HEI HEALTH EFFECTS INSTITUTE

Acute Effects of Carbon Monoxide Exposure on Individuals with Coronary Artery Disease

The HEI Multicenter CO Study Team (In Alphabetical Order):
Elizabeth N. Allred, Eugene R. Bleecker, Bernard R. Chaitman, Thomas E. Dahms, Sidney O. Gottlieb, Jack D. Hackney, Denise Hayes, Marcello Pagano, Ronald H. Selvester, Sandra M. Walden, Jane Warren

Includes the Report of the Institute's Health Review Committee

Research Report Number 25

The Health Effects Institute (HEI) is a nonprofit corporation founded in 1980 to assure that objective, credible, high-quality scientific studies are conducted on the potential human health effects of motor vehicle emissions. Funded equally by the U.S. Environmental Protection Agency (EPA) and 27 automotive manufacturers or marketers in the United States, HEI is independently governed. Its research projects are selected, conducted, and evaluated according to a careful public process, including a rigorous peer review process, to assure both credibility and high scientific standards. HEI makes no recommendations on regulatory and social policy. Its goal, as stated by former EPA Administrator William D. Ruckelshaus, is "simply to gain acceptance by all parties of the data that may be necessary for future regulations."

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STATEMENT FROM THE BOARD OF DIRECTORS ON THE HEI MULTICENTER CARBON MONOXIDE STUDY

Eight years ago, we were asked by a group of dedicated individuals in the U.S. Environmental Protection Agency and the automobile industry to help develop a new scientific institution that would be the national center for research on the health effects of automotive pollutants. This center was to be independent of its sponsors, and its objective was to involve the best scientific talent available to work on the potential health problems from automotive emissions. The institution was also to be structured to ensure that its work would be objective and credible to the public.

A few years after the Health Effects Institute (HEI) was established, the HEI sponsors requested that the Institute undertake a study of carbon monoxide effects in angina patients, similar to earlier studies on which the National Ambient Air Quality Standard for carbon monoxide was based. These studies had reported that there was a shortening of the "time to the onset of angina" after exposure to carbon monoxide. The occurrence of angina pectoris, or chest pain, is a health endpoint of concern because it is thought to result from myocardial ischemia, that is, decreased oxygen supply to the heart. Better information on cardiovascular effects of carbon monoxide was needed because doubts had arisen about the reliability of the earlier studies.

Because of the regulatory importance of the proposed study, the HEI Research Committee decided that HEI should have a greater role than customary in the planning and oversight of this study. An ad hoc advisory committee, consisting of three members of the Research Committee and three other experts, was appointed. The objective was to address the same issues as in earlier studies, but to use a new protocol, including the testing of more subjects, so that the results would be more definitive. Another important aspect of the protocol was to utilize changes in each subject's electrocardiogram as an objective indicator of myocardial ischemia, in addition to the subjective indicator (angina pectoris) used in previous investigations. In 1985, after a period of careful planning by several investigators, the ad hoc advisory committee, and the HEI staff, work on the study began.

After the study was finished, the HEI Review Committee—an independent group of distinguished scientists who had no role in the planning or execution of the study—undertook a detailed and rigorous review. This somewhat lengthy and demanding process has produced two high-quality reports. The Investigators' Report—the first of the two reports contained in this document—gives an accurate and detailed account of the conduct of the study, its results, and its significance. The Health Review Committee's Report is a constructive evaluation of the study, its findings, and its implications. Although there are some small differences between the investigators and the Review Committee, the Review Committee and the Board of Directors have both come to the same conclusion: The HEI Multicenter CO Study was a well-planned and well-conducted study, which produced highly reliable results and which added significantly to the knowledge of the health effects of carbon monoxide. The thoughtful planning and execution, illustrated by several unique features (such as the multicenter design and quality assurance plan—both rather rare in environmental health research), paid off handsomely in the end.

The results confirm the hypothesis that exposure to carbon monoxide that produces relatively small increments in blood carboxyhemoglobin levels decreases the time to the onset of myocardial ischemia in exercising males with coronary artery disease. The relatively small increments in carboxyhemoglobin levels studied here occur in some people during the course of their daily activities. The HEI study obviously has not resolved all the scientific questions regarding the cardiovascular effects of carbon monoxide. There are, as discussed in the two reports in this document, several areas where more scientific work would be useful. Nevertheless, the Board believes that the study marks a significant contribution toward resolving scientific issues that are important for public health and regulatory purposes. Even though only an approximate correlation between exercise on a treadmill and normal work or recreational activities is possible, the same results must be expected to occur in people similar to the subjects of this study while climbing two flights of stairs or walking a mile at a slow to moderate pace. Furthermore, although the study sample was by necessity confined to a population of male subjects who met the stringent enrollment criteria, there is little reason to think that the results cannot be extended to most other male, as well as to female, coronary artery disease patients. The results may also be relevant for patients with other forms of cardiovascular diseases.

In addition to the scientific value of the work, we believe that this study demonstrates the strength and the advantages of the HEI research and review processes. Eight years ago, when HEI was founded and we contemplated incorporating this system of checks and balances in our structure, it was not totally clear how the system would work. Today, we believe that it has served both the HEI and the scientific community very well. We hope that this scientific process is one that other scientific organizations will find of interest, and will emulate and improve.

Archibald Cox (Chairman)
William Baker
Donald Kennedy
Charles Powers

ABBREVIATIONS

ATPS	ambient temperature and pressure, saturated with water vapor
CAD	coronary artery disease
CO	carbon monoxide
COHb	carboxyhemoglobin
ECG	electrocardiogram
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
GC	gas chromatography
JH	Johns Hopkins University School of Medicine
MET	basic metabolic equivalent
MI	myocardial infarction
NAAQS	National Ambient Air Quality Standards
NBS	National Bureau of Standards
O_2Hb	oxyhemoglobin
P_{O_2}	partial pressure of oxygen
ppm	parts per million
QA	Quality Assurance
RLA	Rancho Los Amigos Medical Center
SD	standard deviation
SEM	standard error of the mean
STL	St. Louis University School of Medicine
STPD	standard temperature and pressure, dry
$\dot{V}_{O_2}$	oxygen consumption

Acute Effects of Carbon Monoxide Exposure on Individuals with Coronary Artery Disease

The HEI Multicenter CO Study Team: Elizabeth N. Allred, Eugene R. Bleecker, Bernard R. Chaitman, Thomas E. Dahms, Sidney O. Gottlieb, Jack D. Hackney, Denise Hayes, Marcello Pagano, Ronald H. Selvester, Sandra M. Walden, Jane Warren

ABSTRACT

The purpose of this study was to determine, using more objective evidence than that reported in previous studies, whether or not exposures to carbon monoxide that produce approximately 2% or 4% blood carboxyhemoglobin levels cause an exacerbation of myocardial ischemia during a progressive exercise test. The objective measurements were based on the development of electrocardiographic evidence of ischemia. In addition, time to onset of angina pectoris was studied.

Male subjects, ages 35 to 75, with stable exertional angina pectoris and positive exercise treadmill tests with reproducible ischemic ST-segment changes in their electrocardiograms, were studied. In addition, each subject fulfilled at least one of the following criteria of coronary artery disease: angiographic evidence of at least a 70% occlusion of one or more major coronary artery; prior documented myocardial infarction; or a positive exercise thallium test.

Each subject was evaluated on four separate occasions, a qualifying visit and three blinded test visits, which involved exposure (in random order) to air without added carbon monoxide and to air that contained carbon monoxide concentrations calculated to produce approximately 2.2% or 4.4% carboxyhemoglobin, measured by gas chromatography, at the end of the exposure period. These immediate postexposure target levels were set 10% higher than the desired postexercise carboxyhemoglobin levels of 2.0% and 4.0% because exercise while breathing room air results in loss of carbon monoxide. The actual one-minute postexercise levels reached were 2.0% ± 0.1% (mean ± standard error of the mean) and 3.9% ± 0.1%.

On each test day, the subject performed a symptom-limited exercise test on a treadmill, was exposed for approximately one hour to air or to one of two levels of carbon monoxide in air, and then performed a second exercise test. Time to the onset of ischemic ST-segment changes and time to the onset of angina were determined for each exercise test. The percent difference for these endpoints on the pre- and postexposure exercise tests was determined, and then the results on the 2%-COHb-target day and the results on the 4%-COHb-target day were compared to those on the control day.

Data from the 63 subjects who completed the three test visits and met all protocol criteria were analyzed. There were 5.1% ($p = 0.01$) and 12.1% ($p \leq 0.0001$) (trimmed mean) decreases in the time to development of ischemic ST-segment changes after the 2%- and 4%-COHb-target exposures, respectively, compared to the control day. The 90% confidence intervals were 1.5% and 8.7% for the 2%-COHb target, and 9.0% and 15.3% for the 4%-COHb target. There were also 4.2% ($p = 0.027$) and 7.1% ($p = 0.002$) (trimmed mean) decreases in time to the onset of angina after the 2%- and 4%-COHb-target exposures, respectively, compared to the control day. The 90% confidence intervals were (0.7%, 7.9%) and (3.1%, 10.9%), respectively. Thus, any percentage reduction in time to endpoint between 1.5% and 7.9% at the 2%-COHb-target exposure is consistent with both the ST and the angina analyses; and similarly, any reduction between 9.0% and 10.9% at the 4%-COHb-target exposure is consistent with both the ST and the angina analyses. These overlaps in confidence intervals for the ST and angina endpoints are compatible with the view that both endpoints are measuring aspects of the same phenomenon.

In addition, a significant exposure-response relationship was found for the individual differences in the time to ST endpoint for the pre- versus postexposure exercise tests at the three carboxyhemoglobin levels ($p \leq 0.0001$). For this range of carboxyhemoglobin levels (0.2% to 5.1%), a 3.85% ± 0.63% decrease in time to ST endpoint occurred for every 1% increase in carboxyhemoglobin.

These findings demonstrate that low levels of carboxyhemoglobin produce significant effects on cardiac function during exercise in subjects with coronary artery disease.

