjects exhibiting preventricular contractions (PVC's) on one or more test days.

(CO/SL) and clean air at high altitude (CA/HA) exposures were approximately equivalent (-9.1% and -11.4%, respectively). The CO at high altitude (CO/HA) exposure caused a significant -18.0% change in the time to onset of angina compared to CA/SL exposures. There was no significant interaction between the atmosphere and altitude factors. Approximately the same pattern of changes in maximal exercise time was seen in the subgroup with ST↓ or PVC's on one or more tests, and for the entire group of subjects.

Only 6 of the 18 subjects exhibited clinically significant ST↓ (≥ 0.1 mV) prior to the end of the maximal exercise on all 4 test days, although a larger number (12) showed ST↓ of ≥0.5 mV on one or more test days. As an index of the effect of exposure on development of ST↓, the mV depression for this group of 12 subjects were summed over the number of minutes during and post-exercise in which depression occurred, yielding a value for integrated ST depression in mV•min. There were no significant altitude or atmosphere-related integrated ST↓ effects detected using ANOVA and the differences in group mean values were not statistically significant (Table 2).

Twelve subjects exhibited PVC's during or after exercise on one or more of the test days, but only one subject had PVC's on all 4 test days. The total number of PVC's observed for this sub-group were approximately doubled following exposures to CO or to high altitude, with and without CO (Table 2), however the differences were not significant at the $\alpha = 0.05$ level. The effects seen after CO exposures tended to be greater than those observed during clean air exposures at either altitude (39 events vs. 25 events, respectively), but this was not a significant difference.

Hemodynamic Parameters

Heart rate and blood pressure data are summarized in Table 3 for values at the end of exercise. Effects of atmosphere and CO exposure were not statistically significant

Table 3. Results of Symptom Limited Exercise Tests - Hemodynamic Parameters at End of Exercise

Subject	Heart Rate (min^{-1})				Systolic Blood Pressure (mm Hg)				Diastolic Blood Pressure (mm Hg)			
	CA/SL	CO/SL	CA/HA	CO/HA	CA/SL	CO/SL	CA/HA	CO/HA	CA/SL	CO/SL	CA/HA	CO/HA
1 [a,b]	140.	140.	145.	145.	170.	168.	160.	160.	78.	58.	48.	64.
2 [a,b,c]	100.	100.	98.	94.	158.	158.	161.	ND	80.	93.	98.	ND
3 [a,b,c]	80.	64.	58.	67.	148.	120.	143.	144.	64.	70.	65.	88.
4 [a,b,c]	145.	130.	145.	140.	142.	148.	160.	170.	91.	80.	92.	70.
5 [a,b,c]	100.	122.	120.	110.	200.	202.	201.	194.	90.	67.	105.	112.
6 [a]	98.	100.	100.	98.	170.	219.	ND	120.	80.	104.	ND	80.
7 [b,c]	130.	140.	140.	135.	ND	178.	198.	230.	ND	80.	90.	80.
8 [a,b,c]	80.	80.	94.	94.	224.	210.	ND	190.	90.	110.	ND	106.
9 [a,b,c]	92.	92.	90.	92.	174.	255.	ND	169.	69.	132.	ND	78.
10 [c]	80.	82.	84.	78.	174.	179.	ND	198.	128.	73.	ND	80.
11 [a,c]	78.	76.	82.	78.	150.	158.	146.	158.	88.	70.	70.	64.
12 [b]	140.	145.	140.	150.	190.	184.	160.	170.	88.	84.	80.	80.
13 [b]	140.	120.	123.	140.	168.	172.	178.	190.	90.	86.	90.	109.
14 [a]	80.	80.	87.	141.	154.	176.	172.	217.	88.	73.	68.	88.
15 [b,c]	130.	140.	120.	140.	188.	190.	180.	190.	68.	84.	82.	84.
16 [a,b]	100.	85.	100.	100.	170.	172.	168.	152.	90.	68.	70.	80.
17 [a]	150.	145.	ND	140.	170.	170.	178.	ND	90.	90.	90.	ND
18 [a,c]	100.	130.	100.	110.	160.	140.	178.	164.	90.	90.	90.	90.
All Subjects Mean ± SD	109±26	110±27	108±25	115±27	171±20	178±31	171±17	176±28	86±14	84±18	81±15	85±14
Angina Subjects[a] Mean ± SD	103±25	104±26	103±24	110±26	170±28	177±36	168±17	167±26	84±9	85±21	81±17	84±15

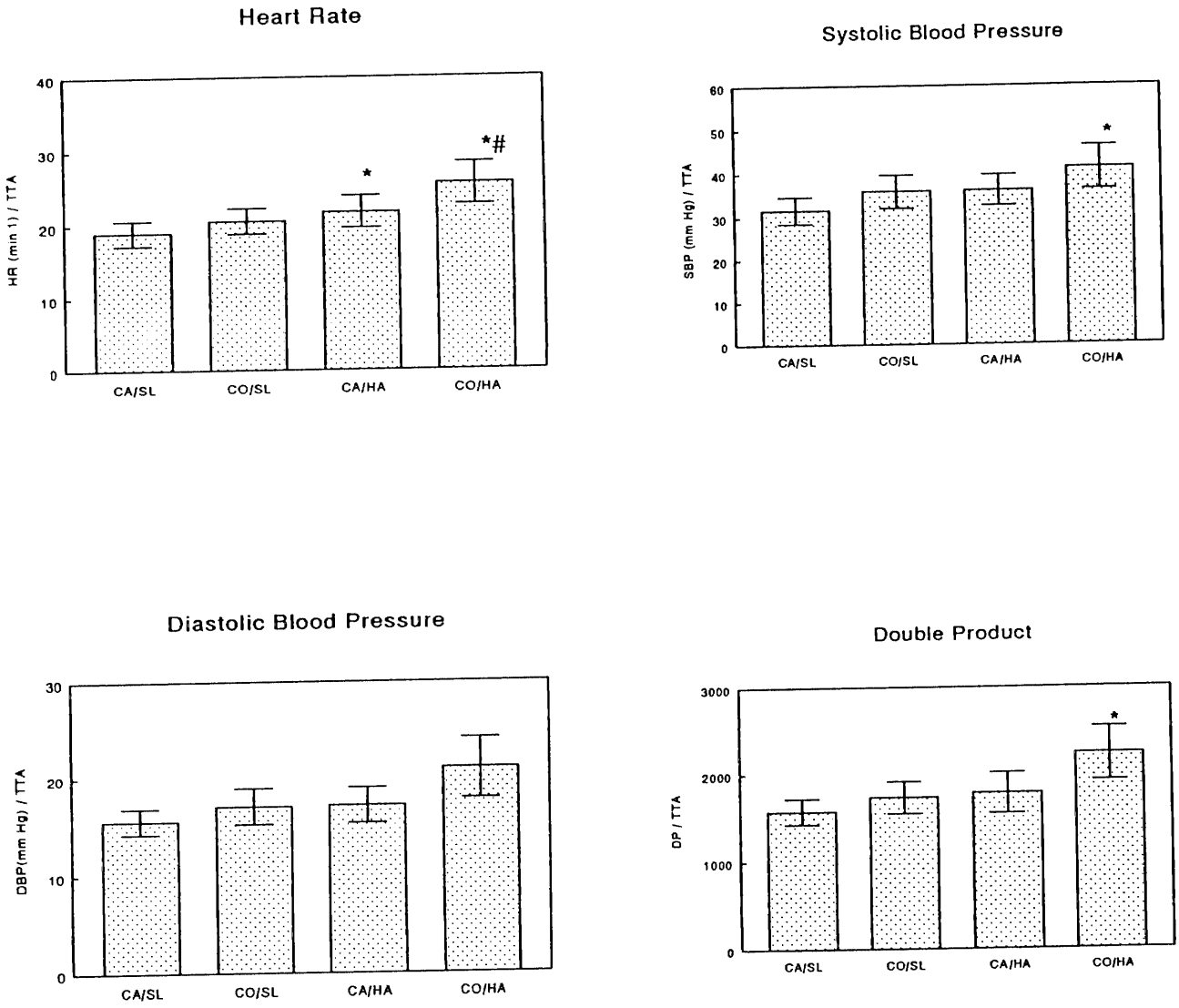
Notes: a - subjects with angina on all 4 test days; b - subjects exhibiting ST segment depression (ST↓) on one or more test days;
c - subjects exhibiting preventricular contractions (PVC's) on one or more test days.

