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The objective of this project was to determine the subclinical effects of carbon monoxide (CO) on individuals with ischemic heart disease. Studies were conducted to improve procedures for measuring carboxyhemoglobin (COHb), and to characterize responses in CO-sensitive subjects. Measures of COHb (1 to 3%) were obtained using a CO-Oximeter (IL 282; Instrumentation Laboratories). Eighteen subjects were exposed to 50 or 100 ppm CO to raise COHb levels to 3%. Times to onset of angina and significant ST segment depression were measured. Time to onset of angina was reduced by 100 ppm CO, but not by 50 ppm CO. Times to significant ST segment depression were reduced more following exposure to 50 ppm CO than after 100 ppm CO. Eleven CO-responsive subjects were exposed to purified air or 100 ppm CO, at sea level or simulated high altitude (to simulate pO_2 at 2000 m elevation). Exposure to CO reduced the time to onset of angina by 5% at sea level and by 9% at high altitude. At high altitude, about 12% more blood must be pumped to achieve the same exercise state. While the number of subjects tested was too small to conclusively assess the potential health risks of high altitude CO exposure, the results demonstrate the potential for significant health risks.

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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Carbon monoxide (CO) reversibly reacts with proteins, competing with oxygen for binding sites on hemoglobin and myoglobin molecules, compromising the ability of these proteins to transport oxygen. Small decreases in hemoglobin oxygen-carrying ability resulting from 3 to 5% carboxyhemoglobin (COHb) levels can significantly reduce the ability of sensitive individuals to perform exercise; such effects have been documented over the past several years. Low-level CO exposure reduced the time to onset of angina in men with stable angina pectoris and reduced the maximum aerobic capacity achieved in normal individuals during exercise. There are, however, many areas of uncertainty in clinical studies of CO health effects. Among these are: the large amount of subject-to-subject variability in physiologic responses to CO; reported biases in the spectrophotometric methods used for measurement of COHb in blood in several previously reported studies; interactions between medications a subject takes for control of his angina and the response of that subject to CO; and high variability in rate of uptake of CO between subjects. The goal of this study was to improve methods to more accurately and reliably determine the subclinical effects of low level CO exposure on cardiac function in individuals with ischemic heart disease. To accomplish this objective, we undertook development studies to improve the analytical chemical methodology for measuring low-level carboxyhemoglobin, we evaluated additional potentially more sensitive endpoints and modified exposures and exercise test protocols for detecting CO-induced changes in the heart, and we established criteria to better characterize those subjects who were most likely to be sensitive to CO exposure under different environmental conditions. We then integrated the results of our work and applied them to determine the feasibility of testing whether there was significant interaction between CO-induced hypoxia and hypoxia related to high altitude exposure. The latter point is based upon predictions of models which have not been validated by laboratory studies.

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SCOPE AND PURPOSE

This project was multifaceted in that it evaluated and used several different techniques, with the overall goal of developing methods which could be applied to performing efficient and cost-effective studies of the effects of carbon monoxide on the health of sensitive individuals residing in California (and other areas as well). To this end we investigated the appropriateness of various methods of determining CO exposure and dose, we considered whether studies of populations other than individuals with exercise-induced angina were warranted at this time, we compared effects of CO exposure at two different dose rates on a group of subjects with ischemic heart disease, we tested echocardiography, a previously untried endpoint (with respect to air pollution health effects studies), as an objective measure of the onset of exercise-induced angina pectoris, and we developed information which allowed us to design a pilot study regarding a question of regulatory interest in California, whether or not exposure to CO at high altitude (such as at Lake Tahoe) might pose additional risks for sensitive populations. The pilot study was conducted, and the results obtained provided sufficient information to design a more extensive study which would have a reasonable possibility of successfully answering this question.

SUMMARY AND CONCLUSIONS

This multidisciplinary and multitask effort was undertaken to address several areas of uncertainty in clinical studies of CO health effects. Among these were: the large amount of subject-to-subject variability in physiologic responses to CO; reported biases in the spectrophotometric methods used for measurement of COHb in blood in several previously reported studies; interactions between medications a subject takes for control of his angina and the response of that subject to CO; and high variability in rate of uptake of CO between subjects. The goal of this study was to improve methods to more accurately and reliably determine the subclinical effects of low level CO exposure on cardiac function in individuals with ischemic heart disease. To accomplish this objective, we undertook development studies to improve the analytical chemical methodology for measuring low-level carboxyhemoglobin, we evaluated additional potentially more sensitive endpoints and modified exposures and exercise test protocols for detecting CO-induced changes in the heart, and we established criteria to better characterize those subjects who were most likely to be sensitive to CO exposure under different environmental conditions. We then integrated the results of our work and applied them to determine the feasibility of testing whether there was significant interaction between CO-induced hypoxia and hypoxia related to high altitude exposure. The latter point is based upon predictions of models which have not been validated by laboratory studies. This concern is of regulatory interest in mountainous communities which have the potential to experience high levels of CO derived from mobile sources.

The steps taken in the first year involved the improvement of analytical methodologies, and an evaluation of exposure and exercise test protocols. Eighteen carefully selected subjects were examined after exposure to CO at 50 ppm to bring COHb levels to about 3%, and the times to onset of angina and to significant ST segment depression were compared with those observed with the same subjects exposed for a shorter period of time to 100 ppm, but brought to the same 3% COHb level. Group mean averages for these two variables were not significantly different between exposure conditions. Time to onset of angina was reduced following the 100 ppm exposure but not following the 500 ppm exposure, however time to significant ST segment depression was reduced more following the 500 ppm exposure than after the 100 ppm exposure. Overall the data do not indicate that 100 ppm exposures to 3% COHb concentrations bias the results of exposure studies with subjects with coronary artery disease.

With regard to evaluating analytical methodologies, an extremely sensitive gas chromatographic method for analysis of CO content of blood and breath samples was installed in our laboratory. We were thus able to evaluate measurements made using the Instrumentation Laboratories CO-Oximeter (IL 282) after the latter instrument was carefully calibrated. Our findings were that in our hands the IL 282 provided accurate and precise estimates of COHb, even at levels in the 1 to 3% COHb range. We attribute this to the care, consistency and attention to detail we used in preparing standards and in calibrating both instruments.

During the final year of the study, we applied the findings from our initial studies to a pilot study of the effects of high altitude, low level CO exposure. A group of 11 subjects

with coronary artery disease and who were responsive to CO were recruited (most from among participants in our earlier studies). We used exercise test protocols and software developed and perfected in year 1 and an improved IL 282 calibration procedure and developed and tested a system for simulating high altitude exposures.

Each subject was exposed to four atmospheric conditions: purified air at sea level; purified air at simulated high altitude (partial pressure of oxygen reduced by addition of purified nitrogen gas to the inspired airstream - conditions were designed to simulate breathing at 2000 m elevation); 100 ppm CO at sea level (to a blood COHb concentration of 3%); and 100 ppm CO at simulated high altitude. Carbon monoxide at sea level reduced the time to onset of angina by about 5% whereas exposure to CO at high altitude decreased time to onset of angina by 9%. Cardiophysiological results suggested that high altitude alone presented a stress to these subjects; the heart was required to work harder and to pump about 12% more blood to accomplish the same amount of physical exercise.

The number of subjects, while too small to conclusively assess the potential health risks associated with high altitude CO exposure, was sufficient to demonstrate that using the methods and the subject screening criteria developed, a full-scale study could provide convincing evidence of potentially significant health risks associated with high altitude CO exposures.