INTRODUCTION

REGULATORY BACKGROUND OF THE STUDY

A study of the effect of carbon monoxide (CO) exposure on subjects with stable angina pectoris was undertaken at three clinical centers to determine whether or not relatively low levels of carboxyhemoglobin (COHb), which might result from exposure to CO in areas with traffic congestion, would result in myocardial ischemia. The purposes were to test the hypothesis that even low levels of COHb can affect cardiac function in individuals with documented coronary artery disease, and to use objective electrocardiographic (ECG) parameters to extend earlier studies that were used to justify the National Ambient Air Quality Standards (NAAQS) for CO.

Authors are listed alphabetically because many people had varied roles in this study and it was impossible to list authors according to the level of contribution. Questions or comments can be addressed to Jane Warren at Health Effects Institute.

The NAAQS for CO, promulgated by the U.S. Environmental Protection Agency (EPA) in 1971, are set at levels of 9 parts per million (ppm) for an eight-hour averaging time and 35 ppm for a one-hour averaging time. Extrapolation of these exposure levels to COHb levels is useful because COHb provides a biological marker of exposure that represents an accurate measurement of CO dose in these studies, and it enables the comparison of results among studies that employed different exposure regimens. According to estimates by the EPA (U.S. Environmental Protection Agency 1984), a typical adult involved in moderate activity could have a COHb level of approximately 2.0% after a one-hour exposure to CO at 35 ppm. An eight-hour exposure to 9 ppm CO, with light or moderate activity, was projected to result in about 1.4% COHb. In the ambient atmosphere, CO levels would usually fluctuate over the eight-hour averaging time. Various patterns of CO exposure that result in a 9-ppm eight-hour average would produce COHb concentrations in the range of 1.4% to 1.9% in typical adults.

Originally, the CO standards were based on human neurobehavioral studies by Beard and Wertheim (1967). These investigators reported impairment in the ability to discriminate time intervals at COHb levels as low as 1.8%. Although later studies did not support alterations in vigilance at levels below about 5% COHb (U.S. Environmental Protection Agency 1979), other data suggested human effects at COHb levels of 2% to 3%. Studies by Aronow and Isbell (1973) and by Anderson and coworkers (1973), which showed a decrease in the time to the onset of angina (chest pain) during exercise in subjects with coronary artery disease, were used to justify the NAAQS for CO.

Aronow and Isbell (1973) reported a statistically significant reduction in time to angina in a group of 10 subjects with coronary artery disease who had average COHb concentrations of 2.7% after a two-hour exposure to 50 ppm CO. The study design involved comparing the time to onset of angina during exercise tests administered before and after exposure either to "clean air" or to air containing elevated levels of CO. These subjects showed an average decrease in the time to angina of 36.7 seconds (from 224.3 ± 43.6 to 187.6 ± 36.2) between the pre- and postexposure exercise tests on the CO-exposure day, compared to an average decrease of 3.7 seconds on the "clean-air" day. In a later study, Aronow (1981) reported a similar decrease in the time to angina during exercise in 15 subjects after exposure to 50 ppm CO for one hour, resulting in average COHb levels of only about 2.0%. In that study, there was an average decrease of 32.5 seconds (from 321.7 ± 96.0 to 289.2 ± 88.0) after CO exposure, compared to an average increase of 5.8 seconds after exposure to clean air.

Other evidence for an effect of CO at COHb levels less than 3% was provided by a study of 10 subjects with angina by Anderson and coworkers (1973). This study had a somewhat different design than the Aronow studies, in that there was no preexposure exercise test. On different days, each subject was exposed to "normal" air, air with 50 ppm CO, or air with 100 ppm CO for four hours, and had average postexposure blood COHb levels of 1.3%, 2.9%, and 4.5%, respectively. Subjects were able to exercise for an average of about 325 seconds after exposure to clean air, but average exercise time was significantly shorter, about 264 seconds, after either CO exposure.

Given the small number of subjects and the subjective nature of the endpoint (angina pectoris) in the Aronow and Anderson studies, further investigation of the health effects of CO was indicated. In addition, an expert committee convened by the EPA concluded that the EPA should not consider the Aronow results as definitive evidence of the biologic effects of CO, but might consider using them in developing a margin of safety (U.S. Environmental Protection Agency 1984). Because of the potential importance of reports showing CO effects on subjects with angina pectoris in the justification of the NAAQS for CO, a study was sponsored and managed by the Health Effects Institute (HEI) to provide more objective evidence of the effect of CO exposure in subjects with coronary artery disease. Specifically, the study was designed to answer the following question: "Does CO exposure that produces 2% or 4% COHb decrease the time to onset of ischemia during exercise, as documented by ECG ST-segment changes and angina?"

SCIENTIFIC BASIS OF THE STUDY

Carbon monoxide's toxicity is thought to be caused by its ability to bind tightly to hemoglobin, forming COHb. This reaction not only decreases the oxygen-carrying capacity of the blood, but also shifts the oxyhemoglobin (O_2Hb) dissociation curve to the left (Roughton and Darling 1944). The shift to the left means that oxygen is released less readily from circulating hemoglobin, thereby reducing oxygen delivery to peripheral tissues. The net consequence of these effects is to produce a state of relative hypoxia in the tissues.

Low levels of COHb are generally not associated with adverse effects in normal individuals. However, there are several conditions that may make an individual more susceptible to the adverse effects of CO exposure. These include (a) exposure to high altitude, where ambient oxygen tension is reduced; (b) anemia, where the oxygen-carrying capacity of the blood is decreased; (c) chronic lung disease, where gas-exchange abnormalities cause hypoxemia; and (d) coronary artery disease, in which blood flow to the myocardium is restricted. The last condition is the focus of this study.

The hypothesis tested in this study is that exposure to CO resulting in low levels of COHb reduces exercise tolerance in subjects with coronary artery disease. The rationale for this hypothesis is based upon the inability of an individual with coronary artery disease to increase coronary blood flow sufficiently to meet an increased level of myocardial oxygen consumption. Increased myocardial oxygen demand is normally met primarily by increasing coronary blood flow because the extraction of oxygen by cardiac muscle, even at rest, is near maximum. As a subject exercises, myocardial oxygen demand

increases in order to maintain an adequate cardiac output. As long as the increasing myocardial oxygen demands are met, the heart continues to function normally. At the point where myocardial blood flow cannot meet oxygen demands, the myocardium becomes ischemic. Altered cellular metabolism associated with myocardial ischemia results in the development of chest pain or characteristic ECG changes, or both. Symptoms of myocardial ischemia (angina pectoris) occur in individuals with coronary artery disease at specific levels of exercise and limit their exercise capacity. It is possible that increased levels of COHb may produce ischemic symptoms at lower levels of work because of the reduced ability of the blood to carry oxygen to the myocardium.

STUDY DESIGN

In this study, two endpoints were used to assess adverse effects. The time to development of an ST-segment change in the ECG (Figure 1) that exceeded a specific threshold level (see Exercise Treadmill Testing section under Methods) will be referred to as either "onset of ST endpoint" or as "onset of ST-segment change." The ST endpoint was chosen because it is an objective indicator of myocardial ischemia. The second endpoint was the time to onset of angina pectoris. This provides an indicator of symptomatic myocardial ischemia and was the primary cardiac endpoint used in previous studies.

Because CO-uptake rates vary among individuals, exposure to a fixed concentration of CO for a fixed time period results in a wide range of COHb concentrations in the blood (Appendix O). In this study, in order to produce similar levels of COHb in all subjects, the CO exposure concentration was individually adjusted based on experimentally determined uptake rates.

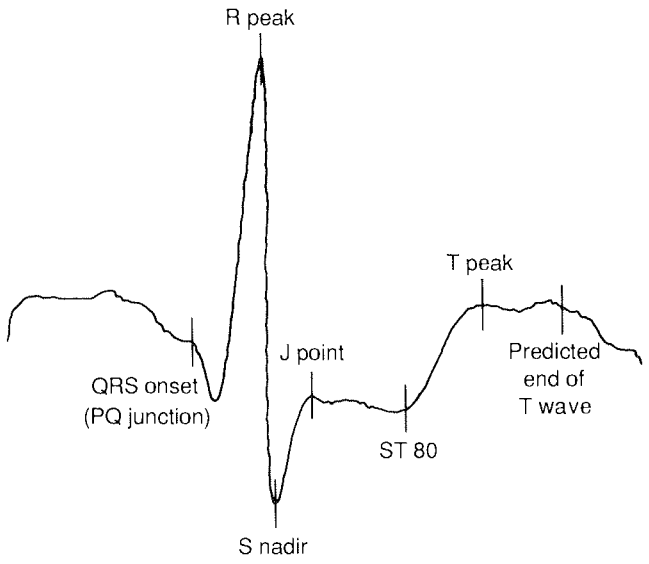

Figure 1. Trace of a normal ECG pattern displaying ST segment.

In addition, exposure times were varied between 50 and 70 minutes to allow for daily variation in individual uptake rates.

Subjects enrolled in this study had coronary artery disease with stable exertional angina and reproducible exercise-induced ST-segment changes. To ensure an adequate sample size, 20 to 25 subjects were recruited at each of three centers and testing was completed on the 63 subjects who met all criteria. A standardized protocol was developed, and the same experimental methods were followed at each center. In addition, periodic audits evaluated adherence to the protocol and reliability of the recorded data.

The basic design of this study was to use repeated exercise tests on the same day, in order to minimize variability in the endpoints (Starling et al. 1984). On each test day, an exercise test was performed before and after the exposure period. Each subject was exposed on three separate days in an environmental chamber to air or to one of two different levels of CO in a random double-blind fashion to produce 2%, 4%, or control levels of COHb. For each test day, the difference in results of the pre- and postexercise tests for the two endpoints (time to ST endpoint and time to angina) was determined, and then these differences on the 2%- and 4%-COHb-target days were compared to the results on the control day. This analysis is preferable to a direct comparison of the time to endpoint on different test days because of known day-to-day variation of these two endpoints in these subjects.