(ANOVA) and there were no significant changes in group mean heart rate, systolic blood pressure or diastolic blood pressure at the end of exercise among the four test conditions; heart rates averaged for all subjects were 109 ± 26 min^{-1} following CA/SL exposures and 110 ± 27, 108 ± 25 and 115 ± 27 min^{-1} following CO/SL, CA/HA and CO/HA exposures, respectively. There were no differences in systolic or diastolic blood pressure values on any of the four test exposure days. However, since the duration of exercise, and total work performed, was less on the test exposure days than the clean air days, the heart rate and systolic blood pressure data suggest that subject's cardiac output was greater relative to work load on the CO exposure days to accomplish the same amount of work as performed on the CA/SL control day. To examine this finding statistically, 2-way repeated measures ANOVA was performed on the ratios formed by dividing HR, SBP, DBP and double product (DP) by the duration of exercise, in min. The ratios normalized these four hemodynamic parameters with respect to exercise duration. The group mean values and significant differences among these means are summarized for the subjects with angina on all 4 test days in Figure 1. Normalized HR, SBP and DP were all significantly increased, relative to CA/SL control values, following the CO/HA exposure; the group mean values for CO/SL and CA/HA exposures were approximately equal and slightly, but not significantly, greater than the CA/SL control value. The values for DBP followed the same pattern, but none of the exposures produced statistically significant changes, relative to the CA/SL control values. ANOVA demonstrated significant ($p \leq 0.02$) main effects of CO exposure on all four normalized hemodynamic parameters. A significant main effect of altitude ($p = 0.01$) was observed for the ratio HR/TTA, but there were no significant altitude x exposure interactions for any of the four normalized parameters.

Respiratory Exchange Parameters

Respiratory exchange data measured at the end of exercise are summarized in Table 4. Oxygen uptake (L min^{-1}) at the time of onset of angina was reduced 3% following either CO/SL or CA/HA exposures and by 11% following CO/HA exposure for the angina sub-group. Carbon dioxide (CO_2) elimination rates were reduced 6%, 3% and 12%, respectively. These changes (as did the changes in hemodynamic parameters) probably

Figure 1. Changes in Hemodynamic Parameters Normalized With Respect to Duration of Exercise to the Onset of Angina (Mean ± S.D.)



Notes: Subjects with angina on all 4 test days

* = different from CA/SL ($P < 0.05$)

\# = different from all other group mean values ($p < 0.05$)

Table 4. Results of Symptom Limited Exercise Tests - Respiratory Physiological Parameters at End of Exercise

	Oxygen Uptake ($\dot{V}_{O_2}$)				Carbon Dioxide Exhaled ($\dot{V}_{CO_2}$)				Respiratory Quotient ($\dot{V}_{O_2}/\dot{V}_{CO_2}$)			
Subject	CA/SL	CO/SL	CA/HA	CO/HA	CA/SL	CO/SL	CA/HA	CO/HA	CA/SL	CO/SL	CA/HA	CO/HA
1 [a,b]	1.18	1.35	0.97	1.07	1.38	1.54	1.24	1.30	1.16	1.14	1.28	1.22
2 [a,b,c]	1.93	1.47	1.77	1.41	2.33	1.48	2.04	1.50	1.21	1.00	1.16	1.06
3 [a,b,c]	0.93	0.81	0.70	0.98	0.95	0.85	0.77	0.64	1.02	1.05	1.11	0.64
4 [a,b,c]	ND	0.91	0.93	0.88	ND	1.08	1.08	1.01	ND	1.19	1.16	1.14
5 [a,b,c]	1.04	1.06	1.08	0.80	1.14	1.21	1.15	0.90	1.09	1.15	1.07	1.12
6 [a]	1.01	ND	0.80	1.05	1.16	ND	0.89	1.22	1.14	ND	1.17	1.16
7 [b,c]	1.31	1.31	1.03	1.26	1.29	1.63	1.37	1.86	0.98	1.24	1.32	1.48
8 [a,b,c]	ND	1.73	1.87	1.97	ND	1.94	2.33	2.37	ND	1.12	1.25	1.21
9 [a,b,c]	1.09	0.91	1.19	1.08	1.22	1.05	1.41	1.26	1.12	1.14	1.19	1.17
10 [c]	0.94	0.90	0.92	0.88	0.95	0.99	1.10	1.01	1.01	1.11	1.08	1.26
11 [a,c]	1.03	1.07	1.16	0.60	1.14	1.10	1.19	0.77	1.11	1.03	1.03	1.27
12 [b]	1.25	1.21	1.12	0.95	1.50	1.42	1.51	1.22	1.21	1.17	1.34	1.31
13 [b]	1.31	1.28	1.13	1.03	1.47	1.50	1.33	1.26	1.12	1.17	1.18	1.22
14 [a]	1.06	0.93	0.86	0.70	1.24	1.10	1.02	1.00	1.17	1.17	1.19	1.42
15 [b,c]	0.86	0.68	0.86	ND	0.85	0.92	1.14	ND	0.99	1.36	1.33	ND
16 [a,b]	1.48	1.19	1.34	1.30	1.44	1.02	1.23	1.22	0.98	0.86	0.93	0.94
17 [a]	1.28	1.19	ND	1.04	1.55	1.49	ND	1.27	1.21	1.25	ND	1.16
18 [a,c]	0.96	1.13	ND	0.94	1.22	1.33	ND	1.19	1.28	1.18	ND	1.27
All Subjects Mean ± SD	1.17±0.27	1.13±0.26	1.11±0.32	1.06±0.31	1.30±0.34	1.27±0.30	1.30±0.40	1.23±0.40	1.11±0.09	1.14±0.11	1.17±0.11	1.18±0.19
Angina Subjects[a] Mean ± SD	1.18±0.29	1.15±0.27	1.15±0.38	1.06±0.35	1.34±0.37	1.27±0.30	1.31±0.47	1.20±0.42	1.14±0.09	1.11±0.11	1.14±0.10	1.14±0.19

Notes: a - subjects with angina on all 4 test days; b - subjects exhibiting ST segment depression (STI) on one or more test days; c - subjects exhibiting preventricular contractions (PVC's) on one or more test days.

reflect the fact that exercise durations, hence total work performed, was reduced by each of the exposures as compared to the CA/SL control exposure. These differences, although consistent with the differences in time to onset of angina, were not statistically significant. On the average, the respiratory exchange ratio, R, was slightly, but not significantly, greater following the three test exposures as compared to the control exposure. Again, it is important to note that for the same amount of work, the R values would have been greater for the test exposures relative to the CA/SL control.

Oxygen uptake, respiratory exchange ratio and minute ventilation at the beginning and end of exercise are plotted vs. time for all subjects in Figures 2, 3 and 4, respectively. The work rate was set to 30 watts initially and was increased 10 watts at the end of each minute of exercise, so that the exertion level was increased approximately linearly during exercise. The lines in the figures represent least squares fits of the data for each of the 4 atmosphere exposures. The solid lines represent sea level exposures and the dashed lines represent simulated high altitude exposures. The intercept at time = 0 is the resting value measured immediately prior to the beginning of exercise at 30 watts.