GENERAL BACKGROUND

Despite many studies which have been performed, our understanding of the mechanisms by which CO exerts its influence on man's health is still incomplete. CO reversibly reacts with proteins, competing with oxygen for binding sites on hemoglobin and myoglobin molecules, compromising the ability of these proteins to transport oxygen. It is still, however, not clear how very small decreases in hemoglobin oxygen-carrying ability resulting from 3 to 5% carboxyhemoglobin levels can cause the effects which have been observed over the past several years. It is useful to briefly review the data which have been examined in the establishment of the current ambient air quality standard for CO.

Initially, the National Ambient Air Quality standard was established based upon neurobehavioral data. Psychomotor performance, measured in terms of visual threshold (McFarland, et al., 1944), flicker fusion frequency (Lilienthal, et al., 1946), and time perception (Beard and Wertheim, 1966), was reported to be degraded at levels of CO exposure sufficient to elevate carboxyhemoglobin (COHb) levels in the blood to about 5%. Later attempts to validate results at CO concentrations near ambient levels produced equivocal results, and the health effect basis for an ambient CO standard was shifted to findings of reduced exercise tolerance and inducement of anginal pain in subjects with coronary artery disease.

Exercise tolerance in relatively healthy individuals was reported to be impaired after subjects breathed 100 ppm CO for one hour, increasing COHb from an average of 1.7% at baseline to 3.95% after exposure, and decreasing mean exercise time from 11.63 minutes to 11.04 minutes (Aronow and Cassidy, 1975). Horvath, et al., (1975) also reported that CO exposure reduced exercise tolerance in healthy individuals; approximately 4.9% and 7.0% reductions in time to exhaustion were observed when COHb levels reached 3.3% and 4.3%, respectively. These investigators also reported that aerobic power, measured as the maximum rate of oxygen uptake (V_{02} max), declined linearly in response to CO exposure, and that for COHb levels greater than 4%, the percent decrease in V_{02} max could be computed as:

$$V_{02} \text{ max (\% decrease)} = 0.91 \text{ (\% COHb)} + 2.2 \quad \text{.....(1)}$$

Aronow and Isbell (1973) reported that exposure to low levels of CO significantly reduced the ability of individuals with cardiovascular disease to exercise. Subjects exposed to 50 ppm CO for two hours increased their COHb levels from an average baseline of 1.0% to a level of 2.7% and the time of onset of exercise-induced angina pectoris was shortened from 3.74 minutes (observed after subjects breathed clean air) to 3.13 minutes (a 16% decline observed after CO exposure). A study by Anderson, et al. (1973) showed 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%. While these data indicated a consistent pattern of adverse health effects induced by low-level CO exposure, the quality of the data reported by Aronow and his colleagues was questioned. An EPA review panel examined the available Aronow data base and found that, because records were inadequately

kept, it was not possible to validate Aronow's findings, leaving the health-related basis for the ambient CO standard on somewhat unstable ground. This state of affairs has sparked an intense effort on the parts of several teams of investigators, supported by several different institutions, to carefully re-examine the question of the effects of CO on human health.

Sheps, et al., (1985), reported that they did not observe significant effects of CO on a group of subjects with ischemic heart disease at a 4% COHb level, however this group did observe significant CO-induced effects in a subsequent study (Sheps et al., 1988) at 6% COHb. Kleinman, et al., (1986) exposed 26 subjects with ischemic heart disease (IHD) to 100 ppm CO for one hour, raising subjects' carboxyhemoglobin (COHb) levels from a baseline of about 1.6% to a level of 2.9% COHb. Following CO exposure, the exercise time to the onset of angina was reduced an average of 7% ($p<0.05$), compared to the subjects' performance after breathing clean air. In addition, their symptom-limited V_{02} max was reduced, and the duration of their anginal pain was increased. In a further analysis of these data, Kleinman et al. (1989) reported that in a subset of subjects who exhibited ST-segment changes prior to the onset of angina, the time to ST-segment depression of 0.1 mV was reduced by 12% ($p \leq 0.05$). A large multicenter study, conducted under the sponsorship of the Health Effects Institute, of the effects of CO at 2% and 4% COHb levels on subjects with coronary artery disease using inducement of anginal pain and changes in electrocardiographs as endpoints, demonstrated significant dose-dependent effects of CO on cardiovascular function. The results of these three studies demonstrate that the effects of CO in the 2% to 4% COHb range are likely to be small and difficult to detect, unless the characteristics of the study population are quite homogeneous. They also point out that there is a relatively wide range of responses to CO among individuals with ischemic heart disease. Given this diversity, it is possible that there may be individuals that might be termed "responders" and those that might be termed "non-responders." Differences in findings among the four studies discussed above, may well be attributed to differences in the individuals making up the subject populations that participated. For example, Kleinman and his associates found statistical associations between the types of medications (and medication dose levels) subjects were taking to control their angina and their ability to exercise after breathing CO. There were also substantial inter-subject differences in the rate of uptake of CO; overweight (body mass)95 kg) subjects achieved substantially lower COHb levels after 1-hr exposures to 100 ppm CO than did subjects of normal weight. It is extremely important that we understand the mechanisms behind these inter-subject differences, especially if we intend to compare results among several different experiments. Developing these types of understandings may allow us to perform clinical studies in a more efficient, cost effective manner, by improving our ability to accurately characterize the subjects who participate in tests, thereby increasing the ratio of "signal" to "noise," and perhaps permitting studies to be performed using fewer experimental subjects.

Another possible explanation for individualistic differences in response to the effects of CO might be inaccuracies in the determination CO dose, as measured by the % saturation of COHb. As part of the quality assurance effort in the HEI multicenter study, the reliability of the IL 282 CO-Oximeter (a spectrophotometric detector system) at levels below 5% COHb was questioned. This instrument, or other similar devices, had been used as the primary source of dose information in many of the past clinical and epidemiological CO

health effects studies. The HEI investigators reported that there were serious biases between the CO-Oximetry data from specific instruments and data obtained using a gas chromatographic (GC) method.

While it appeared, by 1987, that there would likely be sufficient grounds to support the current Federal and California State ambient air quality CO standards for sea-level conditions, none of the available studies addressed the possibility that exposure to CO under other environmental conditions of relevance to residents of California, such as high altitude. There have been some data to support the contention that high altitude exposure to CO might pose additional risks to susceptible populations. The most convincing evidence came from mathematical and computer models that evaluated the potential physiological impact of such exposures (Collier and Goldsmith, 1983; Dawson, unpublished data). Clinical studies with young, healthy individuals exposed in environmental chambers at sea level and at 7000 ft altitude did not demonstrate significant differences. No studies, however had been performed in which the participants were selected from among a population expected to be extremely sensitive to the effects of CO. Since the primary mechanism for the CO-induced effects is related to reduction in the efficiency of delivery of oxygen to stressed cardiac muscle, one could expect this population to also show effects when exercising at high altitude. The potential was, therefore, that there might be an additive or worse effect when such subjects exercised under high altitude conditions after being exposed to CO.

There was a need, therefore to perform clinical studies to determine whether or not joint altitude and CO effects would be present. Prior experience, however, indicated that in order to maximize the probability of obtaining either convincing positive (or convincing negative) results, many of the problems encountered in previous studies needed to be overcome. To address these problems a multidisciplinary group of researchers with interests in evaluating the potential health effects of CO was assembled and a project consisting of interrelated tasks was developed and executed, culminating in two pilot clinical studies in which the developed methods were applied and evaluated. The final results were then used to plan and initiate a full-scale study of the joint effects of CO - high altitude exposure; which is currently underway.