It should be noted that methodological differences in the measurement of COHb in various studies make comparisons of results difficult. In the HEI study, two different types of instruments were employed. All samples were measured by CO-oximetry, and critical samples were also measured by gas chromatography (GC) at a central reference laboratory. Gas-chromatography measurements are considered more accurate (see Methods and Appendices), but CO-oximetry was also used for two reasons. First, the CO-Oximeter could be used for rapid analysis of COHb levels at each center to determine CO exposure conditions. In addition, it provided values that could be compared to those reported in other studies. In this study, COHb levels measured by GC were lower than those measured by CO-oximetry. This difference, which is referred to as the "offset," was approximately 0.5% to 1.2% COHb, depending on the individual center and the level of COHb. The relevance of the COHb levels and the CO exposure levels will be discussed in the Implications of the Findings section.

SPECIFIC AIMS

The primary goal of this study was to investigate the effect of exposure to CO, at levels producing 2% and 4% COHb, on an objective indicator of myocardial ischemia, the time to onset of ST-segment changes in the ECG. The effect of CO exposure on time to angina pectoris, a symptomatic indicator of myocardial ischemia, was also evaluated.

METHODS

ORGANIZATION OF THE STUDY

Many groups participated in this study, as shown in Figure 2. Their roles are described briefly below.

Test Centers

Investigators at three medical centers—Johns Hopkins University School of Medicine, Rancho Los Amigos Medical Center, and St. Louis University School of Medicine—participated in designing the study, writing the protocol and standard operating procedures, and recruiting and testing the subjects. Selection of the three teams was based upon their responses to a request for proposals issued by HEI in September 1983.

Reference Laboratory

A Reference Laboratory, operated at St. Louis University School of Medicine, provided samples for comparing CO-Oximeter readings at the three test centers. The laboratory was also responsible for gas-chromatographic measurements of CO on key blood samples from the three test centers.

Statistical and Data Management Center

Investigators at the Statistical and Data Management Center at the Harvard School of Public Health participated in designing the study, writing the protocol, designing the data-collection forms, and performing the data analysis. They also processed and analyzed data sent on forms from the test centers.

Test Centers
Francis Scott Key Medical Center, The Johns Hopkins University School of Medicine, Eugene R. Bleecker, Sidney O. Gottlieb, Sandra M. Walden

Rancho Los Amigos Medical Center, Jack D. Hackney, Ronald H. Selvester, Robert B. Pearson

St. Louis University School of Medicine, Thomas E. Dahms, Bernard R. Chaitman, Robert Wiens

Reference Laboratory
St. Louis University School of Medicine, Thomas E. Dahms

Statistical and Data Management Center
Harvard University School of Public Health, Marcello Pagano, Elizabeth N. Allred

Quality-Assurance Group
Arthur D. Little, Inc., Denise Hayes, Andrew Sivak

Advisory Committee
John W. Tukey (Chairman), Stephen Achuff, Stephen Ayres, Joseph D. Brain, Steven Horvath, Roger O. McClellan

Project Manager
Health Effects Institute, Jane Warren

Figure 2. Major participants in the study.

Quality Assurance Team

A group at Arthur D. Little, Inc., was responsible for ensuring the quality of data generated in this study. This group participated in reviewing the protocol and standard operating procedures for the study. The Quality Assurance (QA) Officer performed site visits at the test centers, Reference Laboratory, and Statistical and Data Management Center in order to determine that the protocol and standard operating procedures were being followed and that data were reliable and traceable. They conducted the final quality assurance audit on the data at the end of the study.

Advisory Committee

An ad hoc advisory committee, composed of three members of the HEI Health Research Committee and three additional experts, was formed in August 1983 to assist the HEI in selecting investigators, planning the study, and providing guidance throughout the study.

Project Manager

The HEI project manager, with the guidance of the advisory committee and the Health Research Committee, participated in designing the study and writing the protocol and standard operating procedures, facilitated communication among other participants in the study, and worked with the investigators to address problems that arose during the course of the study. She was also responsible for managing the preparation of the final report.

SUMMARY OF EXPERIMENTAL PROTOCOL

Overview

The goal of this study was to evaluate the effect of 2% and 4% COHb on subjects with coronary artery disease at each of three centers. For entrance into this study, the subjects had to be men who did not smoke, ages 35 to 75 years, with stable exertional angina pectoris and a positive exercise treadmill test showing ST-segment changes in the ECG suggestive of myocardial ischemia. These subjects also had documented evidence of coronary artery disease characterized by at least one of the following: angiographic evidence, prior myocardial infarction, or positive exercise thallium test with unequivocal perfusion defect.

The study consisted of four visits for each subject: a base-line qualifying visit (visit 1) and three test visits (visits 2, 3, and 4). The protocol performed during each visit is outlined in Figure 3 and illustrated in Figure 4. On each test day, the subject was screened for elevated base-line COHb levels, performed a symptom-limited exercise test on a treadmill, after recovery was exposed in an environmentally controlled chamber for approximately one hour to room air or CO in room air, and then performed a second exercise test. The exposure conditions for test visits were randomized (see Appendix D)

among room air and two ambient levels of CO. These levels were chosen in order to produce 2.2% and 4.4% COHb before exercise, and approximately 2.0% and 4.0% COHb at the end of exercise, as determined by gas chromatography, assuming a 10% decrease in percent carboxyhemoglobin (%COHb) during exercise. Two main indicators were used to evaluate myocardial ischemia during both exercise tests each day. These are time to ischemic ST endpoint on the ECG and time to angina.

The protocol was approved by the Institutional Review Board at each test center.

Visit 1

The qualifying visit included obtaining the subject's informed consent, followed by a complete medical history and physical examination and a base-line 12-lead ECG. If the subject qualified by the inclusion-exclusion criteria described below, a blood sample was taken to determine whether or not the subject's base-line blood COHb level was above the level specified by the exclusion criterion. This requirement is identical to that in visits 2, 3, and 4, and is discussed in detail below. A symptom-limited exercise treadmill test, using a modified Naughton protocol (Raider 1973), was then performed. After the exercise test, the subject rested until his ECG returned to base-line values. The subject then entered the environmental chamber for a one-hour sham exposure period prior to a second, identical, exercise test. Qualification for entry into the study consisted of reproducible results on the two exercise tests: Both tests had to be positive with respect to both ST endpoint and angina; in addition, the difference in duration of the two exercise tests had to be 150 seconds or less.

Subjects who met the entrance criteria were then exposed in the environmental chamber to 150 ppm CO for 60 minutes. The subjects remained seated during the exposure. Venous blood samples were collected every 15 minutes and analyzed immediately for %COHb by CO-oximetry. These data were used to determine the subject's CO uptake constant; this was used on subsequent visits to calculate the atmospheric CO levels required for each subject to reach the desired COHb level.

Qualifying Visit (1)
History, physical examination
Informed consent
Base-line blood tests (including COHb)
Exercise treadmill test 1A
Recovery period
Sham exposure (60 minutes)
Exercise treadmill test 1B (to verify reproducibility by comparison with 1A)
Recovery period
Standard 150-ppm CO exposure (60 minutes)

Test Visits (2, 3, and 4)
Interim history, physical examination
Base-line COHb
Exercise treadmill tests 2A, 3A, 4A
Recovery period
Exposure (in random order to 4%-COHb target, 2%-COHb target, or room air for 50 to 70 minutes)
Exercise treadmill tests 2B, 3B, 4B

Figure 3. Protocol for qualifying and test visits.

Visits 2, 3, and 4

Subjects who met the reproducibility requirements during the initial visit returned for three test visits. These visits were separated by at least 72 hours, and all occurred within a four-week period. The subjects were instructed to continue their cardiac medications throughout the study. Every effort was made to maintain consistency of dose and time of use before a test visit. Subjects were also instructed to refrain from eating or drinking for two hours before coming to the laboratory.

Upon arrival at the laboratory, the subject's base-line COHb level was measured by CO-oximetry to assure that it was at or below the equivalent of the GC level of 1% (see below). A pretest interim history and 12-lead ECG were done at each visit to verify that the subject was clinically stable. The subject then performed a symptom-limited exercise test and was allowed to recover. The original protocol permitted a subject to complete the test visit whether or not he achieved sufficient angina or ST-endpoint changes during the first exercise test. As a precaution, the protocol was amended, effective February 18, 1986, to minimize the potential failure to achieve the cardiac endpoints. When a subject did not achieve the required level of angina or ST-endpoint on the preexposure exercise test of visits 2, 3, or 4, the exposure and second exercise test for that day were not done, and the visit was rescheduled. If the subject failed to achieve one or both of the endpoints on two successive visits, he was dropped from the study. This resulted in rescheduling two subjects and dropping one subject from the study.

The subjects were exposed to room air, to the lower level of CO, or to the higher level of CO after recovering from the first exercise test. The subjects and the personnel responsible for the exercise testing and the cardiovascular monitoring were

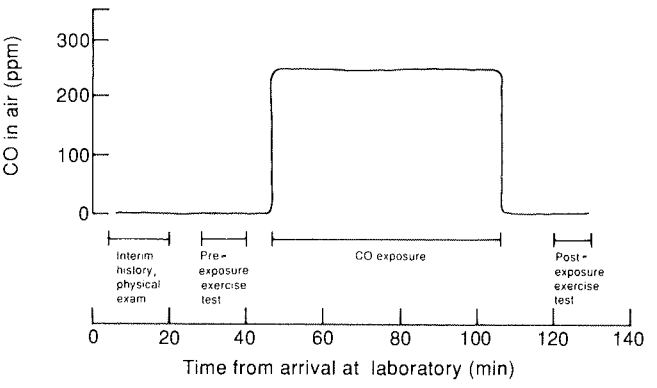

Figure 4. Illustration of protocol for test visit on 4%-COHb-target day.

blinded as to the exposure conditions. The exposure conditions were selected to produce no change in %COHb, an increase to 2.2% COHb, or an increase to 4.4% COHb, as measured by GC. These COHb targets are 10% greater than the desired levels at the end of the second exercise test, which were 2.0% and 4.0% COHb, to compensate for the loss of CO during exercise. In order to attain a relatively constant level of %COHb at the end of exposure, the level of CO in the chamber for each individual was varied according to the uptake constant determined during the qualifying visit (visit 1). An additional variable, duration of exposure, was also used to help attain the desired level of %COHb. On the 2%- and 4%-COHb-target days, the actual duration of exposure was based upon the results of the analysis of COHb by CO-oximetry of blood samples collected at 30 and 40 minutes of exposure. On the air-exposure days, the length of exposure of the subject in the chamber was randomly determined prior to the subject's arrival at the laboratory. All of the other procedures used on the CO-exposure days were followed on the air-exposure days to maintain double-blind conditions. After the exposure, the subjects exercised, using the same protocol, for a second time. The mean time from the end of the exposure period to the beginning of the second exercise test was 17 minutes (SD = ± 10.2; SEM = ± 0.6).