Exercise at high altitude resulted in a greater increase per unit time for all three parameters, as compared to exercise at sea level. Oxygen uptake (VO_2) at rest was decreased by about 12% following CO exposures relative to clean air at both sea level and high altitude conditions but this decrease was not statistically significant. The slopes of the VO_2 vs time curves (Figure 2) for all three test atmospheres were slightly, but not significantly, greater than that for the CA/SL control exposure. High altitude exposures tended to increase the slopes of the respiratory exchange ratios curves (22% relative to the slopes following sea level exposures); these differences approached but did not attain statistical significance (Figure 3). High altitude exposure increased the slope of minute ventilation change with time (Figure 4) about 24% relative to sea level exposure, significant at the $p \leq 0.05$ level. The slopes of the minute ventilation curves were not affected by CO. There was considerable subject-to-subject variation in all three parameters. The average minute ventilation at rest in clean air at high altitude was 10% greater than that in clean air

Figure 2. Oxygen Uptake During Exercise

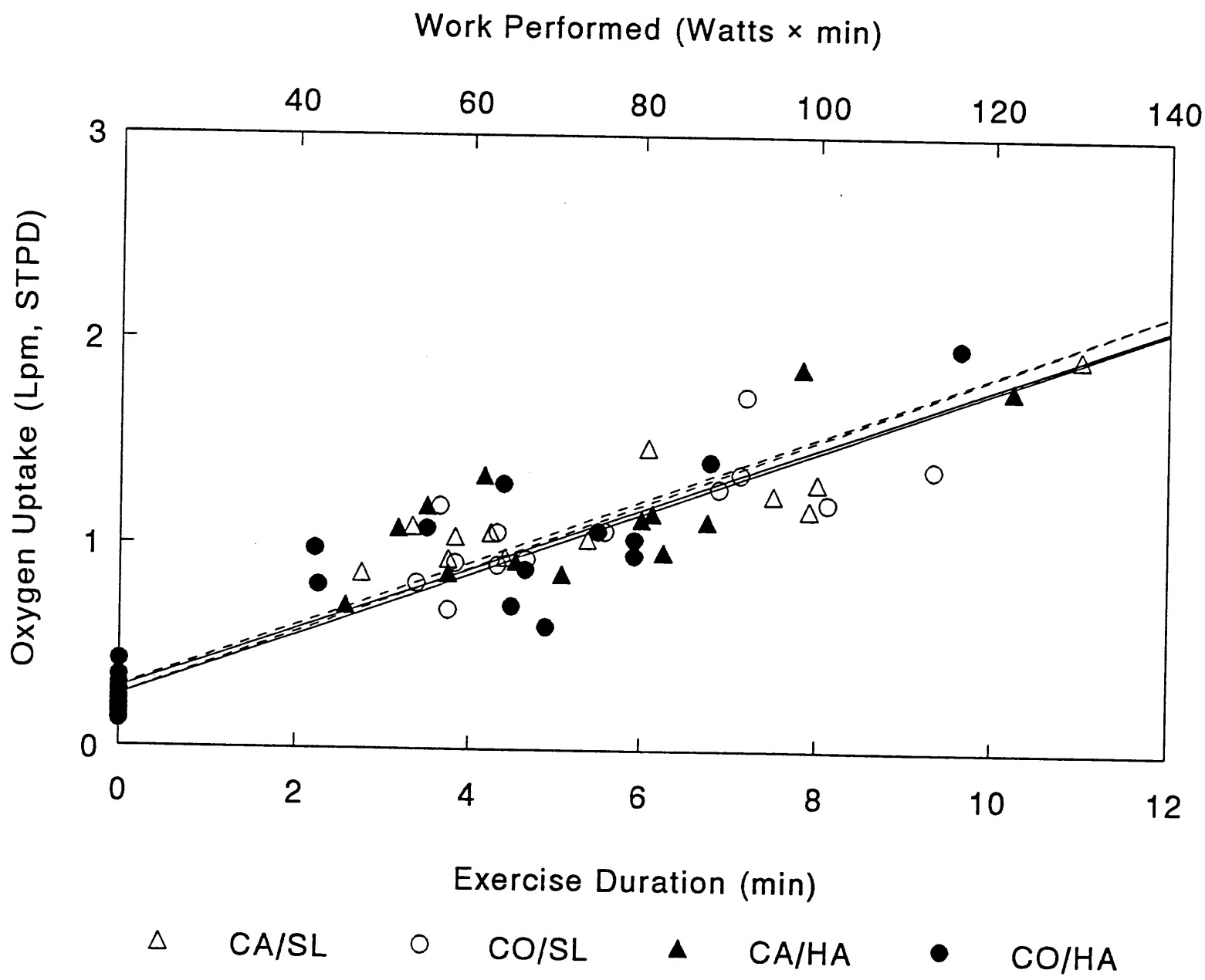


Figure 3. Respiratory Exchange

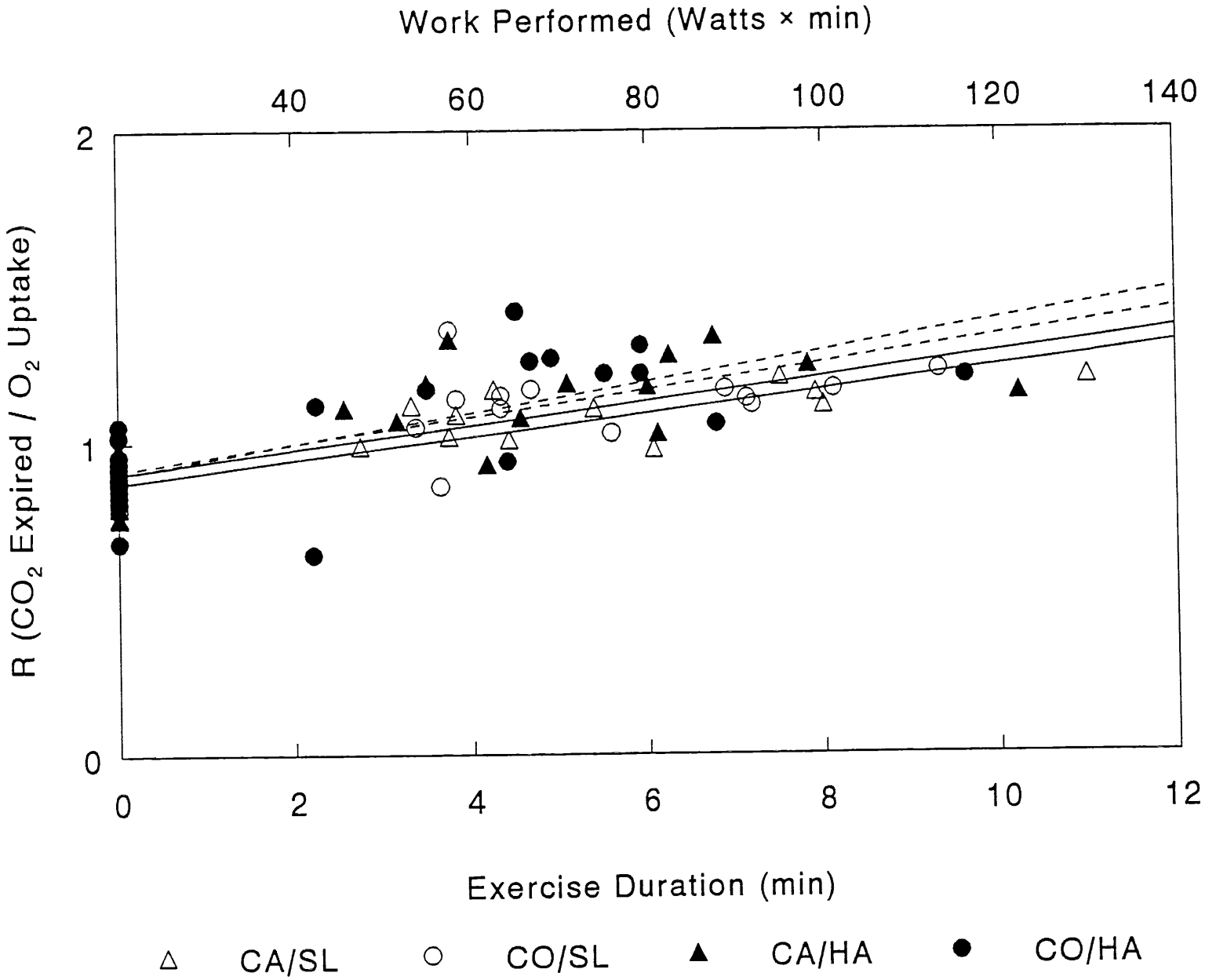
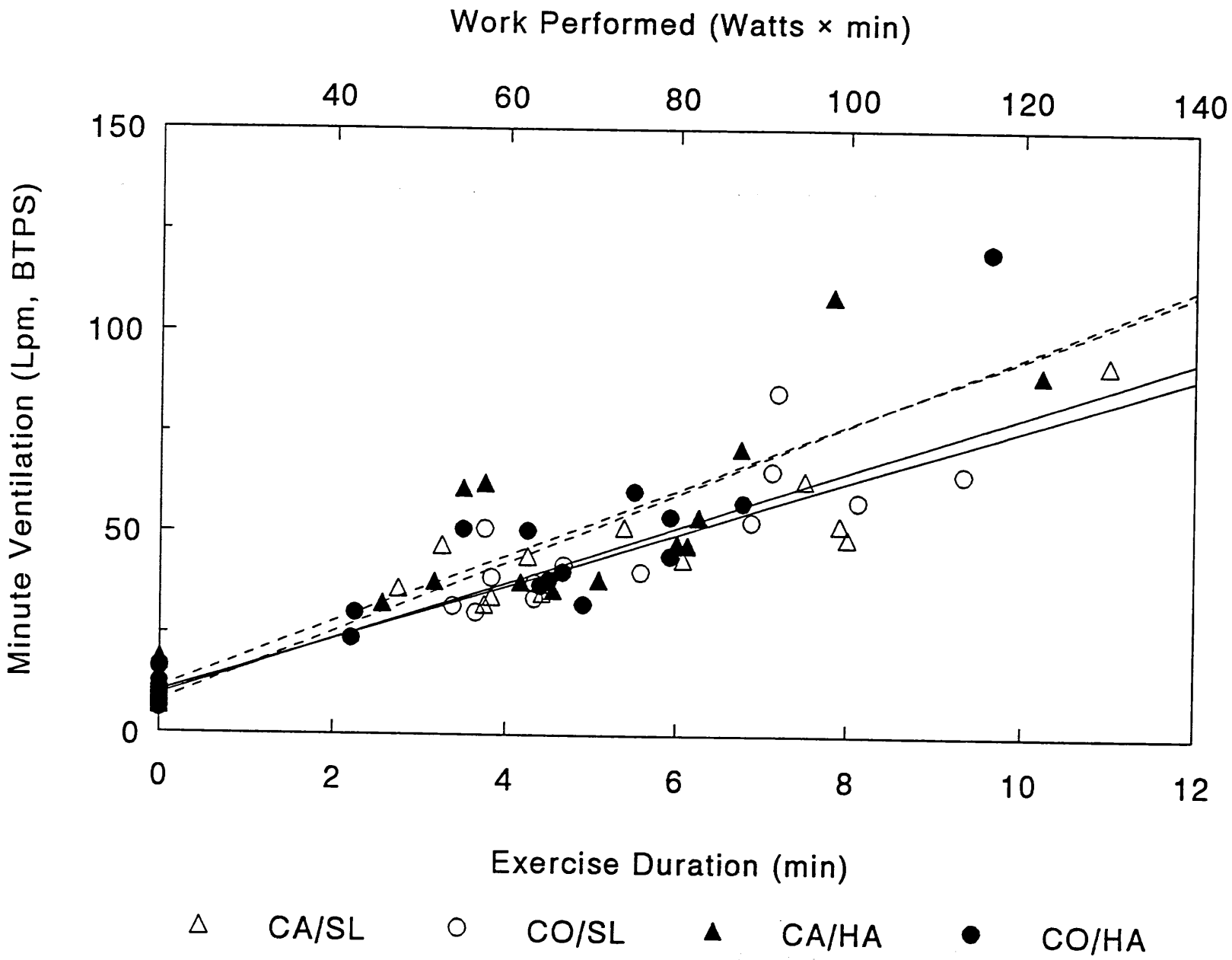