SPECIFIC TASKS AND THEORETICAL APPROACHES

I. Application of the IL 282 CO-Oximeter to measure low levels of COHb:

The IL 282 CO-Oximeter has been widely used to directly measure blood COHb levels because of its ease of sample handling, speed, and low cost as compared to the more time consuming and technically difficult manometric (Van Slyke) and gas chromatographic (GC) techniques. Comparisons of IL 282 and GC measurements on blood samples from 106 subjects demonstrated that IL 282 data from an instrument housed at the HEI Multicenter CO Study's central laboratory (St. Louis) correlated strongly with GC data from an instrument at that same laboratory but showed consistent bias (Allred et al. 1989a). IL 282 readings were about 1% COHb greater than the GC values, over the range 1% to 4.4% COHb (GC). Standard samples sent to each of the laboratories participating in the HEI Multicenter Study quality assurance program were evaluated vs. St. Louis GC readings on the same samples. Similar results were found; the data correlated strongly but demonstrated significant bias, ranging from 0.47% COHb to 0.89% COHb. These results indicated that the magnitude of observed bias was instrument specific. Furthermore, in systematic examinations, blood oxygenation levels appeared to influence the results of blood COHb measurements by the IL 282 (Allred et al., 1989a) and there may also be individual-specific factors that could impair accuracy of result from different subjects (Lambert et al., 1988). These phenomena may be explained by (1) interindividual differences in the absorbance of light by hemoglobin in its oxy-, deoxy-, met-, and carboxy-states, and (2) interindividual differences in the concentration of non-hemoglobin species, including drugs, distributed in the plasma and bound to blood proteins.

Researchers in attendance at the EPA/GRI Human Exposure Assessment Workshop (held at Harvard, October 1986) familiar with the IL 282 suggested that some of the observed variability in low level measurements may have been caused by the present protocol for zeroing and spanning the instrument. Before each use, the IL 282 is spanned for total hemoglobin using a standard dye of approximately 15.0 g/100 ml and then is checked, but not spanned, for carboxyhemoglobin measurement using a bovine serum control at approximately 98% COHb. The instrument self-zeroes on each sample cycle. Concerns were raised that there are no multiple points of span, and that the sole span point was 98% COHb, far above the 0.5 to 5% range of interest in the nonsmoking subjects participating in laboratory and field studies.

In conducting our study, the traditional gas chromatographic analytical techniques were not available because the interfacing hardware was no longer manufactured, and there was some limited evidence (due to our occasional participation in sample intercomparisons with Dr. Dahm's laboratory) that our IL 282 instrument was less biased than some other instruments. We therefore incorporated a multipronged approach. We planned to acquire, install and test a new sensitive GC technique which used a reducing gas detector to measure the CO content of very small samples of blood, to improve upon the existing methods of IL 282 calibration by developing multiple levels of standards, in the concentration ranges of interest to environmental needs, and to examine in a more systematic way individual factors that might lead to inaccuracies in measurements in a given individual's blood.

II. Response of Volunteers with Ischemic Heart Disease to Low-Level CO at Two Different Dose Rates:

While several investigators have reported significant changes in time to onset of angina in subjects exposed to low levels of CO (Aronow and Isbell, 1973; Anderson, et al., 1973), the findings remain controversial. Recent studies by Sheps, et al. (1986), showed no CO effect at 4% COHb on several cardiopulmonary functions, including time to angina, and by Kleinman, et al. (1989), showed small CO effects on time to onset of angina, heart rate, blood pressure, and time to onset of ST-segment depression. The discrepancies in findings point to a need for better understanding of the characteristics that might identify CO-sensitive subjects and improve the uniformity of experimental groups, and also to a need to develop more sensitive endpoints and better experimental protocols to improve the probability of seeing effects, if and when they occur, by reducing "background noise" imposed by non-uniformity in subject treatment. This is especially important now, since the difficulty and expense of recruiting suitable human volunteers has greatly escalated due to improvements in medications and treatments available to individuals suffering from ischemic heart disease. This task was therefore designed to improve experimental protocols with respect to dose rate, administration of exercise stress, endpoint evaluations and subject selection, with the goal of developing techniques that could be applied to subsequent more definitive studies.

Some information in the published literature suggested a difference between effects observed at high and low exposure concentrations, that is, that there may indeed be a dose rate-related component to CO dose/response relationships. Anderson, et al., (1973) compared effects in subjects with coronary artery disease, of 4-hr CO exposure at 50 ppm (to 2.8% mean COHb) with those observed after 4-hr exposure to 100 ppm (to 4.5% mean COHb). They reported that the effects at 50 ppm were not significantly different from those at 100 ppm, although both CO exposures adversely affected exercise performance relative to clean air. This experiment does not give any direct evidence for the effect of dose rate, since both dose rate and final COHb level varied. Bear and Grandstaff, (1975) examined the effect of CO on vigilance in non-smoking, normal subjects after exposures to 0, 50, 175, or 250 ppm CO, achieving COHb levels of <1%, 1.8%, 5.2% and 7.5%, respectively. Responses at 50 and 175 ppm were significantly decreased ($p < 0.05$), compared to clean air; the responses at 250 ppm were not significantly different from those in clean air. O'Donnell, et al., (1971) performed tests on time discrimination in subjects after exposures to 0, 50, and 125 ppm CO. They showed greater effects at 50 ppm than at 125 ppm. Taken alone, any one of these studies might just indicate some experimental inconsistency; taken as a group they suggest the influence of an unknown factor in the mechanism of action of CO, possibly that which is related to the rate at which CO is introduced into the body. Since most clinical studies of CO health effects had been conducted at high concentrations, relative to the standard, it was important that the observed effects be compared with effects at levels closer to ambient concentrations. Also, since there has been a great deal of intersubject variability in response, it was important to detect subtle changes in physiology and to develop additional sensitive, and possibly more objective, endpoints.

The exercise protocol itself contributes to test variability. In previous tests, the protocol used in several of the Aronow et al. studies was replicated. This involved an initial exercise load of 50 watts, with the load increased by 25 watts at three minute intervals. In the current study, we instituted a more gradual test in order to improve resolution of the work rate at which anginal pain is induced, and to improve the interpretability of the gas exchange physiological data being collected. In our modified protocol, the initial work rate was set at 30 watts and the work rate was increased by 10 watts at 1 minute intervals. This protocol provided approximately the same level of work intensity, over the average exercise period, but provided smoother transitions between work rate increments. We also used cardiac ultrasound imaging and flow monitoring to look for wall motion abnormalities of the left ventricle and perturbations of cardiac blood flow which should be indications of the direct effects of ischemia on the heart, and which might occur before anginal pain or ST segment changes. Wall motion changes, if detected, would provide an objective endpoint which could be used in conjunction with subject reports of anginal pain, and which would be especially valuable for those subjects who had coronary artery disease, but did not experience angina (silent ischemia). Echocardiography continuously produces images of the left ventricular walls and cavity, while Doppler ultrasonography (collinear with the echocardiographic signal) reliably produces simultaneous blood flow signals from the ascending aorta and from within the heart.