Venous blood samples were collected to monitor the CO-exposure conditions. Carboxyhemoglobin levels were measured in each subject during visits 2, 3, and 4 at the times indicated in Table 1 and illustrated in Figure 5: arrival at the laboratory (sample 1); within one minute of completing the first exercise test (sample 2); after 30 minutes of exposure (sample 3); after 40 minutes of exposure (sample 4); at the end of exposure (sample 5); and within one minute of completing the second exercise test (sample 6). All samples were evaluated by CO-oximetry at the individual test centers. In addition, key samples were sent to the St. Louis Reference Laboratory for analysis by GC. Samples 2 and 6, which were drawn at the end of exercise tests 1 and 2, respectively, were analyzed by GC in all subjects throughout the study. Beginning August 29, 1985, a protocol amendment was introduced to require analysis by GC of sample 5 (end of exposure). Only CO-oximetry measurements were performed on samples 1, 3, and 4.

The COHb levels by CO-oximetry were related to the GC targets by an offset that represented the average difference between GC and CO-Oximeter measurements. The initial offset value between the GC and CO-Oximeter was based on a small number of pilot studies. Later, it was modified based on comparisons made with a larger number of observations from early results (Table H.1 in Appendix H). The initial protocol utilized a constant offset of 0.9%; thus, CO-oximetry target levels of 3.1% and 5.3% were used for GC targets of 2.2% and 4.4%, respectively. An amendment on May 3, 1985,

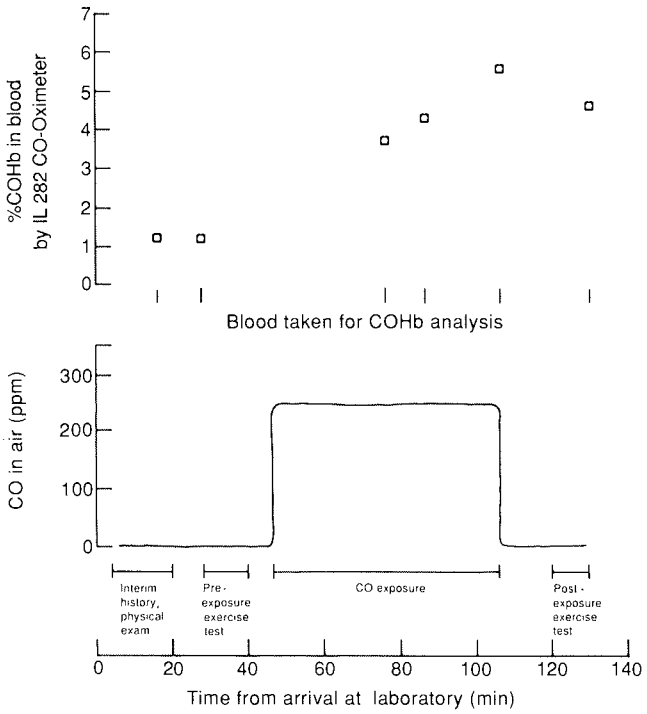

Figure 5. Sampling of blood during test visit. Carboxyhemoglobin levels in all samples were determined by CO-oximetry. In addition, samples 2, 5, and 6 were analyzed for CO content by GC methods at the Reference Laboratory.

Table 1. Blood-Sample Collection and Analysis for Visits 2, 3, and 4

Sample Number	Collection Time	Analysis	
		CO-Oximetry	Gas Chromatography (at Reference Lab)
1	When subject arrives at lab	Immediately	No
2	At end of exercise test 1 (within one minute)	Immediately	Yes
3	At 30 minutes of CO exposure	Immediately	No
4	At 40 minutes of CO exposure	Immediately	No
5	At end of CO exposure	Within one hour	Yes[a]
6	At end of exercise test 2 (within one minute)	Within one hour	Yes

[a] The original protocol did not require GC analysis of sample 5. An amendment, with an effective date of August 29, 1985, added GC analysis of sample 5. Prior to that date, seven subjects were studied.

decreased the offset to 0.5%, and on August 29, 1985, another amendment increased the offsets to 1.0% at the 2%-COHb-target exposure and 1.3% at the 4%-COHb-target exposure. Only seven subjects were studied prior to this amendment. Thus, during the major part of the study, the target end-of-exposure COHb levels were 3.2% and 5.7% by CO-Oximeter, corresponding to 2.2% and 4.4%, respectively, by GC. Although the protocol amendments affected the range of COHb levels achieved in experimental exposures, they did not affect the accuracy of the COHb measurements.

In order to assure that the subjects did not encounter high levels of CO prior to arriving at the laboratory, sample 1 was collected and immediately analyzed. The initial upper limit for sample 1 was 1.8% COHb by CO-oximetry, which was assumed to represent approximately 1% COHb by GC. The upper limit was raised to 2.0% by a protocol amendment, effective September 24, 1985, because experimental results at that point indicated a higher offset between GC and CO-Oximeter measurements than had been assumed based on preliminary comparisons. A June 23, 1986, amendment changed the limit again, based on data showing that blood O_2Hb levels affect COHb readings on the CO-Oximeter (see Appendix I). The new cut-off levels were 2.0% with O_2Hb levels of 60% or less, 2.2% with O_2Hb levels of 61% to 80%, and 2.4% with O_2Hb levels above 80%.

Samples 3 and 4 were used to project the subject's COHb level at the end of exposure at visits 2, 3, and 4. Each subject was exposed to a level of CO that should have resulted in the desired COHb level after 60 minutes, judging from his uptake constant as measured at visit 1. If CO uptake measured at 30 and 40 minutes was faster than expected, then the exposure period was shortened accordingly. If uptake was slower, the exposure period was lengthened. Exposures were limited to either 50, 55, 60, 65, or 70 minutes in length.

DESCRIPTION OF SUBJECTS

Subject-Selection Criteria

The selection criteria for the study population were chosen with the goal of obtaining a group of men who do not smoke and who have objective evidence of coronary artery disease, stable angina, and evidence of reproducible, exercise-induced myocardial ischemia (angina and ischemic ST changes on the ECG) during exercise treadmill testing. The following subject inclusion and exclusion criteria define the study population.

Inclusion Criteria

1. Men, ages 35 to 75 years. (Men were chosen to reduce the risk of false-positive exercise test results that are more common in women [Weiner et al. 1979; Val et al. 1982].)
2. Stable exertional angina pectoris.
   a. Duration of three months or more.
   b. Angina class II or greater (Canadian Cardiovascular Society Classification; Campeau 1976).
3. A positive exercise treadmill test for myocardial ischemia, defined by:
   a. Exercise-induced ST-segment depression or elevation on the ECG compared with the rest tracing, as defined in the Specific Definitions section under Methods; and
   b. Associated exercise-induced angina. (The presence of angina was required in order to address the outcome variable of time to onset of angina, which was used in prior studies.)
4. Additional evidence of coronary artery disease, demonstrated by one or more of the following criteria:
   a. Angiographic evidence of coronary artery disease with one or more major coronary artery with diameter narrowing of 70% or more.
   b. Prior myocardial infarction, documented by at least two of the following:
      - Typical chest pain lasting one hour or more.
      - New Q waves enduring 0.04 seconds or more, or T-wave inversions lasting longer than one week on the 12-lead ECG.
      - Creatine kinase (serum) rise greater than twice normal with 10% or more MB isoenzyme fraction.
   c. Positive exercise thallium test with unequivocal perfusion defect.

   (Items a, b, and c were included to provide additional objective evidence of coronary artery disease to exclude subjects with false-positive exercise studies.)
5. Ability and willingness of the subject to provide informed consent.
6. Permission of the subject's primary physician.
7. Stable antiischemic medical regimen for the study duration, or no medication. (Except for digoxin, exclusion criterion 7, there were no limitations regarding medical therapy other than that all medications were to be continued in unchanged doses and at standard dosing intervals throughout the study period.)
8. Ability to exercise for at least three minutes using the modified Naughton protocol, with reproducible total exercise duration on two tests performed at visit 1. (The reproducibility requirement demanded that the total exercise duration times be within 150 seconds, in order to exclude subjects with significant variations in serial exercise performance, in whom variant angina might be present and in whom the effect of CO exposures would be difficult to interpret.)

Exclusion Criteria

1. Cigarette, cigar, or pipe smoking within three months (by history). (Current smokers were excluded because of chronic high-COHb levels.)