Figure 4. Minute Ventilation



at sea level, which is consistent with reported data for minute ventilation changes at a comparable altitude (Chiodi, 1963). CO exposure tended to decrease resting minute ventilation; this effect was considerably more pronounced at simulated high altitude (19%) than at sea level (7%).

Red Blood Cell Fatty Acid Composition

In order to partially verify that subjects had not significantly altered their eating patterns during the course of the study we analyzed blood samples from 6 of the subjects for red blood cell fatty acid profiles at the beginning and end of their series of test exposures. The results, which are summarized in Table 5, indicate that there were no significant shifts in fatty acid profiles over the 4 or more weeks that these subjects were followed. The relative fatty acid composition was normal for this group of individuals.

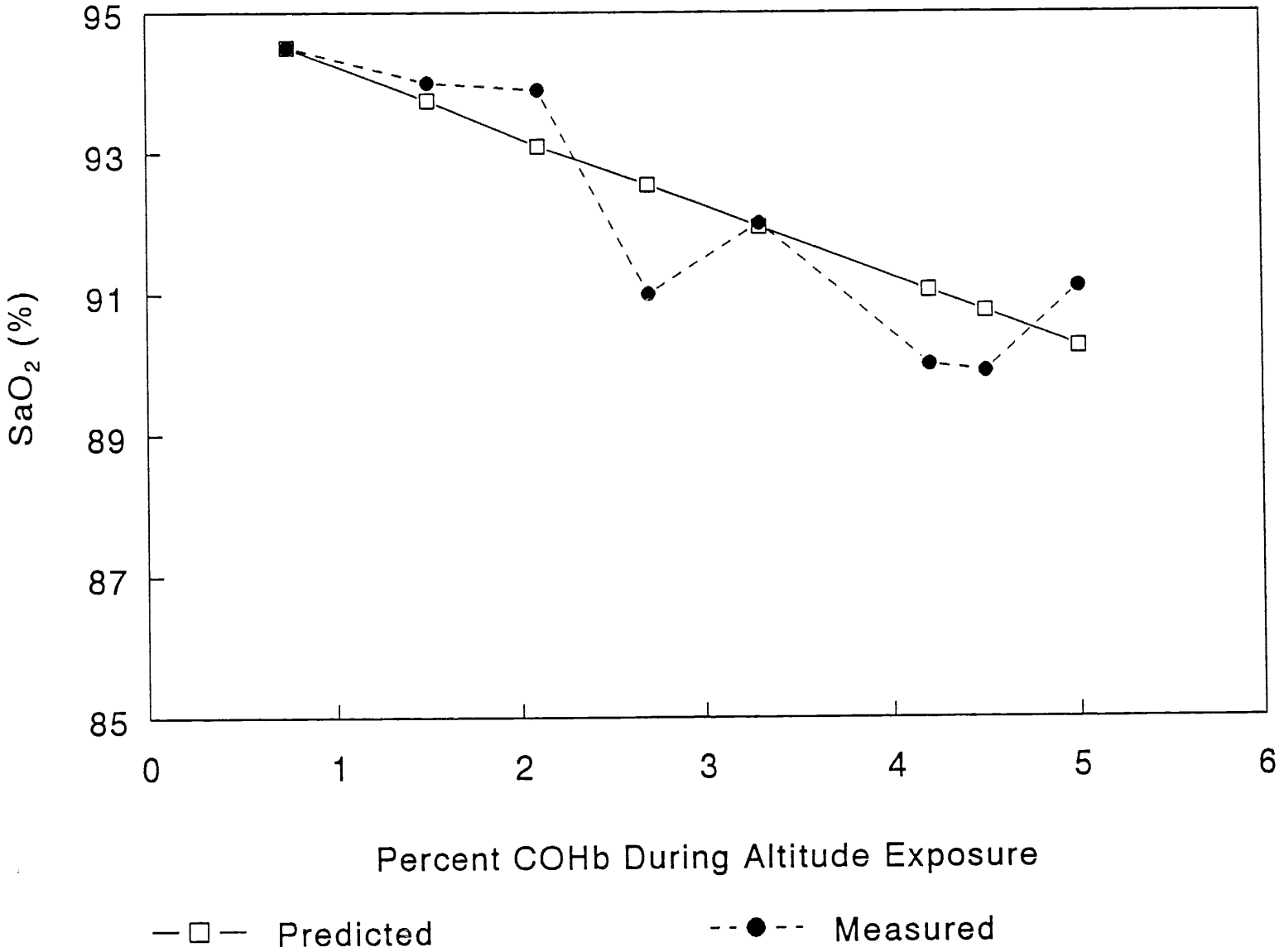
Validation of the altitude simulation

An arterial catheter was emplaced in one volunteer for sequential arterial blood sampling. Following the start of breathing the high altitude gas mixture, SaO_2 rapidly decreased from the average sea level value of 96.8 ± 0.2% to 94.5 ± 1.3%, in the range of normal values for individuals at sea level (96.3 ± 0.3%) and 2 km elevations (93.2 ± 1.0%), respectively (Chiodi, 1963). A relatively high concentration of CO was then administered (250 ppm) and serial arterial blood samples were taken at 5 min intervals for the next 30 min. As shown in Figure 5, the $\%SaO_2$ decreased in response to the increase in COHb and the net measured reduction in $\%SaO_2$ was predicted to within ± 2% by subtracting the %COHb from the initial $\%SaO_2$.