III. Response of individuals with coronary artery disease (CAD) to low level CO exposure at high altitude:

As stated earlier in the section on background, there is some evidence that effects of CO on sensitive, exercising individuals, might be worse during exposures at high altitude than at sea level conditions. This is a situation of regulatory interest in the State of California. We designed and implemented a pilot study with a relatively small group of suitable volunteer subjects. The study incorporated the findings on subjects selection, exercise protocol, dose determination and endpoint evaluation from earlier tasks in this program, and information obtained from results of other investigators, as those results became available. To test the hypothesis that the effects due to CO on the ability of subjects with ischemic heart disease to exercise would be different at high altitude than at sea-level, we exposed volunteers to purified air, with and without low-level CO, and to purified air in which the percent of oxygen was reduced to simulate conditions at 2000 meters elevation, again with and without low-level CO. This experimental design allowed us to test for direct effects of CO and altitude and for interactions (additivity, synergy, or antagonism) between altitude and CO. The study had a randomized cross-over repeated measures design which incorporated four exposures for each subject: purified air at sea level, CO at sea level, purified air at altitude, CO at altitude. This study was planned as a feasibility study and satisfied several objectives. First, it allowed us to further develop clinical test methods for performing efficient and effective health effects studies. Second, it functioned as a scoping study in that it allowed us to refine our target CO doses and altitude levels for a subsequent, definitive study. Third it enabled us to build a group of potential volunteers for subsequent studies.

LIMITATIONS OF THE WORK

This project was initiated at a time when there were several research efforts ongoing directed at developing a better understanding of the toxicology of CO. A primary goal was to develop techniques which would improve the quality of CO clinical health effects studies. Several of the questions which were initially addressed in this project represented alternative approaches to improving data quality in a practical manner. Within our study, several factors were often examined in parallel. In some instances, lines of inquiry were abandoned if it appeared that a better, more practical alternative had been made available. Thus, for instance, we abandoned our development of multipoint standards for calibration of the IL 282 when such standards became available commercially. In another instance, we terminated efforts to develop independent spectrophotometric methods for measuring hemoglobin components to be used in subjects whose blood contained putative interfering substances, because none of the subjects studied yielded problamatical blood samples. We also kept abreast of developments in other ongoing studies. By so doing we were able to make the best use of our resources by not overly duplicating efforts already under investigation by other investigators, such as the dependence of IL 282 COHb values on blood oxygen saturation levels, which was systematically evaluated by the HEI investigative team. This also allowed us to be responsive to regulatory needs by shifting our resources to bear on areas of interest to residents of California which were not being addressed in other studies, such as investigating the effects of high altitude CO exposure.

In reviewing the data produced by this study, it is important to keep in mind that the investigations were exploratory in nature, rather than being designed to produce data for immediate regulatory application. Even so, the high altitude CO study data are suggestive of important possible effects (although the sample size was small) and have been extremely valuable in setting the conditions for subsequent testing which should yield convincing effects (whether they be positive or negative).

EXPERIMENTAL METHODS

Subject Recruitment and Selection:

Male subjects with stable angina pectoris and without evidence of pulmonary disease or anemia were recruited for this study. Subjects from the previous study (Kleinman, et al., 1986) were contracted and additional subjects were identified from records at the VA Hospitals in Long Beach and Loma Linda, California, and from outpatient cardiology clinics at the University of California, Irvine's Medical Center in Orange, California.

Stable angina was defined as pain or discomfort in the area of the chest (with or without radiation to other areas), precipitated by exertion of more than usual effort and relieved by rest or sublingual nitroglycerine, and with no recent changes in frequency, duration, time of appearance or precipitating factors (Lee, 1980). All subjects were non-smoking for at least 6 months prior to study and had pulmonary functions in the range normal for individuals of their age, weight, height, and sex. All subjects provided informed consent before any laboratory procedures were undertaken (including screening tests).

As part of the laboratory screening process, the subject's pulse rate, blood pressure and temperature were taken and the subject was asked to complete a questionnaire which elicited information relevant to the subject's health, smoking habits, living conditions, and other factors which might relate to the subject's exposure to CO in his own environment. Pulmonary function was tested using a Collins Spirometer. Forced vital capacity (FVC), forced expiratory volume at 1.0 second ($FEV_{1.0}$), and the ratio $FEV_{1.0}/FVC$ were determined. The mid-expiratory flow rate was also measured. Subjects with FVC and $FEV_{1.0} \geq 80\%$ of those predicted (Morris, et al., 1971) for comparable individuals in good health, were acceptable candidates for this study.

A physician reviewed the medical history and performed a brief physical examination prior to exercise testing. The exercise stress test performance was monitored by a physician, and controlled by the same exercise physiology technician, in all cases. The screening test exercise protocol was the same as that followed for the exposure tests as described below.

Exposure testing of Subjects:

The tests were designed to minimize the possible influence of confounding factors such as variations in the taking of medication, differences in subject's carbohydrate-loading before exercise tests, and possible uncontrolled exposures to CO during transit to the laboratory on exposure test days. Subjects were telephoned on the evening prior to each exposure test and reminded to take their medication on schedule and to record the time. The subjects were instructed not to eat anything in the morning (a light, standardized snack was provided at the laboratory). Subjects were picked up on the morning of each test and transported to the laboratory in a van equipped with a clean air "tent" to minimize the effects of extraneous CO exposure even when the subject was driven to the laboratory during

heavy traffic conditions.

On arrival at the laboratory, the subjects received a routine chest examination; blood pressure and heart rate were checked; a resting ECG was obtained; and a series of pulmonary function tests were performed. The results were evaluated, samples of blood and breath were collected and analyzed to determine the subject's baseline COHb level, and if there were no contraindications to proceeding with the exercise test, the subject was given a light snack consisting of fruit, cheese, a muffin, and juice or other non-caffeinated beverage, and then prepared for the exposure.

For CO dose rate comparisons, the subjects were exposed, at rest, to either clean air or CO (50 or 100 ppm). The 50 ppm exposure was continuous for two hours but the 100 ppm dose was administered intermittently with clean air for 15 minute periods so that the integrated dosage time was about 1 hour but the total exposure time was the same as for 50 ppm, 2 hours. The target dose level for both exposure concentrations was 3% COHb, as measured by the IL 282 CO-Oximeter.

For the high altitude exposure study we chose 100 ppm CO as a dosing regimen, based upon the greater control and reliablility to attain target COHb levels shown in the earlier study. The subjects were exposed to four atmospheres: clean air at sea level (purified room air), clean air at simulated 2000 meters elevation (purified air into which pre-purified nitrogen gas was metered in to reduce the inspired oxygen fraction to 16.8%), CO (100 ppm, sea level), and CO (100 ppm, simulated 2000 meters elevation). The total exposure time was 2 hr and CO was administered intermittantly. The total CO exposure duration was regulated within the 2 hr period by measuring blood COHb levels and titrating the subject to a pre-exercise test target value of 3.3% by calculating the end-exposure time using the Haldane equation. If the subject reached the target dose before the 2-hour exposure time was completed, the subject was given a maintenance dose of CO to hold the blood %COHb level at 3.3%. At the end of the exposure period, the subject was given a second resting ECG, blood and breath samples were taken to evaluate the post-exposure COHb level, and the subject began the exercise stress test.

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), 30, 60, 90 and 120 minutes of exposure, and after exercise. Blood samples were analyzed for concentrations of total hemoglobin, oxyhemoglobin, carboxyhemoglobin (COHb), methemoglobin, and the volume percent of oxygen in blood using the IL 282 CO-Oximeter. Samples were also analyzed using a gas chromatograph with a reducing gas detector system. The COHb data were kept separate from the exercise test and cardiographic data to maintain blind assignment to CO exposure.