2. Unstable angina, defined by new onset or change in pattern of angina within three months, or symptoms of angina at rest. (These patients were excluded because of their high risk and unpredictable clinical course.)
3. Myocardial infarction within the previous three months.
4. Symptomatic congestive heart failure and congenital or significant valvular heart disease. (Excluded to minimize risk and to obtain a more homogeneous study population for repeated exercise testing.)
5. Previous coronary artery bypass surgery within six months. (Early postbypass patients were excluded because of complications of recovery and pericardial reaction.)
6. Resting ECG abnormalities that may interfere with ST-segment interpretation during exercise, defined by the exclusion of subjects with resting ECGs that meet the following Minnesota code classifications (Prineas et al. 1982): left ventricular hypertrophy (3-1, 3-2, 3-3, 3-4); ST depression (4-1, modified to read 2.0 mm); inverted T waves (5-1); A-V conduction defects (6-1, 6-2, 6-4, 6-8); interventricular conduction defects (7-1, 7-2, 7-4); tachyarrhythmias (8-2, 8-3, 8-4, 8-5, 8-6); and large amplitude T waves (9-6).
7. Digoxin therapy or uncorrected hypokalemia.
8. Significant pulmonary disease defined by a forced expiratory volume in one second (FEV_1) less than or equal to 50% predicted for age, height, and gender (Appendix A), or previously documented resting hypoxemia (arterial partial pressure of oxygen [P_{O_2}] less than or equal to 60 mm Hg), or oxygen saturation less than 90%. (The performance of arterial blood gas or ear oximetry was not required, but could be performed at the investigator's discretion if significant pulmonary disease was suspected.)
9. Uncontrolled hypertension (systolic pressure greater than 170 mm Hg or diastolic pressure greater than 100 mm Hg at rest). (It was felt that items 8 and 9 potentially limited exercise performance significantly, and thus volunteers exhibiting either characteristic were excluded).
10. Anemia (hemoglobin less than 10 g/dl).
11. Thyrotoxicosis or other uncontrolled endocrine disease.
12. Symptomatic cerebrovascular disease, including recent stroke or symptoms suggestive of transient ischemic attacks.
13. Other significant debilitating systemic disease.
14. Inability or contraindication to perform exercise treadmill testing or to return for follow-up visits.
15. Initial venous COHb level at visit 1 greater than the exclusion criterion, as described in the Summary of Experimental Protocol section under Methods. (The CO-oximetry exclusion criterion was chosen to represent 1% or less by GC.)
16. Presence of a permanent pacemaker.

Subject Recruitment Strategy

Subjects were recruited from the three test centers and included outpatients in the cardiology clinics, patients undergoing clinical exercise treadmill testing, patients scheduled for cardiac catheterization, and past and present participants in cardiac rehabilitation programs. Male subjects with positive exercise treadmill tests were further screened for smoking history, and potential candidates were then approached for visit-1 screening after permission from the subject's physician was obtained. Initial screening of subjects was performed by the study investigators, with the assistance of research technicians and nurses.

Subject Enrollment and Exclusion

Seventy-six subjects, who reportedly met all visit-1 criteria, were enrolled in the study. Data from 63 of them were used in the main data analysis, reported in the Results section of this report. The reasons for exclusion of subjects are summarized below and in Figure 6.

Seven of the 76 enrolled subjects did not complete the test visits, leaving a total of 69 subjects who completed the three test visits. Of those not completing the protocol, two dropped out on medical advice, one by subject preference, and four because they did not meet protocol criteria at test visits. Of the 69 subjects who completed the four visits, six were excluded from the main data analysis. Five subjects at Rancho Los Amigos Medical Center were disqualified because they did not meet visit-1 criteria. Four of them were disqualified at the initial cardiology consensus meeting because their ST changes on the visit-1 exercise tests were not sufficient. These four subjects were disqualified prior to data analysis. One was disqualified because he did not meet the reproducibility requirement for the two exercise tests on visit 1 and should not have been included in the randomization.

At the St. Louis University School of Medicine, there were three instances when CO-Oximeter values for sample 1 were higher than the allowable level specified by the final protocol. Two of these samples were from subject 309 and one was from subject 327. Results for subject 309 were not included in the main data analysis. Subject 327 was excluded from the 2% analysis because the entry criterion was exceeded on the 2%-COHb-target-exposure day.

Figure 6 summarizes the exclusion of subjects from the study and the main data analysis. Data from 63 subjects were used in the main analysis of the effect of 4% COHb on time to angina, and data from 62 subjects were used in the analysis of the effect of 2% COHb on time to angina. Because one subject provided no ST data, there are only 62 and 61 subjects in the 4%- and 2%-COHb-target analyses of time to ST, respectively. The range in time from enrollment in the study to study completion was 10 to 35 days.

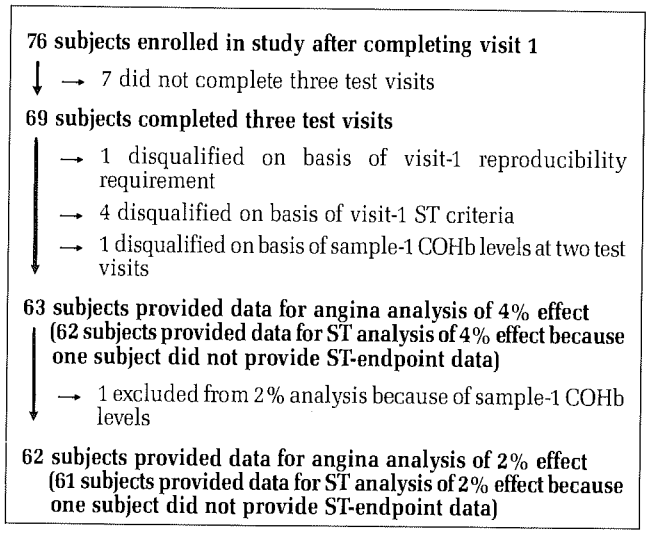

Figure 6. Summary of subject exclusion from study and main data analysis.

Individual subject data for the six subjects who completed the three test visits, but were not included in the main analysis, are included in Appendix B; analysis of data from the 69 subjects is included in Appendix C.

Characteristics of Study Population

The characteristics of the study population are presented in Tables 2, 3, and 4. Table 2 describes the criteria met by each subject with respect to evidence of coronary artery disease. Table 3 presents information about general medical and cardiac history and medication use, as well as demographic information. Table 4 summarizes quantitative information on age, height, weight, and cardiac and pulmonary function.

Sixty-three male, nonsmoking subjects with a mean age of 62.1 ± 8.1[1] years, with stable angina pectoris and positive exercise treadmill tests that showed ischemic ST-segment changes, completed the protocol. In addition, as indicated in Table 2, each subject fulfilled at least one other criterion for coronary artery disease: angiographic evidence, prior myocardial infarction, or a positive exercise thallium test. The majority of subjects was classified as having Class II angina (Canadian Cardiovascular Society; Campeau 1976). Ten had prior coronary artery bypass surgery. Thirty-three subjects had a history of previous myocardial infarction, and of this group 26 had objective evidence of myocardial infarction, as defined by inclusion criterion 4. Twenty-seven subjects had a positive thallium perfusion scintigraphic study, and 40 had previous cardiac catheterization that demonstrated obstructive coronary artery disease. The mean number of anginal episodes per week at study entry was 4.6 (range of 0 to 63) and the mean number of nitroglycerin tablets taken per week was 2.3 (range of 0 to 30). Although 31 subjects reported a history of hypertension, no subject had uncontrolled hypertension during the study.

Coronary angiography had been performed in 43 subjects, although it was not a necessary prerequisite for study inclusion. Forty subjects had at least one vessel with 70% or more diameter obstruction by visual estimation. Thirteen subjects had single-vessel coronary artery disease, 15 had double-vessel coronary artery disease, and 12 had triple-vessel coronary disease. No subject had significant left main coronary disease.

Information on resting ECG abnormalities is summarized in Table 3. Although a significant number of subjects had resting P- and T-wave abnormalities typical for subjects with coronary artery disease, only a small number had minor resting ST-segment elevation (five) or ST-segment depression (seven). In no case was the degree of resting ST-segment changes felt to be incompatible with the interpretation of ischemic ST-segment changes produced during exercise. Pulmonary function assessed by spirometry demonstrated a mean FEV_1 of 88% of predicted values, and a mean forced vital capacity (FVC) of 90% of predicted values for age and size. Thus, significant obstructive or restrictive pulmonary disease was not found in the study population.

Although none of the subjects had smoked for at least three months prior to study entry, 51 had a history of past cigarette smoking. Forty-eight of these had stopped smoking more than 12 months before study entry, one had stopped six to 12 months prior to study entry, and two had stopped three to six months prior to study entry. The low-COHb levels in venous blood samples upon arrival at the testing center provided evidence of compliance with the nonsmoking requirement. No subject who entered the study had to be excluded on the basis of suspected resumption of smoking during the study period.

Most subjects were receiving stable doses of beta-adrenergic blockers, nitrates, or calcium channel antagonists during the study period. The number of subjects using each type of medication is reported in Table 3. All subjects were carefully instructed to take their usual cardiovascular medications at the same time on the study days, and testing was scheduled at a standard time of the day for each subject in order to ensure a uniform time interval between the administration of cardiovascular medications and exercise testing.

All subjects underwent physical examination at study entry. As shown in Table 4, they had a mean weight of 83.0 ± 10.2 kg and a mean height of 176.1 ± 7.2 cm. Their mean resting heart rate was 63.3 ± 10.9 beats per minute. Mean systolic blood pressure was 131.2 ± 20.8 mm Hg (sitting), and diastolic blood pressure was 77.5 ± 8.6 mm Hg (sitting). No patient had evidence of congestive heart failure; no S3 gallops, rales, or evidence of elevated venous pressure were detected. An S4 gallop was detected in six subjects, three subjects had systolic murmurs, and one had a diastolic murmur; all of these murmurs were interpreted to be clinically insignificant. Three subjects had evidence of cardiac enlargement and four had abnormalities of lung fields on chest x-ray examination.

Although the major clinical characteristics were comparable at the three testing centers, several minor differences were noted. There was a higher percentage of subjects in profes-

[1] Throughout the Methods section, mean ± standard deviations are presented.

Table 2. Criteria of Coronary Artery Disease by Which Each Subject Qualified for the Study[a]

Subject	Coronary Artery Disease Indicator		
	Angiography (≥ 70% Lesion)	Myocardial Infarction (Objective Evidence)	Positive Thallium Test
Johns Hopkins			
101	+		
102			+
103	+		
104	+	+	+
105	+		
106	+	+	
107	+	+	
108	+		
109		+	
110	+	+	+
111		+	
112	+		
113		+	
114	+		
115	+	+	+
116		+	
117	+	+	
118		+	
121		+	
122	+		
124	+	+	
125		+	
Subtotal	14	13	5
Rancho Los Amigos			
201	+	+	
205	+		
207	+	+	+
213	+		
214	+		
215	+	+	
218	+		
221			+
222	+	+	
227	+		
229	+		
Rancho Los Amigos (continued)			
234		+	
235			+
236	+		
237	+	+	
239			+
241			+
243		+	
Subtotal	12	7	5
St. Louis			
301	+		
303	+		
304	+	+	+
306			+
310	+		
311	+	+	+
317	+	+	+
318	+		+
323	+	+	+
324	+	+	
325			+
326	+	+	+
327	+		+
328	+		
330	+		
332			+
333	+		+
334			+
335			+
336			+
337			+
338			+
339			+
Subtotal	14	6	17
Total	40	26	27

[a] Any one of these three criteria was required for entry. In addition, each subject had stable exertional angina pectoris and a positive exercise treadmill test, as required by the protocol. A plus sign (+) means that the subject fulfilled the criterion. Information is not available to distinguish an absence of information from failure to meet the criterion.

sional occupations enrolled at Rancho Los Amigos Medical Center (50%) than at Johns Hopkins University (9%) and St. Louis University (39%). There were minor differences in measured electrolyte values, including sodium, potassium, and chloride, but these values were within normal limits at each center.