Table 5. Red Blood Cell Fatty Acid Composition For 6 Selected Subjects (% of Total Fatty Acid Content)

Fatty Acid Class (carbon atoms:double bonds)	Mean ± SD	
	Beginning of Study	End of Study
Saturated		
16:0	20.3 ± 1.4	20.4 ± 2.2
18:0	13.2 ± 0.5	13.3 ± 0.5
Mono-Unsaturated		
18:1	13.9 ± 1.3	14.6 ± 0.8
Poly-Unsaturated		
18:2	10.8 ± 1.8	10.6 ± 1.8
20:4	12.4 ± 0.8	11.7 ± 1.8
22:6	3.8 ± 1.1	3.6 ± 1.2

Figure 5. Arterial O_2 Sat. (2.1 km)
With Increasing Carboxyhemoglobin Levels



Discussion

The primary objective of this study was to examine the effects of high altitude CO exposures on sensitive individuals at an altitude which was relevant to that in which substantial numbers of people are exposed and at a COHb concentration which was within the range experienced by many individuals. A limitation of this study relates to relates to the fact that the study was performed in a laboratory and not at actual high altitude. The approach to simulating high altitude in this study was to reduce the partial pressure of oxygen in inspired air to the level equivalent to that at 2.1 km altitude by metering purified nitrogen into the inspired air. The study could appropriately address altitude-induced hypoxemia, but could not incorporate effects which might be due to barometric pressure and gas viscosity differences. We did, however, we did establish, however, that the simulated 2.1 km conditions (FiO_2 = 16.1%) did produce changes in arterial oxygen saturation (SaO_2) in our labaoratory subjects which matched the changes observed in volunteers under actual high altitude conditions.

The specific conditions chosen for this study were designed to match the levels of reduction in oxygen carrying capacity by the bloodstream for the cases of CO alone, and altitude alone in order to directly compare the effects of altitude-induced hypoxic hypoxia with CO-related chemically induced hypoxia, and then the joint effects of altitude and CO exposure were assessed. In this experiment the oxygen carrying capacity of blood was reduced by approximately 4% by either exposing subjects to 100 ppm CO for 2 hr, or by reducing the fraction of inspired O_2 from a normal sea level value of 20.94% to a value equivalent to the inspired O_2 content of air at 2.1 km altitude, 16.1%. Both methods of inducing hypoxia reduced the $\%SaO_2$ to approximately 94% from the normal baseline value while breathing purified air at sea level, which averages about 98%. As shown in Table 6, the changes in group means of TTA and normalized HR, blood pressures and DP, for either CO/SL or CA/HA exposures relative to CA/SL exposures, were approximately equivalent, to within the limits of measurement error. The joint exposure of subjects to CO at high altitude resulted in group mean changes from sea level clean air control exposures which

were approximately equal to the sum of effects measured under CO/SL and CA/HA conditions (for those parameters which demonstrated significant main effects of atmosphere, altitude, or both). ANOVA did not demonstrate any significant interactions between the factors altitude and exposure. These findings are consistent with an additive, but not synergistic, effect of CO and high altitude, under the experimental conditions used in this study. Furthermore, given the observed equivalence (at equal reductions in SaO_2) and additivity of responses, the effects of CO exposure may be discussed in terms of reduction of blood oxygen carrying capacity, which simplifies the context in which CO and high altitude exposures should be examined for the purpose of comparing results of the present study with those reported by other investigators.

The adverse effects of CO exposure have been documented in air quality criteria documents published by the U. S. Environmental Protection Agency (1979;1984), and in a Technical Staff Report published by the California Air Resources Board (Batchelder, 1989) in support of the California State standard for CO. The health effects literature published since these documents were compiled has been critically reviewed (Kleinman, 1992). Relatively small decreases in hemoglobin oxygen-carrying ability resulting from 2% to 6% carboxyhemoglobin levels cause measurable decreases in exercise tolerance in both normal and sensitive individuals, and may possibly increase the risks of coronary artery disease and cardiac arrhythmias. Exercise tolerance in normal subjects was impaired after breathing 100 ppm CO for one hour, increasing COHb from 1.7% at baseline to 3.95% after exposure (Aronow and Cassidy, 1975). Mean time to a maximal exercise effort was decreased about 5%, from 11.63 minutes to 11.04 minutes. Horvath, et al., (1975) reported that exercise tolerance was reduced by about 6% in CO-exposed, healthy individuals at COHb levels of about 3 to 4%. Individuals with coronary artery disease were found to be especially sensitive to the effects of CO. Aronow and Isbell (1973) reported that exposure to low levels of CO significantly reduced exercise capacity of individuals with coronary artery disease. Subjects exposed to 50 ppm CO for two hours increased their COHb levels from a baseline of 1.0% to a level of 2.7% and reduced the time to onset of exercise-induced angina pectoris from 3.74 minutes (observed after subjects breathed clean air) to 3.13 minutes (observed after CO

Table 6. Summary of ANOVA and Tukey Multiple Comparison Test Results

	2-way Repeated Measures ANOVA (p values)			Group Mean Comparisons (% Change from Control)		
Parameter	CO	Altitude	Interaction	CO/SL	CA/HA	CO/HA
TTA [a]	0.047	0.026	NS	-9	-11	-18*
INT ST↓ [b]	NS	NS	NS	0	-25	+0
PVC [c]	NS	NS	NS	+88	+63	+113
HR/TTA [a,d]	0.002	0.010	NS	+11	+13*	+30**
SBP/TTA [a,d]	0.016	0.099	NS	+15	+12	+20
DBP/TTA [a,d]	0.015	0.178	NS	+11	+9	+22
DP/TTA [a,d]	0.006	0.098	NS	+16	+12	+28*
$\dot{V}_{O_2}$ [a]	NS	NS	NS	-3	-3	-10
$\dot{V}_{CO_2}$ [a]	NS	NS	NS	-6	-2	-10
R [a]	NS	NS	NS	-3	+0	+0

Notes: a - subjects with angina on all 4 test days; b - subjects with ST segment depression (ST↓) on one or more test day; c - subjects with preventricular contractions (PVC's) on one or more test day; d - heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and double product (DP = SBP x HR) normalized with respect to amount of work performed up to the onset of angina by dividing by the duration of exercise (TTA).

* significantly impaired relative to sea level clean air ($p < 0.05$).