The CO-Oximeter was calibrated daily against standards (Instumentation Laboratories) which consisted of dye solutions with colligative properties similar to human blood and with light absorption characteristics similar to hemoglobin, and with human blood-derived standards (IL Multi-four Standards) which provided values in the range 2%, 25%, 50% and 97% COHb. The GC-RGA system was calibrated against gravimetric standard gas mixtures.

Exposure concentrations were prepared dynamically by metering carbon monoxide (1% in air) from a cylinder of compressed gas into a flow of clean, HEPA filtered air. The gases were mixed in a chamber and delivered to the subject who wore a low-deadspace respiratory mask (Vac-U-Metrics). 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 non-dispersive infrared absorption units (Dasibi Model 3003 and Beckman Model 302) which are calibrated daily against certified CO gas mixtures of known concentrations. Regular quality assurance assays were performed by the California Air Resources Board personnel, using standards traceable to the National Bureau of Standards.

Exercise Test Protocol:

Exercise tests were performed on an electro-magnetically-braked cycle ergometer (Gould-Goddart). No more than 15 minutes were allowed from end-exposure to the start of the test and the time of day was consistent between test, ± one hour. A low-deadspace respiratory mask was fitted and carefully sealed over the subject's nose and mouth (with tape, if necessary) to eliminate leaks, and the subject was instructed to breathe quietly and normally. Respiratory gases were monitored continuously; oxygen by an electrochemical detector (Ametek S-3A) and carbon dioxide by an infrared analyzer (Beckman LB-2). These gas monitors were calibrated before each test against certified, NBS 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 microcomputer; the computer provided "real time" measurements of minute ventilation (V_e), oxygen uptake (V_{02}), and carbon dioxide output (V_{CO2}) every 30 seconds. Twelve-lead electrocardiographic tracings (Marquette MAC I) were taken during the last 15 seconds of each minute during the exercise test. The numbers of abnormal heart beats, changes in ST segments at 0.06 sec past the J-point, T-waves, and heart rate were determined from the ECG tracings. Blood pressure was taken during the last 30 seconds of each three minute interval during the exercise test.

The exercise test consisted of three segments; the warm-up period (five minutes), the graded exercise phase to the point of onset of angina pectoris, and the recovery period (eight minutes). The five-minute warm-up was begun after allowing sufficient time to equilibrate the gas sampling instrumentation to the subject's expired breath. The warm-up allowed the subject to become acclimated to the test facility while sitting on the cycle ergometer. At the end of five minutes, the subject was instructed to pedal the ergometer to 60 rpm, as indicated by a meter in the subject's view, and to continue pedaling until he felt the initial onset of anginal pain. The initial work rate was 30 watts and was increased by 10 watts at one minute intervals until the termination of exercise by the subject at the start of his angina. At this point the subject stopped pedaling, was asked to indicate, by

nodding his head if he was feeling his typical anginal pain, and the time-to-angina, blood pressure, pulse rate, and respiratory gas exchange data were recorded. The subject remained seated on the cycle for a further eight minute recovery period during which he indicated when the pain stopped; monitoring continued during recovery.

Statistical Analyses:

Data from these studies were incorporated into a data base over the course of data acquisition. CO exposure and blood carboxyhemoglobin values were kept separately from the physiological and cardiographic data. When all of the data were reduced, the CO exposure and COHb information were integrated with the overall data set and statistical analyses were performed using BMDP statistical programs. Data were analyzed using repeated measures analyses of variance. Data comparing two analytical methods for determining blood COHb concentrations (CO-Oximetry vs. Gas Chromatography) were compared using least squares regression analysis.

RESULTS AND DISCUSSION

I. Application of the IL 282 CO-Oximeter to measure low levels of COHb:

The first year of study focused initially on answering the most important of the questions raised about the use of the IL 282 as a tool for toxicologic research--does the IL 282 yield biased estimates of COHb% at levels below 5% of saturation. We found that for the most part the IL 282 and the GC-RDA analytical methods yielded comparable results over the range of COHb concentrations.

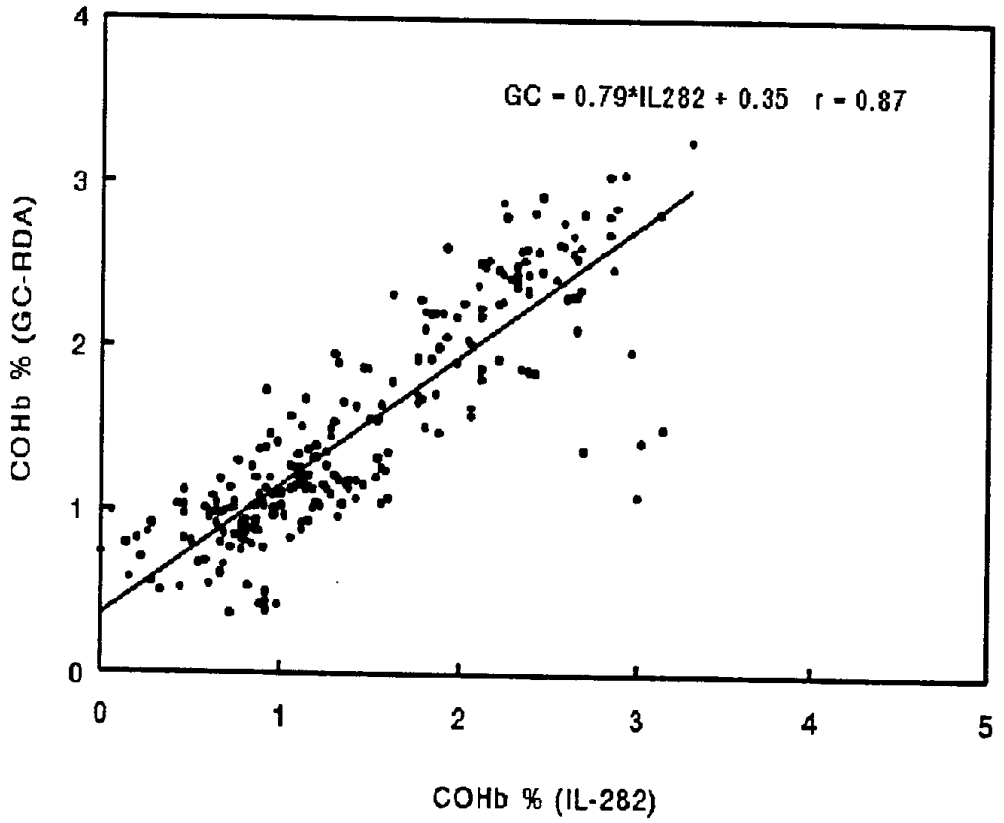
Figure 1 presents a scatterplot of all paired samples for the IL 282 and GC-RDA. The best fit linear least squares regression line is shown. The data illustrate generally good agreement between the two instrumental methods (correlation coefficient, $r = 0.87$; $p \leq 0.001$). There is a statistically significant intercept shown in the graph, indicating that the IL282 was biased slightly low, equivalent to 0.354% COHb, for concentrations below about 1.7% COHb. The slope of the curve, however, was 0.79, thus at the high end of the data set (3% COHb) the discrepancy between the two methods was 0.35% COHb with the IL282 higher than the GC. At the median concentration (about 1.7% COHb for this study) there was no significant difference between the IL282 and GC values. Other investigators have reported fixed biases between IL 282 and GC methods (Allred et al.,1989a), however the agreement observed in our study is better than that observed by the Allred group. The differences in results between these methods can be due to several factors. We used standards at 4 different COHb concentrations in generating our IL282 calibration curves. This appears to provide more accurate results than the prior method, which used a single point calibration. It appears that the direction of the bias varies with individual IL282 machines (Allred et al., 1989a). Anecdotally, we found during the course of our experiment that the calibration of our IL282 suddenly changed. This was found to be caused by a change in the alignment between the matched photodetectors which provide the instrument's readings. We found that modification of this allignment could alter the apparent sign and magnitude of the bias between analytical methods.