Table 3. Base-Line Characteristics of the Study Population (63 Subjects)

Characteristics	Number of Subjects
Heart Disease	
Stable angina pectoris	63
Angina class II	60
class III	3
History of angina at rest	7
History of myocardial infarction	33
Prior coronary artery bypass surgery	10
Information on coronary angiography	43
≥ 70% lesion	40
Single-vessel CAD[a]	13
Double-vessel CAD	15
Triple-vessel CAD	12
Left main CAD	0
Resting electrocardiogram	
P-wave abnormality	9
T-wave abnormality	21
Anterior MI[b]	10
Inferior/posterior MI	11
Resting ST-segment elevation	5
Resting ST-segment depression	7
Positive thallium stress test	27
Disease History	
Hypertension	31
Diabetes mellitus	10
Cerebrovascular disease	4
Peripheral vascular disease	2
Valvular heart disease	1
Pulmonary disease	2
Thrombophlebitis	1
Hepatic disease	1
Renal disease	3
Gout	10
Neoplastic disease	1
Peptic ulcer	4
Hyperlipidemia	12
Smoking	
Currently nonsmoking	63
Prior smoking (cigarettes)	51
When stopped smoking	
3 to 6 months prior to study	2
6 to 12 months prior to study	1
more than 12 months prior to study	48

Characteristics	Number of Subjects
Medications	
Beta-blockers	38
Nitrates (oral)	30
(patch)	6
Calcium antagonists	40
Diuretics	13
Antiarrhythmics	4
Antihypertensives	7
Other cardiac	7
Demographic Information	
Occupation	
Professional	20
Clerical	6
Laborer	31
Other	6
Employment status	
Full-time	25
Part-time	3
Not employed	4
Disabled	5
Retired	26
Education	
Grade school	5
High school	34
Some college	15
College graduate	6
Graduate school	2
Unknown	1
Marital status	
Single	5
Married	50
Divorced/separated	3
Widower	4
Unknown	1
Religious affiliation	
Protestant	30
Catholic	19
Jewish	1
Other	11
Unknown	2

[a] CAD = coronary artery disease.

[b] MI = myocardial infarction.

Table 4. Quantitative Base-Line Subject Characteristics

Characteristics	Mean ± SD[a]
Age	62.1 ± 8.1
Weight (kg)	83.0 ± 10.2
Height (cm)	176.1 ± 7.2
Angina duration (months)	65.5 ± 64.8
No. angina episodes/week	4.6 ± 9.4
No. nitroglycerin tablets/week	2.3 ± 5.5
Resting heart rate (bpm)	63.3 ± 10.9
Blood pressure (mm Hg)	
Systolic (sitting)	131.2 ± 20.8
Diastolic (sitting)	77.5 ± 8.6
Systolic (standing)	130.4 ± 17.9
Diastolic (standing)	78.1 ± 9.4
Spirometry	
FEV_1 (liters)	2.8 ± 0.5
FEV_1 % predicted	88.3 ± 18.3
FVC (liters)	3.7 ± 0.7
FVC % predicted	90.0 ± 15.7

[a] SD = standard deviation.

CARBON MONOXIDE EXPOSURE AND MONITORING

Carbon Monoxide Exposure Methods

The procedures used for exposing the subjects to CO were intended to result in end-of-exercise COHb values of 2.0% or 4.0% as determined by GC. In order to attain these levels of COHb in the subject's blood, the target levels after the exposure were set at values 10% greater than the desired end-of-exercise levels, that is, 2.2% and 4.4% COHb. Subjects exercised while they breathed room air that contained no more than 8 ppm CO, which resulted in the elimination of some of the body burden of CO taken up during the exposure phase. The subjects at all three centers were exposed in room-sized chambers (700 to 2,000 cubic feet) for approximately one hour. The chamber concentrations were adjusted for each subject based on his CO-uptake data obtained at visit 1, but were fixed for the duration of each exposure. Due to variability in a given subject's CO-uptake rate, the length of each exposure was also varied, with the goal of attaining end-of-exposure levels of %COHb as close to the target levels as possible. The effectiveness of the exposure protocol in narrowing the range of COHb values is discussed in Appendix O.

Each individual's CO-uptake rate was determined at the end of the qualifying v' it to the laboratory (visit 1). The subjects were exposed to 150 ppm CO for one hour, and blood samples were drawn prior to, and every 15 minutes during, the exposure period. These samples were analyzed immediately, by CO-oximetry, for %COHb. The %COHb values were plotted against duration of exposure, and the 60-minute uptake rate was computed based upon a linear regression of the slope, with the units of change in %COHb per hour. This value was divided by the actual level of CO to which the subject was exposed (approximately 150 ppm), resulting in an uptake-rate constant expressed as %COHb per hour per ppm CO.

On test visits, the level of CO in the chamber required to increase the %COHb from the sample 2 (preexposure) level to the target level was computed by the following equation: *CO concentration (ppm) = [(target %COHb) - (sample 2 %COHb)]/(uptake rate constant)*. The range of uptake rates and the CO chamber concentrations are reported in Appendix N.

Since the uptake-rate constant was determined on the basis of only one exposure, allowance for variability in the uptake rate on subsequent visits was provided for by measuring uptake at 30 and 40 minutes and varying the length of exposure from 50 to 70 minutes. The appropriate length of exposure to CO was established graphically by linear extrapolation from a plot of the COHb levels at 0, 30, and 40 minutes (Figure 7).

To minimize learning or training effects on the study results, the order of exposure among the three possible conditions of exposure was randomized. The Statistical and Data Management Center assigned a randomization order for each subject as he qualified for entrance into the study (successful completion of visit 1). The effectiveness of the randomization process for determining the order of exposure to air, 2%-COHb target CO level, and 4%-COHb target CO level is summarized in Table 5. A comparable number of subjects received each of the three exposures on each of the testing days.

The CO-Oximeter provided a meti od of rapidly analyzing blood samples for %COHb, as was required to monitor the

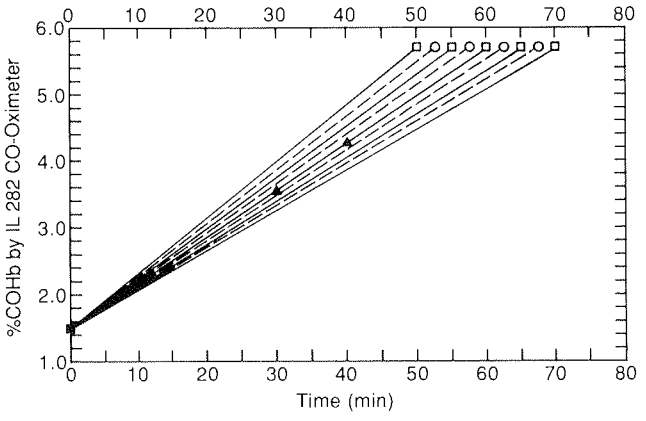

Figure 7. Determination of length of exposure for a given subject. The preexposure sample was analyzed and plotted at time 0. This point was connected to the possible end-of-exposure times of 50, 55, 60, 65, or 70 minutes (□ and solid lines). Additional decision lines were also drawn at 52.5, 57.5, 62.5, and 68.5 minutes and the initial data point (○ and dashed lines). The values of COHb obtained at 30 and 40 minutes were then plotted on this graph (▲). Since these data points fell between the 57.5- and 62.5-minute decision lines, the exposure was carried out for 60 minutes.

Table 5. Results of Randomization of Order of Blinded Exposures to Purified Air, Carbon Monoxide to 2%-COHb Target, and Carbon Monoxide to 4%-COHb Target

Exposure	Number of Subjects		
	Visit 2	Visit 3	Visit 4
Combined			
Air	20	24	19
2% COHb	20	22	20
4% COHb	23	16	24
Johns Hopkins			
Air	7	8	7
2% COHb	8	8	6
4% COHb	7	6	9
Rancho Los Amigos			
Air	5	7	6
2% COHb	4	7	7
4% COHb	9	4	5
St. Louis			
Air	8	9	6
2% COHb	8	7	7
4% COHb	7	6	10

CO exposures. Because %COHb values obtained by GC and CO-oximetry differ, an average offset was used to determine CO-oximetry target values. For most of the study, an offset of 1.0% was used for the 2.2% GC target and 1.3% for the 4.4% GC target, giving CO-Oximeter target levels of 3.2% and 5.7%, respectively. The ability to attain the CO-Oximeter target values is a validation of this technique. Relevant data are presented and discussed in Appendix M.

Monitoring of Atmospheric Carbon Monoxide

The levels of CO breathed by the subjects in this study were measured at all stages of the experiments. This included measuring the background laboratory CO levels to which the subjects were exposed during the exercise tests as well as the chamber CO concentrations during the exposure phase. Carbon monoxide levels were determined with the use of non-dispersive infrared analyzers (Bendix Model 3501-5CA, or Beckman Model 866) that provided a continuous recording of the CO concentration. Prior to use each day, the instruments were calibrated with commercially available standard gases that had been analyzed by the suppliers (Airco, Detroit, MI; or Scott Specialty Gases, San Bernardino, CA) and were shown to be ± 1% of the reported concentration based upon National Bureau of Standards (NBS) standards. In addition, cylinders of CO that had been standardized according to the EPA protocol (U.S. Environmental Protection Agency 1979) were used for daily calibration and to confirm periodically the concentration of the NBS traceable gases. The EPA traceable gases not only met the requirements for the NBS gases, but also had to show consistent values (within 1%) on repeated analyses at least 24 hours apart. All of these gases were contained in aluminum cylinders treated with an antioxidant to prevent loss of CO in the cylinders over time. The zero-CO gas mixtures used were nitrogen without carbon dioxide for the Bendix instruments and nitrogen with carbon dioxide for the Beckman analyzer.