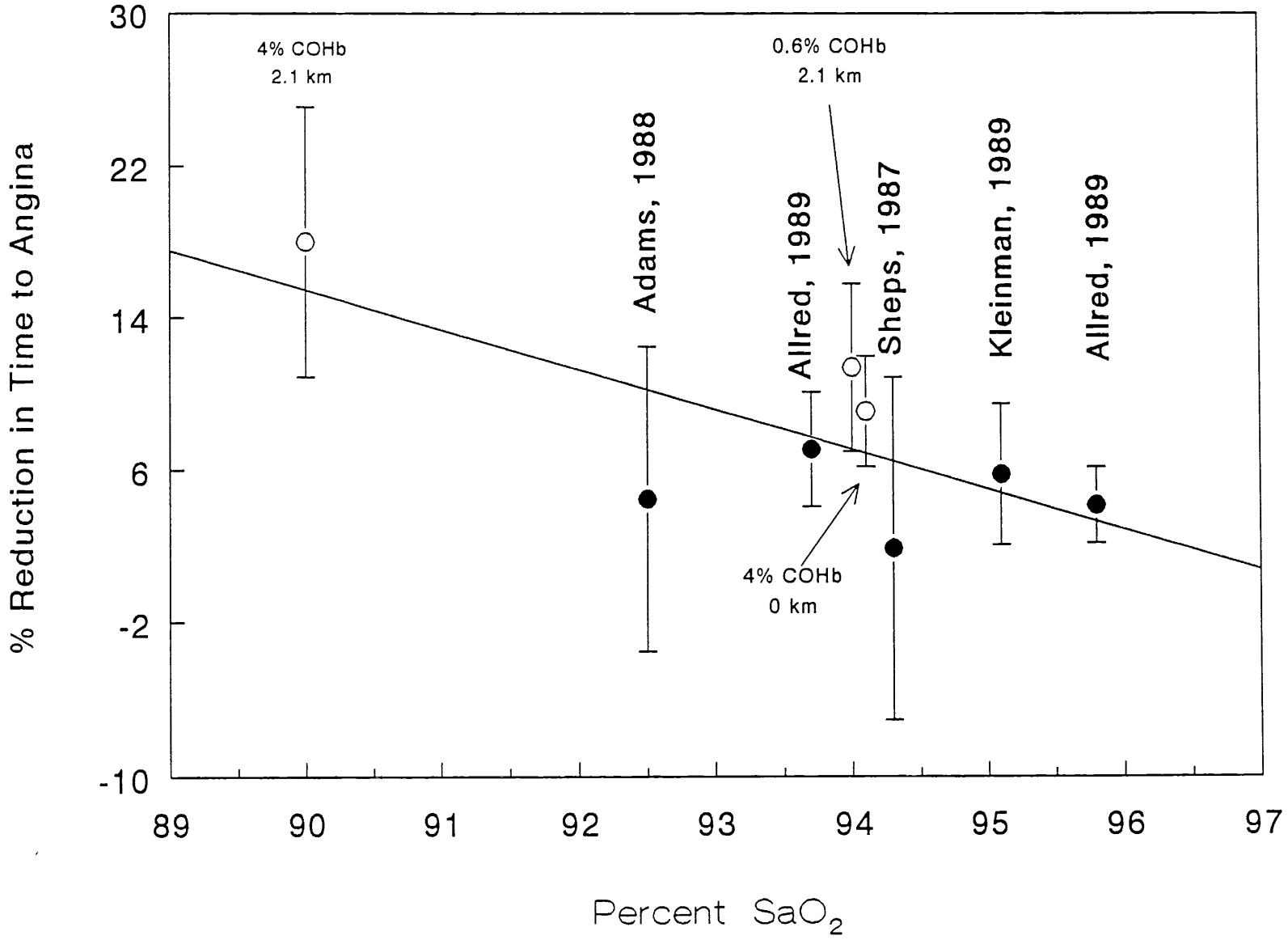
** significantly impaired relative to all other exposures ($p < 0.05$).

exposure). A study by Anderson et al., (1973) reported a decreased exercise tolerance in persons with stable angina pectoris following exposure to CO sufficient to cause a mean increase in COHb of 1.6%. Sheps et al. (1987) exposed 30 subjects with ischemic heart disease, aged 38 to 75 yrs, to CO (100 ppm) or air, during a 3-day, randomized, double-blind protocol, to achieve an average post-exposure COHb concentration of 3.8% on the CO exposure day (COHb on the air exposure day averaged 1.5%). After exposure to either CO or air, subjects performed an exercise stress test. This study showed small, but not significant, decreases in time to onset of angina (1.9%) and maximal exercise time (1.3%) in CO vs. air exposures. Times to significant ST decreases, double product (DP; heart rate x systolic blood pressure) at significant ST depression, and maximal DP were similar for both conditions. (Double products have been shown to correlate with measured myocardial O_2 consumption during dynamic exercise (Kitamura et al., 1972)). The change in ejection fraction (rest to maximal) was slightly lower for CO exposures (air = 3.5%, CO = 2%; $p = 0.049$). The authors concluded that there were no clinically significant effects of low-level CO exposures resulting in COHb concentrations of 3.8%. Adams et al. (1988) subsequently extended the above study to an average post-exposure COHb concentration of 5.9%, during exercise, using an identical protocol and 30 subjects (22 men, 8 women; mean age 58 yrs). The level of submaximal ejection fraction was significantly higher after air, when compared to the CO exposure (3.3%; $p \leq 0.05$) and the change in ejection fraction, from rest to submaximal exercise, was significantly lower after CO exposure, compared to air exposure (air = 1.6% and CO = -1.2%; $p \leq 0.05$). No statistically significant exposure-related differences were seen for either maximal ST-segment depression, time to onset of significant ST-segment depression, or maximal DP. The authors concluded that exposures to CO resulting in COHb concentrations of about 6% significantly impaired exercise performance in subjects with ischemic heart disease. Although we did not quantitate ejection fraction in this study, the finding of higher cardiac rates and pressures at lower workloads after the combined CO and high altitude exposure in our study could reflect decreased left ventricular ejection fraction, relative to controls. Kleinman et al. (1989) exposed 24 nonsmoking male subjects with stable angina and positive exercise tests to 100 ppm CO or air to achieve an average COHb concentration of 2.9%, during exercise, on the CO exposure day. In

subsequent exercise tests, the time to onset of angina was decreased after CO exposure (5.9%; $p = 0.046$) relative to air exposure. The duration of angina was longer after CO exposure compared to air exposure (8.3%), but this change was not statistically significant. Oxygen uptake at the angina point was slightly reduced after CO exposure compared to air exposure (2.2%; $p \leq 0.04$), but the increase in oxygen uptake with increasing workload was similar on both exposure days. Allred et al. (1989) exposed 63 men with documented coronary artery disease to air, 117 ppm CO or 253 ppm CO, on three separate days in a randomized, double-blind protocol, followed by an incremental treadmill exercise test. Average COHb concentrations of 2.2% and 4.3%, during exercise, were achieved on the two CO exposure days (2.0 and 3.9%, respectively, at the end of exercise). The time to onset of angina was significantly reduced by CO exposure, in a dose-dependent manner (4.2% at 2% COHb, $p = 0.054$; 7.1% at 4% COHb, $p = 0.004$). Linear regressions of time to angina vs COHb concentrations for each subject indicated that time to angina decreased 1.9 ± 0.8 percent for every 1 percent increase in COHb ($p \leq 0.01$). The time to onset of 1 mV ST segment depression was also reduced by CO in a dose-dependent manner (5.1% at 2% COHb, $p = 0.02$; 12.1% at 4% COHb, $p \leq 0.0001$) compared to the clean air exposure. There was a decrease of approximately 3.9 ± 0.6 percent in time to ST depression for every 1% increase in COHb ($p \leq 0.0001$). There was a significant correlation between the percent change in the time to onset of angina and the time to onset of ST depression ≥ 1 mV ($p \leq 0.0001$).

In general, the TTA changes at 4% COHb or at 4% reduction of SaO_2 from baseline by breathing air with reduced FiO_2 are in good agreement with those observed in the previously reported studies discussed above. A linear least squares fit line of % change in TTA vs. SaO_2 from this and the previous studies described above (Figure 6), has a significant slope and indicates that a 1% reduction in SaO_2 results in a 2.1 ± 0.8% (mean $\pm$ se) reduction in TTA. The effects of CO and altitude on $\%SaO_2$ appear to be additive, therefore TTA will be reduced both by increasing altitude and by increasing COHb concentrations due to CO exposure.

Figure 6. Change in Time to Angina at R Arterial O_2 Saturation (Mean ± S.D.)