Qualitatively, the intra-instrument variability between paired measurements on our IL 282 and GC-RDA instruments (Figure 1) is similar to the inter-instrument variability for paired samples analyzed for either the IL 282 or GC-RDA alone (not shown). The GC-RDA, however, is slightly more precise, as we had anticipated from operating principles of the two devices, than is the IL 282 even though our calibration proceedure was extremely meticulous.

Finding good association between IL 282 and GC-RDA methods caused us to modify the priority accorded to more peripheral examinations such as the spectral evaluation of blood samples, although much of the initial phases of the examination were completed (none of the samples diverged enough to necessitate--or facilitate--a spectral analysis for potentially interfering blood components). In addition, the availability during the previous year of commercially prepared and precise COHb standards reduced our need to develop our own standards using tonometry and standard additions.

The implications of our findings are: (1) that the use of multilevel standards may improve the accuracy of the IL282 measurements; (2) that calibration responses be compiled and inspected on a regular and consistent basis; (3) that abrupt changes in calibration responses should trigger a more complete evaluation of the instrument; and (4) that under these conditions, the IL282 can provide accurate data which can be used for scientific evaluation of CO health effects.

Figure 1. Comparison of GC and IL 282 Carboxyhemoglobin Determinations



II. Response of Volunteers with Ischemic Heart Disease to Low-Level CO at two different Dose Rates:

This study tested the hypothesis that exposure to CO at 50 ppm for approximately 2 hr would produce effects that were not the same as those due to exposure to CO at 100 ppm for 1 hr (individuals exposed at either dose level being titrated to 3% COHb). Eighteen subjects were recruited into the study and informed consent was acquired. Each subject was randomly assigned to one of six groups, representative of the six possible orders of exposing subjects to three atmospheres (clean air, 50 ppm, or 100 ppm) at random. Each subject was tested on three occasions separated on the average by an interval of one week. Each test consisted of an exposure followed by an exercise stress test. The order of exposures for each of the groups was randomized; each subject was exposed to each of the three atmospheres, and each subject acted as his own control. The distribution of subjects among the groups and the order exposures for these subjects are shown in Table 1. This randomized crossover design was executed in a "double blind" manner, controlling for possible biases such as order effects or training effects. Neither the subjects, nor the exercise physiology staff were informed of the exposure atmosphere prior to the end of the study. The design was not completely balanced; changes in subject's schedules occurred and we were unable to optimally accomodate to those changes. Exposures at 50 ppm for 2 hours were inadequate in 16 out of 18 subjects to achieve the 3% COHb target; the group mean $\pm$ SD level was 2.42 $\pm$ 0.39 % COHb. The intermittant 100 ppm exposure yielded a group mean $\pm$ SD level of 2.77 $\pm$ 0.42 % COHb.

The physical characteristics, blood pressure and heart rates of the 18 subjects are summarized in Table 2. As shown in Table 3, all of the subjects experienced angina; the location of typical pain varied between subjects, but was very consistent within each subject. As shown in Table 4, subjects characterized their pain as mild or intermediate in intensity and their perceptions, or description, of how the pain felt showed subject-to-subject differences. Subject history, including time since their most recent myocardial infarction (M.I.) or coronary artery surgery (C.A.S.) is summarized in Table 5. Objective evidence of heart disease and/or ischemia was available for 14 out of the 18 subjects, through previous medical records, as shown in Tables 5 and 6. Of the remaining four subjects, three evidenced probable ischemia by exhibiting both chest pain and ST segment depression during exercise (which has been shown to correlate well with assessment by thallium isotope tomography). Five of the other subjects with objective indications of ischemic heart disease also exhibited ST segment depression, thus 50% of the subjects studied showed cardiographic changes prior to the onset of angina. It is important to note that, within the experimental design, subjects exercised to the point of onset of anginal pain, and then stopped exercising. It is possible, that if exercise had been continued past this point, a greater fraction of the subjects would have shown ST depression.

As shown in Table 6, this group of 18 subjects did not show a significant decrease in the time to onset of angina. The 2 factor analysis of variance for these data did not demonstrate a significant effect of order. Seven of the 18 subjects consistently showed 0.1

mV, or greater, ST segment depression. The time to onset of this level of cardiographic change is shown in Table 7; CO exposure tended to decrease the time to onset and the changes approached but did not attain statistical significance. Our previous study (Kleinman et al., 1989) suggested that the subjects exhibiting ST segment depression represent a population that is sensitive to the effects of CO. This study represented a relatively small number of subjects and attempted to examine changes at COHb concentrations which were relatively low (3%). Analyses of the data using non-parametric methods (not shown) agreed with the results of the ANOVA and did not allow us to delineate any specific subjeect characteristics which could be identified as criiitical in defiiining the response of subjects to CO. In the subjects studied, there was no evidence that subjects with objective evidence of coronary artery disease, those with ST segment depression, or those who were overweight responded in a fashion different from that exhibited by the population as a whole. The time to ST segment depression of 0.1 mV seemed to be a more sensitive indicator of CO effects than was time to onset of angina, however the trends were not statistically significant. Medication useage was extremely variable among this population and there were insufficient numbers of subjects taking specific medications to determine if these medications influenced a given subjects response to CO. It is recommended that such research be performed at a higher COHb conccentration (one which will cause unequivical changes in physiological responses) so that possible interactions can be measured.

Echocardiography was conducted under the supervision of Dr. Davidson with the goal of possibly observing and measuring changes in cardiac wall motion as an effect of exercise-induced ischemia prior to the onset of anginal pain. Equipment was rented from the Department of Medicine and a trained echocardiography technician performed the measurements. The data were stored on videotape for later evaluation and data reduction. The quality of the ultrasonic image varied from individual to individual and it was difficult to maintain image stability during exercise. With the data reduction equipment available to Dr. Davidson at the time of this study, none of the tapes yielded sufficient information to warrent quantitative evaluations. The state of the art of quantitative echocardiography has, however, advanced rapidly, and new computerized systems for evaluation of data stored on videotape are now available. We are currently exploring whether, with the collaboration of investigators in the Cardiology divisions at U.C.I. or U.C.L.A. Departments of Medicine, our tapes can be digitized and read.

The results of this study demonstrated changes in time to onset of angina and time to significant onset of ST segment depressions that were consistent with those of our previous study (given that for the 50 ppm exposure series, the final COHb concentration was 2.4% while that for the 100 ppm exposure series was 2.8%). The finding that time to significant ST segment depression appears to be a more sensitive indicator of CO effects supports the conclusion from our earlier study that subjects who exhibit ST depression prior to the onset of angina may represent a sensitive class of subjects at risk to the effects of CO.