The calibration of the analyzers was carried out using at least three standard gases for each range of expected use of the instrument on CO-exposure days. On air-exposure days, the analyzers were calibrated with at least a zero gas mixture and one standard gas. The gases were monitored until a stable reading was maintained for at least one minute on the chart recording.

At five separate intervals over the two-year course of testing of subjects, the Rancho Los Amigos center coordinated a round-robin analysis, at all three centers, of cylinders with two unknown concentrations of CO specially prepared each time for this task. The results of these comparative tests provided information about the relative values for monitoring atmospheric levels of CO at all centers, and about the functioning of the instruments that were used throughout the course of the study (Appendix L).

Calibration of the analyzers was carried out periodically. During these sessions, the standard gases were calibrated against the EPA protocol gases over all the intended ranges of use of these instruments. It was the general finding that none of the gases varied from one another, regardless of the protocol under which they were produced at the supplier. Also, the gases remained stable for the two and a half years that they were used for this study.

The monitoring during exposure at each center was done in such a way that the gas that was sampled reflected the CO concentration that the subject was inhaling. This was carried out by sampling next to the head of the subject (within one to two feet) at Johns Hopkins and St. Louis. At Rancho Los Amigos, sampling was at a distant site, and the distribution of CO and the mixing characteristics of the chamber were checked every three months to confirm that there were no gradients of CO in the chamber. Similar checks were also performed at Johns Hopkins and St. Louis, at less frequent intervals. Atmospheric levels were continuously monitored during exercise testing, and never exceeded 8 ppm. During the exposure period, a mean or integrated value of the atmospheric CO concentration was reported to the nearest ppm. The longest interval from which averages were computed was 15 minutes.

All subjects were exposed to the various levels of CO in room-sized chambers equipped with CO monitors and with temperature and humidity controls. The temperature during exposure ranged from 65° to 70°F, and the relative humidity ranged from 45% to 65%. The air volume in these rooms was rapidly turned over for control of the CO levels; the exchange rate varied from 15 to 50 times per hour. During the exposures, the seated subjects could not see the chamber operator or gas monitors. The subjects entered the chambers only after the desired level of CO was achieved. Additional information on the chambers at each test center is presented in Appendix Q.

CARBOXYHEMOGLOBIN MEASUREMENTS

Gas Chromatography

Previous studies that investigated the influence of CO on the exercise tolerance of patients with coronary artery disease used spectrophotometric techniques for assessing blood levels of CO (Anderson et al. 1973; Aronow and Isbell 1973; Aronow 1981; Kleinman and Whittenberger 1985; Sheps et al. 1987). Since the physiological effects of CO are thought to be dependent upon the relative loss of the oxygen-carrying capacity of hemoglobin or on the partial pressure of CO in blood, %COHb is the conventional measure. Spectrophotometric methods are not sufficiently sensitive for assessing very low levels of COHb. The error of analysis for the IL 282 CO-Oximeter is reported to be 1.0% COHb (Instrumentation Laboratories 1980), which is greater than the expected resting COHb level in nonsmokers. Therefore, for this study, GC, a more accurate technique for the measurement of CO in blood, was used in addition to CO-oximetry to determine COHb levels (U.S. Environmental Protection Agency 1979). The advantage of the GC method used in this study is that it is precise (resolution better than 0.01 ml of CO/dl of blood) and linear throughout the entire range of potential values of CO (up to 100% COHb), without modifying the analytical technique (Dahms and Horvath 1974).

The CO content of each sample of venous blood was measured by GC by the Dahms and Horvath (1974) technique. By this technique, the CO contained in 200 μl of blood is released from the hemoglobin by denaturation of the hemoglobin, and is then extracted into the headspace of the sealed reaction vial with the use of a vortex. The headspace containing the CO is eluted onto the GC columns. The columns used for separating the CO from the other gases were Porapak Q and molecular sieve in series. The peaks of the gas distribution were measured by thermal conductivity sensors; peak height was used for quantification. The system was calibrated by adding known amounts of CO in place of the blood in a standard reaction vial. All values were corrected to standard temperature and pressure, dry (STPD) conditions. Further information on GC methods is given in Appendix F.

Gas-chromatographic analysis provided a value for the CO content of each sample. The CO content was converted to %COHb by the use of a calculated CO capacity derived from triplicate measurements of hemoglobin by the cyanmethemoglobin technique. The capacity was calculated by the following formula (Eilers 1967): *average hemoglobin (g/dl) × 1.389 ml/g*.

The value for the binding capacity was verified on a monthly basis. Samples of fresh whole blood were saturated with CO by equilibration with 99.5% CO for 30 minutes, followed by equilibration with 1,000 ppm CO balance nitrogen for 10 minutes. This last step reduced the amount of dissolved CO (unbound to hemoglobin) to negligible levels, while maintaining saturation of the hemoglobin with CO. The analysis of these saturated samples by GC provided a measurement of the CO capacity that could be compared to a capacity calculated on the hemoglobin values for these same samples.

Care was taken in the determination of hemoglobin using the cyanmethemoglobin technique in those specimens containing high levels of COHb: The full development of color required four to eight hours. This was determined using a Beckman model spectrophotometer, with measurements every 10 minutes for a 12-hour period. These specimens were pipetted, sealed, and stored in the dark overnight prior to analysis. The measured value averaged 98% of the expected value. Some of this difference could have been due to the presence of methemoglobin, which was not measured. The range of values varied between 93% and 102%. The agreement between the techniques provided the required validation of the expression of the data in terms of %COHb. Full details of this methodological comparison are presented in Appendix E.

Rather than establish this GC technique at three separate locations, samples requiring GC analysis were shipped to the Reference Laboratory in St. Louis for analysis. This was possible because COHb is very stable in a well-sealed syringe. Even though these samples would remain stable for weeks, they all were sent to the Reference Laboratory within three days of collection and analyzed within three days of receipt.

All samples were analyzed in triplicate, or until three results were attained that were within a range of 5% of the average value or 0.02 ml/dl of blood. The values used in all the data analyses from this study represent the mean of the three values for each sample. Hemoglobin determinations were made on each sample. If the samples yielded inconsistent results, they were hemolyzed with dry saponin and reanalyzed. Hemolysis with dry saponin was shown to have no effect on the CO content of blood samples, or on the hemoglobin content, as measured by the cyanmethemoglobin technique.

The GC results from these samples are unaffected by all the factors that may influence the CO-Oximeter values determined for the same samples. Therefore, the GC values were viewed as being a more accurate measurement of body burden of CO than the CO-Oximeter data for the low-COHb concentrations in this study. For most of the study, three blood

samples from each test visit (visits 2, 3, and 4) for each subject were analyzed by GC: the sample taken immediately after the first exercise test (sample 2), the sample collected at the end of the exposure period (sample 5), and the sample collected at the end of the second exercise test (sample 6). Before an amendment of August 29, 1985, the sample collected at the end of the exposure period was not analyzed by GC. The samples at the end of each exercise test were those representing the COHb levels at approximately the time the indicators of myocardial ischemia were measured.

CO-Oximetry

The optical method employed by CO-Oximeters provides a means of obtaining rapid measurements of COHb levels. The time required to analyze a blood sample with sufficient replicates to obtain reliable data is five minutes. The IL 282 CO-Oximeter (Instrumentation Laboratories, Lexington, MA) was used in this study because of the need for immediate information on blood COHb levels and the extensive use of this instrument in this area of research by previous investigators. Rapid measurements were needed to determine if subjects' base-line COHb levels were below the exclusion criterion and to determine the duration of the CO-exposure period. These were samples 1, 3, and 4 during the three test visits, as shown in Table 1. Other samples, which were measured by GC, were also measured by CO-Oximeter.

Four IL 282 CO-Oximeters were used in this study: one at each test center and one at the Reference Laboratory. The standard operating procedure was as described in the IL 282 manual (Instrumentation Laboratories 1980). The instrument operation was checked, at the beginning of each work day, with a control dye solution obtained from a commercial vendor. Every week, the dye solution was analyzed to check the calibration, and the instrument was adjusted if necessary.

Considerable effort was made to investigate the factors affecting CO-Oximeter measurements of %COHb, with the goal of improving the accuracy of measurements and the comparability of values among individuals. Of the many factors investigated (Appendix I), the O_2Hb content of the blood was found to have a major effect on the CO-Oximeter values for %COHb.

The relative operation of the instruments at the three centers was compared with the use of modified whole human blood standards that were prepared specifically for this purpose (see Appendices J and K). A method was developed by the Reference Laboratory for producing a set of fresh, whole-blood samples with known amounts of %COHb determined by GC.

Throughout the study, a set of four blood samples, containing 0.5% to 8% COHb, was provided on ice to the three test-center laboratories every two weeks. The first day of analysis at each laboratory was the day following shipment. The Reference Laboratory carried out daily analyses on the samples for three days, starting with the day of shipment (see Appendix G). This enabled same-day comparison of data collected on these standards, in case the standards showed a change in their optical characteristics that could result in a change in the value for %COHb. The Reference Laboratory included, in the shipment with the samples, the data from the day-1 analysis by both GC and CO-oximetry. This enabled the receiving laboratory to know if there was a problem with its instrument.

These blood standards were not intended to serve as a means of absolute calibration of the instruments, but to provide a means for comparing measurements by the instruments at each of the three test centers and for identifying problems with the instruments. No attempt was made to adjust the data obtained at any center. The results obtained on these standard sample sets were then transmitted to the Statistical and Data Management Center for monitoring and analysis. The results were also transmitted back to the Reference Laboratory for the purpose of trouble-shooting the standardization procedure.