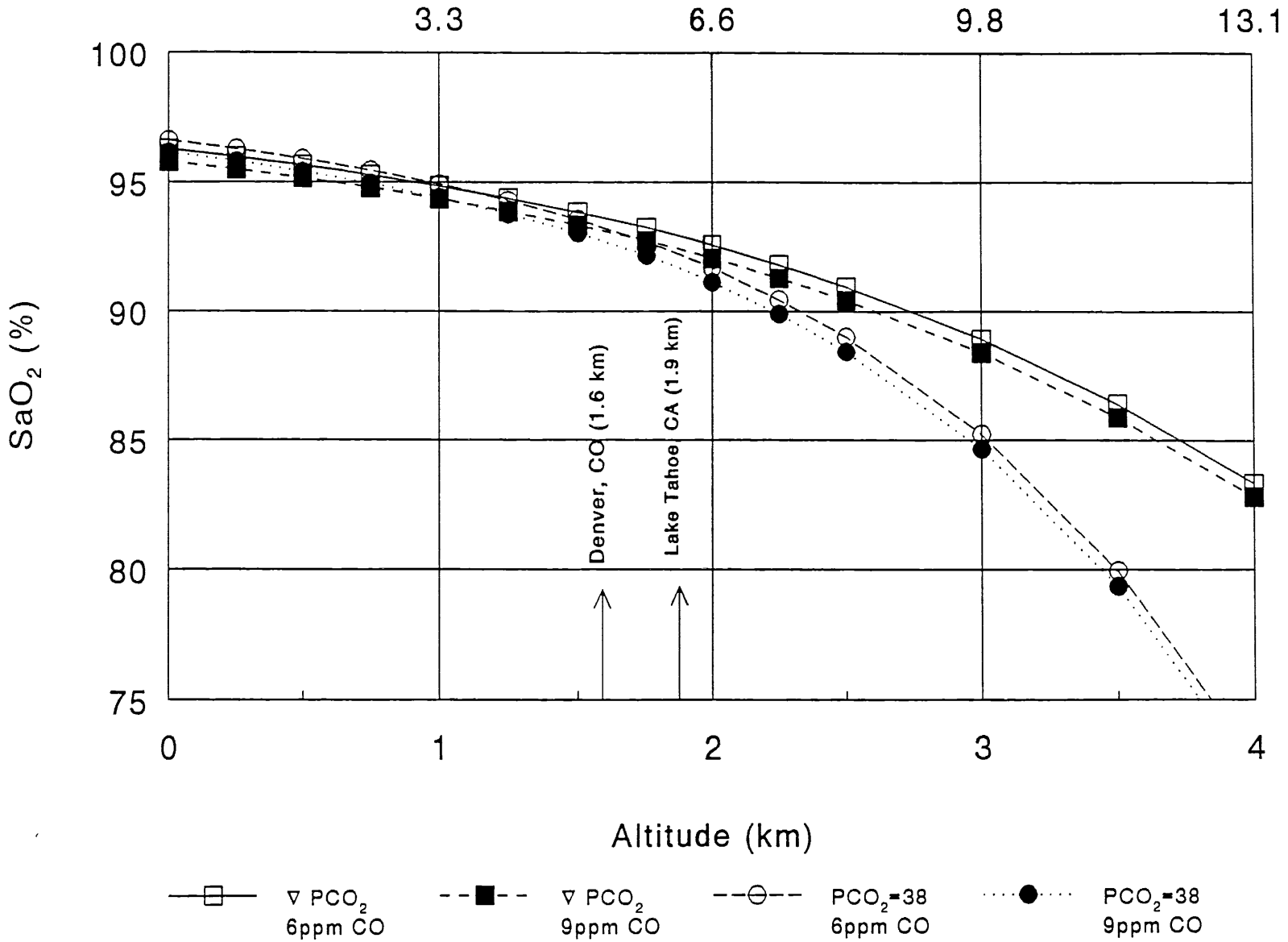
As discussed earlier, Pitts and Pace (1947) estimated that a 1% increase in COHb was equivalent to an increase in altitude of approximately 110m. More recently Collier and Goldsmith (1983) modeled, as a function of altitude, COHb concentrations and $\%SaO_2$ for individuals exposed to CO at the levels of the State of California Air Quality standards (9 ppm at sea level and 6 ppm at the altitude of Lake Tahoe, 1.9 km). The model is based on the Coburn-Foster-Kane equation for changes in COHb as a function of inhaled CO, ventilation, diffusion of CO across the lung epithelium, endogenous CO production and exposure duration. Physiological parameters relevant to O_2 uptake and CO_2 elimination, and other physical parameters were also modeled. The assumptions and algorithms used in the model have been carefully detailed by Collier and Goldsmith (1983). This type of modeling is useful because it allows the comparison of potential effects of applying ambient air quality standards under specific environmental conditions. Using the Collier and Goldsmith model, and the health effects data obtained in this study, it is possible to evaluate potential differences in health effects which might occur as a result of CO exposures at high altitude. Using the same set of assumptions and algorithms, the model was run on an IBM PC, and the results were compared with those published by Collier and Goldsmith. Two modifications were made to the model to better match the conditions of our protocol. The ventilation rate was reduced from 500 L min^{-1} to 250 L min^{-1} (consistent with ventilation rates measured for resting subjects in this study) and the arterial CO_2 tension (PCO_2) was estimated as a linear relationship with altitude (in the previous model PCO_2 was held constant at 38 mm Hg, although Collier and Goldsmith (1983) recognized that PCO_2 would most likely decrease with altitude). The model was applied to estimate the $\%SaO_2$ as a function of both altitude and ambient CO concentration. The specific cases of ambient CO at the California State Air Quality 8 hr Standards, 9 ppm (solid symbols) and 6 ppm (open symbols), applied to sea level and high altitude situations, respectively, are shown in Figure 7. Two sets of curves are shown, depicting the cases of PCO_2 held constant at 38 mm Hg (circles) and PCO_2 reducing with altitude (squares). The linear relationship for PCO_2 decrease with increasing altitude was computed from a least squares fit of data reported by Chiodi for unacclimatized individuals at high altitude. The sets of curves begin to diverge at altitudes above 1.5 km. The assumption of constant PCO_2 causes a greater decrease in

SaO_2 per unit increase in altitude than is seen if PCO_2 decreases. The curves can be used to estimate that, in both models, reduction of ambient CO from 9 to 6 ppm results in a smaller decrease of SaO_2, due to the smaller concentration of COHb resulting from the reduced ambient CO exposure. The magnitude of the difference (COHb is increased from 1.5% at 6 ppm to 2.0% at 9 ppm) is not appreciably changed as a function of altitude. From the linear regression of data in Figure 6, it can be estimated that TTA would be reduced to a 35% greater extent if subjects were exposed to 9 ppm CO than if they were exposed a 6 ppm at 2.0 km altitude (equivalent to reductions in TTA of 4.2 and 3.1 %, respectively, over the effect of altitude alone). If these changes are placed into the context of the effect which would be induced by changing altitude alone, there some differences between to two models tested. In the case of holding pCO_2 constant, the reduction in $\%SaO_2$ due to increasing ambient CO from 6 to 9 ppm at Lake Tahoe would be equivalent to an increase in altitude of 98 m. In the case of allowing for reduction of pCO_2 (which is consistent with an increased expiration of CO_2 due to increased ventilation rates at high altitude), the altitude equivalent difference would be 140m, 43% greater. Viewing Figure 7 in another way, however, one can estimate that for both models breathing air at a CO concentration of 9 ppm at Denver, CO would be approximately equivalent to breathing 6 ppm CO air at Lake Tahoe (increasing altitude by 200 to 300 m). Comparison of resting $\%SaO_2$ reported for unacclimatized individuals at high altitudes (Hurtado and Aste Salazar, 1948; Huang et al., 1987; Bender et al., 1989) with the values in Figure 7 suggest that the model allowing for reduced pCO_2 with altitude provides a better $\%SaO_2$ estimate, than maintaining pCO_2 at a constant value of 38 mm Hg, although previous experiments at simulated high altitude have indicated otherwise (Gong et al., 1984).

This study demonstrated a substantial (60 to 110%), but not statistically significant, increase in the incidence of abnormal heart beats in subjects following CO and simulated high altitude exposure and exercise testing. The subjects in this study were selected on the basis of stable angina, and individuals prone to cardiac arrhythmias were not specifically sought. The cardiac monitoring protocol only recorded EKG's for about 20 sec of each minute of rest or exercise. Our findings are, however, consistent with those of other investigators (Kerin et al., 1979; Carboni et al., 1987; Sheps et al., 1990). Hinderliter et al.

(1989) demonstrated that CO exposure (4% to 6% COHb) was not arrhythmogenic in patients with coronary artery disease and no ventricular ectopy at base line. More recently, however, Sheps et al. (1990) examined a relatively large study population (41 subjects) with some evidence of ventricular ectopy and found that the frequency of single ventricular depolarizations (VPD's) increased by 30%, relative to clean air control values, in CO-exposed patients (6% COHb), and that multiple VPD's increased threefold, relative to air exposure. The subjects who exhibited increased frequencies of multiple VPD's tended to be older, exercised for longer durations, and had higher peak workloads during exercise than those who did not exhibit complex arrhythmias. To summarize, the results of this study demonstrated significant additive, but not synergistic, effects of high altitude and CO exposures for individuals with coronary artery disease. The parameters examined included cardiological measurements (time to onset of angina, ST segment depression and incidence of abnormal heart beats), exercise physiological measurements (oxygen uptake, carbon dioxide expiration, ventilation rates), and hemodynamic measurements (heart rates and blood pressures). The subjects in this study were not acclimated to the simulated high altitude condition and all of the exposures and exercise tests represented acute events. Future research directions might therefore involve other populations at risk to high altitude CO exposure effects (e.g. individuals with cardiac arrhythmias, pregnant women), altitude acclimatized subjects, and chronic exposures.