Table 1. Exposure Dosing Regimens

Exposure Group	A - 1st (ppm)	B - 2nd (ppm)	C - 3rd (ppm)	n
1	0	50	100	2
2	0	100	50	3
3	50	0	100	3
4	50	100	0	2
5	100	0	50	5
6	100	50	0	2

Table 2. Dose-Rate Study: Subject Physical Characteristics

Physical characteristics						
Subject	age	height (in.)	weight (lbs.) (kg)	screen resting HR	screen resting SBP	screen resting DBP
1	63	68	162.5	80	136	74
2	67	70.5	225	68	152	70
3	59	66	162.5	70	166	80
4	63	65.5	177.5	100	152	100
5	63	68.5	194.5	80	104	64
6	63	70.3	204	80	158	90
7	59	69	159.5	80	132	88
8	64	69	164	60	100	62
9	63	67	158	86	126	60
10	68	67	152	56	128	70
11	65	64.5	172	76	132	76
12	58	67.5	191	60	120	70
13	59	66.5	164	64	120	76
14	53	70	205	60	90	60
15	51	68	184	80	122	70
16	43	68	183.5	100	140	84
17	53	67.5	142	46	98	58
18	50	64.5	163	62	110	70
mean	59.1	67.6	175.8 (79.7)	72.7	127.0	73.4
stan dev	1.58	1.8	21.5 (9.7)	14.6	21.6	11.5
smallest	43	64.5	142 (64.4)	46	90	58
largest	68	70.5	225 (102.0)	100	166	100

4 of the 18 subjects (22%) were significantly overweight, as defined by being more than 3 s.e. above the mean body weight in the range of about 194.5 to 225 lbs. (88.2 to 102.0 kg)

Table 3 Location of typical angina pain

subject	chest	left arm	jaw/neck/throat	rt arm
1		x	x	
2	x			
3	x			
4		x	x	
5	x			
6	x			
7	x			
8	x	x		x
9			x	
10	x			
11	x			
12	x			
13	x			
14	x			
15	x	x		
16	x			
17	x			
18	x			
n	16	4	3	1
%	89	22	17	6

Table 4 Type and intensity of typical angina pain

Subject	Type of pain				Intensity of pain		
	sharp	dull	choking	other	mild	intermediate	severe
1			x		x		
2		x			x		
3				x		x	
4	x					x	
5	x	x				x	
6	x	x				x	
7		x			x		
8				x	x		
9				x		x	
10	x				x		
11				x	x		
12				x		x	
13	x	x				x	
14				x		x	
15				x	x		
16				x	x		
17				x		x	
18	x	x			x		
n	6	6	1	9	9	9	0
%	33	33	6	50	50	50	0

Table 5 Cardiological Characterization of Subjects

Subject	years since angina diag.	days since last angina	months since last M.I.	C.A.S.	months since C.A.S.	number of grafts
1	17	1	96	yes	204	2
2	10	2	--	no		
3	5	21	54	no		
4	5	21	--	no		
5	8	2	96	no		
6	25	1	150	no		
7	15	5	--	yes	192	4
8	4	4	--	no		
9	1	7	12	yes	110	3
10	3	1	--	no		
11	10	14	10	no		
12	3	1	28	no		
13	16	--	94	no		
14	15	4	176	yes	180	2
15	10	--	49	yes	67	5
16	1	--	9	no		
17	16	1	60	yes	53	2
18	12	4	--	yes	126	4
mean	9.8	5.93	69.5		133.1	3.14
stan dev	6.6	7.0	54.6		60.6	1.21
smallest	1	1	9		53	2
largest	25	21	176		204	5
n	18	15	12	7	7	7
%			67	39		

Table 6 Cardiology - diagnostic history

Subject	angiography	thallium	angioplasty	bypass
1				x
2				
3	x	x		
4				
5				
6				
7				x
8				
9				x
10				
11	x			
12	x			
13				
14				x
15	x			x
16			x	
17				x
18				X
n	4	1	1	7
%	22	6	6	39

Table 7. Times from start of Exercise to Onset of Angina (TTA) and ST Depression (STi)						
	Time to Angina			Time to 0.1 mV STi		
Subject	CA	50 ppm	100 ppm	CA	50 ppm	100 ppm
1	6.61	8.53	6.25	--	--	--
2	8.27	8.53	8.53	6.0	5.0	4.0
3	5.28	4.75	5.02	3.0	2.0	3.0
4	4.67	5.55	4.28	--	--	--
5	4.38	3.35	3.80	4.4	3.0	3.8
6	4.87	6.00	3.57	--	--	--
7	2.02	2.27	2.73	--	--	--
8	6.68	6.15	7.88	4.0	4.0	5.0
9	7.25	7.98	7.15	4.0	5.0	5.0
10	4.70	4.17	4.17	4.8	3.0	3.0
11	5.50	5.40	5.28	--	--	--
12	4.00	4.60	5.47	2.0	3.0	2.0
13	4.33	5.32	5.62	--	--	--
14	5.62	4.52	5.57	--	--	--
15	5.98	5.60	6.35	--	--	--
16	9.18	8.12	6.50	--	--	--
17	4.00	3.33	3.80	--	--	--
18	6.90	6.16	6.28	--	--	--
Means	5.57	5.57	5.46	4.03	3.57	3.69
SD	1.72	1.32	1.56	1.28	1.13	1.11

TTA = Time to Onset of Angina
STi = Time to Onset of ST Segment Depression of 0.1 mV

III. Response of individuals with coronary artery disease (CAD) to low level CO exposure at high altitude:

This study tested the hypotheses that: (1) the effects of sea level CO exposure would differ from those of high altitude CO exposure; (2) hypoxia due to high altitude exposure would reduce the ability of CAD subjects to exercise; and (3) the joint effects of CO and high altitude exposure would be additive. The approach taken was to simulate high altitude by reducing the oxygen content of inspired air by metering purified nitrogen gas into the breathing supply air of exposed subjects.

A total of 11 subjects were recruited for this study and informed consent was obtained. The screening and recruiting methods have been described previously. The characteristics of these subjects are summarized in Table 8. Four of the subjects had previous myocardial infarctions, but none within the 6 months prior to testing. Three of the subjects had previous coronary artery bypass surgery involving 2 or more vessels. Three of the subjects had prior angioplasty, but none within the 6 months preceeding testing. In addition, four of the subjects exhibited 0.1 mV or more ST segment depression during exercise, prior to the onset of angina. In all, objective evidence of ischemic heart disease was present for 10 of the 11 subjects; all but subject #9. That individual, however, had been diagnosed as having stable angina, and in our testing, developed exercise-induced anginal pain in a repeatable manner.

Each subject was exposed on four occasions separated by not less than 2 days nor more than 21 days. The subjects were exposed for two hours to either purified air (sea level), purified air (simulated 2000 m elevation), 100 ppm CO (sea level), or 100 ppm CO (simulated 2000 m elevation). The 100 ppm CO was administered by metering 1% CO into the subject's breathing air in alternating 15 minute segments, for a total CO exposure of about 1 hour. CO concentrations in the subjects breathing zone were monitored continuously during exposures using calibrated non-dispersive Infra-red absorption CO monitors (Beckman Model 302, Dasibi Model 3003). The inspired oxygen content of the breathing air was monitored continuously during exposure and exercise testing with an electrochemical detector system (Ametek S-3A). The order of the four exposures was randomized for each subject. Blood samples were taken at 30 minute intervals and analyzed using an IL 282 CO-Oximeter (Instrumentation Laboratories). Total CO exposure time was determined using the Halldane equation to estimate total time of uptake required to attain 3% COHb.

The blood carboxyhemoglobin levels attained under each of the four exposure conditions are summarized in Table 9. Under clean air (CA) exposure conditions at either sea level (SL) or simulated 2000 m elevation (HA), pre- to post-exposure COHb concentrations showed a slight, but not significant, increase. This increase might be due to diurnal metabolic differences. Pre-exposure COHb levels did not vary greatly over the four exposure test days for most subjects. One subject (#1) varied more than the others; this subject did not smoke (none of the subjects were smokers), however he was often in the company of smokers. The post-exposure %COHb levels presented in Table 9 are the averages of the concentrations in the end-exposure and the end-exercise blood samples, and represent the average concentration during exercise. The target concentrations for both the SL and HA days were 3% COHb and our group mean averages were within 10% of that

target. In five out of 22 cases, the actual COHb concentrations were outside of the ±10% range.

The means and standard deviations (SD) for measured cardiovascular parameters at the point of angina are summarized in Table 10. The data show several trends, some of which approach statistical significance. Larger numbers of subjects would be required before firmer conclusions could be drawn. For SL conditions, time to angina (TTA) was decreased by about 5% by carbon monoxide, which compares well with the 7% decrease demonstrated in our previous study (Kleinman et al., 1989). Heart rate (HR), systolic blood pressure (SBP) and double product (DP) [HR x SBP] were slightly decreased at the angina point, consistent with the slight reduction in exercise time. Similar, but not identical, CO-related changes were seen for the HA conditions. Time to angina decreased by 9%, but there were no changes in the average HR or SBP. The average DP (which is a measure of blood ejection by the heart, cardiac ejection fraction) decreased by about 9%. The parameter that appears to be most influenced by altitude is the DP, which is consistently lower at SL than at HA. There is an effect of CO; DP for both CO/SL and CO/HA conditions are lower than for the corresponding CA conditions. This CO-related effect, however, is likely due to the reduction in the amount of work performed. The ratio of DP/TTA should be proportional to ejection fraction per amount of work performed. For SL conditions (CA and CO), this ratio equals 0.34 and 0.32, respectively. For HA conditions the ratios for CO and CA conditions are both 0.37, suggesting that under these HA conditions the heart must work harder and pump about 12% more blood to accomplish the same amount of physical exercise.

Gas exchange values measured at the point of angina are summarized in Table 11. Minute ventilation (V_e), on the average, was unchanged by the 4 exposure conditions. The rate of oxygen uptake (V_{O2}), which is proportional to amount of work performed, was slightly decreased in HA exposures but when normalized for the duration of exercise (V_{O2}/TTA), there were no changes seen. Horvath, et al., (1988) performed studies in healthy younger men, and concluded that maximal aerobic capacity did not show changes that could be attributed to combinations of CO and high altitude exposure.

The rate of CO_2 expiration (V_{CO2}) and the ventilation equivalent for CO_2 (V_e/V_{CO2}) were not changed by the exposures. Respiratory quotient (R) and the ventilation equivalent for oxygen (V_e/V_{O2}) were both increased in HA conditions, relative to SL. These findings, while not conclusive because of the small number of subjects tested, suggest that these simulated high altitude exposures modify an individuals ability to perform exercise in a manner that is different from the effects of CO, in contrast with the conclusions of Horvath et al. (1988), based upon testing healthy, younger subjects. The measurements performed in our study, however, are technically difficult. Factors, such as small undetected biases in instrument readings can influence results, despite the care with which instruments are calibrated and checked. It would therefore be important to have multiple independent methods for assessing similar parameters. Thus, in future studies we intend to measure the percent oxygen saturation in arterial blood, as well as the fractions of inspired and expired oxygen to coordinate with our gas exchange measurements and possibly to non-invasively monitor several important cardiological parameters directly, including ejection fraction, cardiac contractility and blood flow, using a new instrument.

Table 8. CO/High Altitude Study - Subject Characteristics

Subject	Age	Weight	Height	Coronary Artery Surgery	Number of Vessels	Number of Myocardial Infarcts	Date of First Myocardial Infarct	Number of Angioplasties	Date of First Angioplasty
1	64	206	71	0	0	2	1962	0	0
2	68	228	71	0	0	0	0	0	0
3	64	165	67	1	2	0	0	0	0
4	64	149	68	1	3	0	0	0	0
5	59	277	73	0	0	0	0	0	0
6	67	147	63	0	0	1	1986	0	0
7	61	147	61	1	7	1	1978	1	1987
8	56	169	64	0	0	0	0	1	1988
9	66	176	68	0	0	0	0	0	0
10	62	155	66	1	3	1	1979	0	0
11	61	177	67	0	0	0	0	2	1986
Means	63	181	67	1	4	1	1976	1	1987
S D	4	41	4	1	2	1	10	1	1
n	11	11	11	4	4	4	4	3	3

Table 9. Blood Carboxyhemoglobin Levels

	CA/SL		CA/HA		CO/SL		CO/HA	
Subject	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	0.96	1.40	1.87	2.15	1.43	3.37	2.10	3.48
2	0.40	1.03	1.10	1.03	1.07	2.98	1.30	2.60
3	1.00	1.13	0.83	1.03	1.10	3.05	1.13	3.02
4	1.33	1.15	1.56	1.07	0.63	2.75	0.90	3.10
5	1.07	1.10	1.33	1.62	0.53	3.17	0.90	3.32
6	0.70	0.82	0.40	0.70	1.20	3.13	0.87	3.63
7	0.97	1.20	1.07	1.13	1.30	2.90	1.17	3.12
8	0.73	0.80	0.77	0.67	0.70	2.88	0.63	3.03
9	0.97	0.93	1.00	1.23	0.83	3.15	0.83	3.27
10	0.67	1.25	0.83	1.08	1.00	3.28	1.67	3.48
11	1.00	1.27	1.13	1.32	0.73	3.40	1.77	3.23
Mean	0.89	1.10	1.09	1.18	0.96	3.09	1.21	3.21
St Dev	0.24	0.19	0.40	0.41	0.29	0.21	0.46	0.28
n	11	11	11	11	11	11	11	11

Table 10: Cardiovascular Parameters at the point of Angina (Mean ± SD)

	CA/SL	CA/HA	CO/SL	CO/HA
Time to Angina (min)	5.97 ± 2.01	6.10 ± 2.42	5.66 ± 1.60	5.56 ± 1.54
Heart Rate	108 ± 25	110 ± 24	102 ± 20	110 ± 16
Systolic Blood Pressure	179 ± 14	178 ± 30	175 ± 24	177 ± 25
Diastolic Blood Pressure	88 ± 16	91 ± 15	90 ± 14	88 ± 14
Double Product ($\times 10^{-4}$)	2.03 ± 0.54	2.23 ± 0.89	1.84 ± 0.50	2.03 ± 0.39

Table 11. Gas Exchange Measurements at the Angina Point

	CA/SL	CA/HA	CO/SL	CO/HA
V_e	46.2 ± 11.5	46.4 ± 19.1	44.4 ± 11.0	47.9 ± 14.4
V_{O2}	1.16 ± 0.24	0.97 ± 0.32	1.15 ± 0.21	1.06 ± 0.29
V_{CO2}	1.28 ± 0.32	1.18 ± 0.43	1.20 ± 0.24	1.22 ± 0.24
R	1.06 ± 0.13	1.15 ± 0.20	1.02 ± 0.14	1.15 ± 0.19
V_e/V_{O2}	39.8 ± 4.6	46.7 ± 10.6	38.3 ± 4.3	45.1 ± 7.3
V_e/V_{CO2}	36.2 ± 3.6	38.7 ± 3.9	37.2 ± 4.9	37.8 ± 3.0

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