Figure 7. Modeled Reduction of SaO_2



References

Adams, K.F., Koch, G., Chaterjee, B., Goldstein, G.M., O'Neil, J.J., Bromberg, P.A., Sheps, D.S., McAllister, S., Price, C.J. and Bisette, J., Acute elevation of blood carboxyhemoglobin to 6% impairs exercise performance and aggravates symptoms in patients with ischemic heart disease. J. Am. Coll. Cardiol. 12: 900-909, 1988.

Allred, E.N., Bleeker, E.R., Chaitman, B.R., Dahms, T.E., Gottlieb, S.O., Hackney, J.D., Pagano, M., Selvester, R.H., Walden, S.M., and Warren, J., Short-term effects of carbon monoxide exposure on the exercise performance of subjects with coronary artery disease, New England J. of Med. 321:1426-1432, 1989.

Anderson, E.W., Andelman, R.J., Strauch, J.R., Fortuin, N.J. and Knelson, J.H., Effect of low-level carbon monoxide exposure on onset and duration of angina pectoris. Ann. Intern. Med. 79:46-50, 1973.

Aronow, W.S and Cassidy, J., Effect of carbon monoxide on maximal treadmill exercise. A study in normal persons. Ann. Int. Med. 83: 496-499, 1975.

Aronow, W.S. and Isbell, M.W., Carbon monoxide effect on exercise-induced angina pectoris. Ann. Int. Med. 79:392-395, 1973.

Astrup P. and Pauli, H.G., eds. A comparison of prolonged exposure to carbon monoxide and hypoxia in man. Scand. J. Clin. Lab. Invest. 24 (Suppl. 103): 1-71, 1968.

Ayers, S.M., Giannelli, S. Jr. and Mueller, H., Part IV. Effects of low concentrations of carbon monoxide. Myocardial and systemic responses to carboxyhemoglobin. Ann. N.Y. Acad. Sci. 174: 268-293, 1970.

Batchelder, J., Scibienski, C. and Saloum, L. Adequacy of the statewide carbon monoxide ambient air quality standard: The impact of recent health effects studies. State of California Air Resources Board, Sacramento, CA, December 1989.

Bender, P.R., McCullough, R.E., McCullough, R.G., Huang, S.Y., Wagner, P., Cymerman, A, Hamilton, A.J. and Reeves, J.T., Increased exercise SaO_2 independent of ventilatory acclimatization at 4,300 m. J. Appl. Physiol. 66: 2733-2738, 1989.

BMDP. Biomedical Computer Programs, Department of Biomathematics, School of Medicine, University of California, Los Angeles, University of California Press, 1989.

Carboni, G.P., Lahiri, A., Cashman, P.M.M. and Raftery, E.B. Mechanisms of arrhythmias accompanying ST-segment depression on ambulatory monitoring in stable angina pectoris. Am. J. Cardiol. 60:1246-1253, 1987.

Chiodi, H., Respiratory adaptation to high altitude. In The Regulation of Human Respiration, D.J.C. Cunningham and B.B. Lloyd, eds., F.A. Davis Company, Philadelphia, PA, 1963.

Coburn, R.F., Foster, R.E. and Kane, P.B., Considerations of the physiological variables that determine the blood carboxyhemoglobin concentrations in man. J. Clin. Invest. 44: 1899-1910, 1965.

Collier, C.R. and Goldsmith, J.R., Interactions of carbon monoxide at high altitude. Atmos. Environ. 17:723-728, 1983.

Environmental Protection Agency. Air Quality Criteria for Carbon Monoxide, EPA Report No. EPA-600/8-79-022, Environmental Criteria and Assessment Office, Research Triangle Park, NC, NTIS No. PB81-244840, 1979.

Environmental Protection Agency. Revised Evaluation of Health Effects Associated with Carbon Monoxide Exposure: An Addendum to the 1979 EPA Air Quality Criteria Document for Carbon Monoxide, EPA Report No. EPA-600/9-83-033F, Environmental Criteria and Assessment Office, Research Triangle Park, NC, NTIS No. PB85-103471, 1984.

Gong, H. Jr., Tashkin, D.P., Lee, E.Y and Simmons, M.S. Hypoxia-altitude simulation test. Amer. Rev. Respir. Dis. 130:980--992, 1984.

Hinderliter, A.L., Adams, K.F. Jr.,Price, C.J., Herbst, M.C., Koch, G. and Sheps, D.S. Effects of low-level carbon monoxide exposure on resting and exercise-induced ventricular arrhythmias in patients with coronary artery disease and no baseline ectopy. Arch. Environ. Health 44: 89-93, 1989.

Horvath, S.M., Raven, P.B., Dahms, T.E. and Gray, D.J., Maximal aerobic capacity at different levels of carboxyhemoglobin. J. Appl. Physiol. 38:300-303, 1975.

Huang, S.Y., Moore, L.G., McCullough, R.E., McCullough, R.G., Micco, A.J., Fulco, C., Cymerman, A., Manco-Johnson, Weil, J.V. and Reeves, J.T. Internal carotid and vertebral arterial flow velocity in men at high altitude. J. Appl. Physiol. 63:395-400, 1987.

Huntsberger, J. and Leaverton, K. Biostatistics, J. Wiley and Co., New York, NY, 1970.

Hurtado, A. and Aste Salazar, H. Arterial blood gases and acid-base balance at sea level and at high altitudes. J. Appl. Physiol. 1:304-325, 1948.

Kerin, N.Z., Rubenfire, M., Naini, M., Wajszczuk, W.J., Pamatmat, A. and Cascade, P.N. Arrhythmias in variant angina pectoris: Relationship of arrhythmias to ST-segment elevation and R-wave changes. Circulation 60:1343-1350, 1979.

Kitamura, K., Jorgenson, C.R., Gebel, G.L., Taylor, H.L. and Wang, Y., Hemodynamic correlates of myocardial oxygen consumption during upright exercise. J. Appl. Physiol. 32:516-522, 1972.

Kleinman, M.T. Health effects of carbon monoxide. In Environmental Toxicants: Human Exposures and Their Health Effects, M. Lippmann, ed., Van Nostrand Reinhold, New York, 1992.

Kleinman, M.T., Davidson, D.M., Vandagrif, R.B., Caiozzo, V.J. and Whittenberger, J.L., Effects of short-term exposure to carbon monoxide in subjects with coronary artery disease. Arch. Environ. Health 44:361-369, 1989.

Larsen, R.I. and Burke, H. Ambient carbon monoxide exposure. Presented at the 62nd Annual Meeting of the Air Pollution Control Assoc., New York, 1969.

Longo, L.D. Carbon monoxide: Effects on oxygenation of the fetus in utero. Science 194:523-525, 1976.

NAS. Carbon Monoxide. National Academy of Sciences, Washington, D.C., 1977.

Pitts, G.C. and Pace, N., The effect of blood carboxyhemoglobin concentration on hypoxia tolerance, Amer. J. Physiol. 148:139-151, 1947.

Roughton, F.J.W. The equilibriumof carbon monoxide with human hemoglobin in whole blood, Ann. N.Y. Acad. Sci. 174 (Part 1): 177-188, 1970.

Sheps, D.S., Adams, K.F. Jr., Bromberg, P.A., et al., Lack of effect of low levels of carboxyhemoglobin on cardiovascular function in patients with ischemic heart disease, Arch. of Environ. Health 42:108-116, 1987.

Sheps, D.S., Herbst, M.C., Hinderliter, A.L., Adams, K.F., Ekelund, L.G., O'Neil, J.J., Goldstein, G.M., Bromberg, P.A., Dalton, J.L., Ballenger, M.N., Davis, S.M. and Koch, G. Production of arrhythmias by elevated carboxyhemoglobin in patients with coronary artery disease. Ann. Intern. Med. 113:343-351, 1990.

Silverman, K.J. and Grossman, W., Angina pectoris: Natural history and strategies for evaluation and management. New Eng. J. Med. 310:1712-1717, 1984.

Snedecor, G.W. and Cochran, W.G. Statistical Methods, The Iowa State University Press, Ames, Iowa, 1980.

Vogel, J.A. and Gleser, M.A. Effect of carbon monoxide on oxygen transport during exercise. J. Appl. Physiol. 32:234-239, 1972.