EXERCISE TREADMILL TESTING

General Considerations

After meeting the inclusion/exclusion criteria described above, the subject was scheduled for the chamber exposure and exercise-test protocol. All subjects were screened by one of the cardiologist principal investigators prior to randomization. Once the subject was entered, the entry was considered final and the subject was not removed from the study at a subsequent date except for the specified criteria described below. In order to minimize the loss of data because of day-to-day variability of the angina and ECG ischemic ST responses, it was deemed prudent to use entry thresholds for the angina and ST endpoints that were above the actual thresholds enumerated in the next section.

At visit 1, both exercise tests were in the absence of CO exposure, as were the first exercise tests during visits 2, 3, and 4. This provided individual reproducibility studies for each subject. A pretest interim history and 12-lead ECG were done at each visit to verify that the angina and ischemic heart disease had been stable. If there was evidence of unstable angina, the subject was excluded from the study.

A venous cannula was inserted for the duration of each day's testing, and a pretest COHb level was determined to document the lack of exposure to significant levels of CO in the recent past. If the COHb level was above the cut-off level and the history established a probable and remediable cause for the elevated COHb level, the subject's visit was cancelled for the day and rescheduled. After the February 1986 protocol amendment, if the subject failed to develop angina or an ischemic ST change on the first test of any visit, the visit was rescheduled and the exposure and second test for that day were not

done. Furthermore, if the subject failed to meet either the angina or ST-change requirement, or the COHb inclusion criteria, on two successive visits, he was dropped from the study.

The Mason-Likar (1966) preexercise supine, sitting, and hyperventilation 12-lead ECG, along with heart-rate and blood-pressure measurements, were the pretest control studies. The three preexercise resting ECGs were acquired to determine if posture- or hyperventilation-induced ST-T-wave changes occurred. The control ECG recorded at each visit, along with an interim history, was used to assure the cardiologist that no new coronary event had transpired since the previous test. The preexercise standing 12-lead Mason-Likar ECG (including -AVR) was the base-line reference for all the ST-segment endpoint measurements of each day's tests.

Specific Definitions

ECG ST-Segment Response. There are three types of ST changes in the ECG that are interpreted as being indicative of myocardial ischemia, referred to collectively in the text as "ST endpoint":

Type 1 ST 80 ms and J-point elevation 1 mm or more, with ST horizontal or upsloping in leads not showing abnormal Q waves.

Type 2 ST 80 ms and J-point depression 1 mm or more, with ST horizontal or downsloping.

Type 3 ST 80 ms and J-point depression 1.5 mm or more, with ST upsloping.

Note that Type 1 is the mirror image, or "reciprocal," of Type 2.

Anginal Chest Pain. The subject was instructed by the tester to report any symptoms and to grade his chest pain on a scale of 1 to 4 as follows:

L-1 Onset of angina, mild, but recognized as the usual angina-of-effort pain or discomfort with which the subject is familiar.

L-2 Same pain; moderately severe, definitely uncomfortable, but still tolerable.

L-3 Severe anginal pain, at a level that the subject will wish to stop exercising.

L-4 Unbearable chest pain, the most severe pain that the subject has felt.

ST and Angina Endpoints. Time of onset of the earliest typical qualifying ST change, measured in seconds from the onset of exercise, was determined. The lead from the visit-1 preexposure tests in which this occurred was entered as the designated lead, along with the type of ST change (1, 2, or 3). The lead and type of ST change generally remained the same in any given subject for all exercise tests, on both qualifying and test visits. In the unusual instance in which the time to onset of a different type of ST response occurred earlier in any lead in a subsequent test, the lead and the type of response were recorded as the time to ST depression or elevation only if this ST response occurred two minutes or more before the usual ST response in the designated preexposure lead.

Type 1 changes were the endpoint in two exercise tests, Type 2 in 455, and Type 3 in 34. In 57 subjects, the same type of ST response was used in all exercise tests. In five subjects, both Type 2 and Type 3 changes were used; in one subject Type 1 and Type 2 changes were used. In 59 subjects, the same leads were used for the ST endpoint in all exercise tests. In four subjects, two leads were used; in two of these subjects, V5 and V6 were used; in one, V4 and V5 were used; and in one, V5 and II were used.

The duration of the ST change, in seconds, in recovery was also recorded for the designated lead. Similarly, time to the onset of angina, measured in seconds, from the beginning of exercise to the onset of the earliest recognized angina (Level 1) was determined, along with the duration of angina, in seconds, in recovery after the cessation of exercise.

Exercise Testing

The exercise treadmill test was performed on a standard motorized programmable treadmill, using a modified Naughton protocol (Raider 1973), as shown in Table 6. Subjects exercised for two minutes at each stage of the protocol; each stage was designed to increase the workload by an estimated 1 MET (basal metabolic equivalent). The maximum workload on the protocol was 11 METs at 18 to 20 minutes. The scale readings for treadmill speed and grade on the electronic programmer were verified on a regular basis by manual measurement of actual treadmill speed and grade. Before each test it was verified that all resuscitation equipment, oscilloscope monitors, blood pressure cuffs, and ECG recording equipment were turned on and working properly. The ECG recording and digitizing system was calibrated before each recording session.

The 12-lead Mason-Likar ECG (including -AVR) was recorded throughout the exercise with a commercially available system (CASE II, Marquette Electronics, Minneapolis, MN) at Johns Hopkins and St. Louis, or with an emulation of this system on a General Automation minicomputer system at Rancho Los Amigos. Selected leads were monitored continuously for rhythm changes by oscilloscope or hard-copy analog tracings, or both, throughout the testing procedure. The Mason-Likar 12-lead ECG was digitally sampled at 250 Hz or higher through buffered inputs and with sample and hold amplifiers that had a frequency response of from 0.05 Hz to at least 120 Hz, with a common mode rejection of 100,000 to 1 or better.

The signal processing consisted of a learning period when a typical cycle was selected (for all 12 leads). A template-matching algorithm was then applied to each of the new 12

Table 6. Modified Naughton Protocol for Exercise Treadmill ECG Test

Stage	2.0-mph Grade (%)	3.0-mph Grade (%)	Duration (minutes)	Estimated METs (units)	Elapsed Time (minutes)
1	0.0		2	2.0	2
2		0.0	2	3.0	4
3		2.5	2	4.0	6
4		5.0	2	5.0	8
5		7.5	2	6.0	10
6		10.0	2	7.0	12
7		12.5	2	8.0	14
8		15.0	2	9.0	16
9		17.5	2	10.0	18
10		20.0	2	11.0	20

ECG incoming signals, with time alignment around a fiducial multilead QRS detector trigger. A running updated complex was generated that included P-QRS-T. The QRS onset and offset (J point) were determined automatically by the program and were displayed for verification. The amplitude of the ST 80 ms after J in the updated beat was plotted every three seconds and was recorded as a hard-copy trend plot for each lead (Figure 8). In addition, the amplitude of the ST 80 ms was printed out in digital form each minute (Figure 9), along with heart rate and the processed 12-lead waveforms of the ECG. The time, to the nearest 10 seconds, from the start of exercise to the onset of ischemic ST, was determined from the trend plots. A template-matching cycle selection and running averaged beat were processed with a continuous measurement and hard-copy plot of ST at 80 ms after the J point. Hard copy of the unprocessed and processed ECG was made at a minimum of one-minute intervals. Both the unprocessed and processed ECGs were manually overread, in order to verify the appropriateness of the cycle selection and signal processing of the ECG, the computer-generated QRS offset (J point), and the ST-80 measurements and trend plots.

The criteria for stopping the symptom-limited exercise tests were: (1) fatigue, shortness of breath, leg pain, or subject's request to stop; (2) Level 2 angina approaching Level 3 and subject's wishing to stop; (3) 3-mm ST-segment shift; (4) hypertensive blood-pressure response (240 systolic or 130 diastolic); (5) drop in blood pressure equal to or greater than 20 mm below peak, confirmed by a second reading within 20 seconds (an elevated resting blood pressure, usually with anxiety, that stabilized without symptoms after an initial drop in the first one to two minutes of exercise was disregarded); (6) significant dysrhythmia, that is, supraventricular tachycardia lasting more than 10 seconds, multifocal preventricular contractions (more than six per minute) if associated with increasing angina or significant ST change, three or more preventricular contractions in a row, or second- or third-degree AV block; (7) symptoms of possible central nervous system dysfunction, such as lightheadedness, dizziness, ataxia, nausea, and pallor; and (8) equipment failure or poor ECG recording that prevented accurate interpretation of rate, rhythm, or ST change.

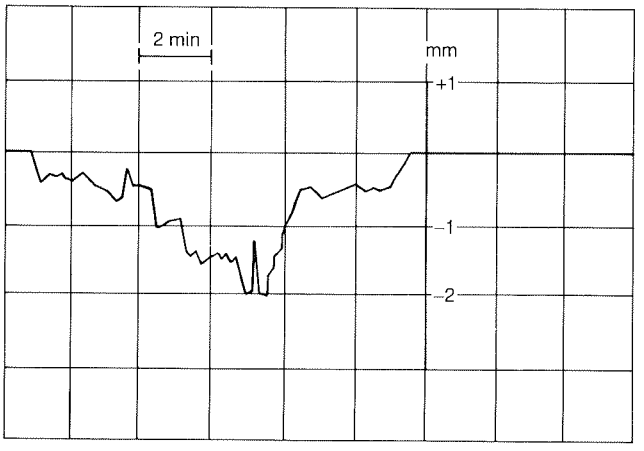

Figure 8. Trend plot of ST-80 measurements for the lead showing the worst ST-segment changes. The time to onset of ST endpoint was measured from this type of plot. Note that maximal ST displacement is 2 mm.

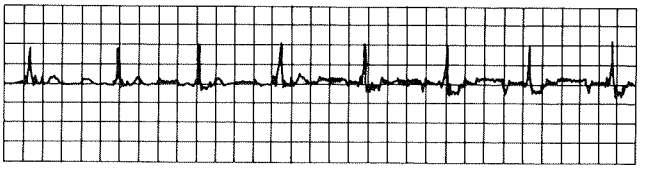

Figure 9. For the lead showing the worst ST-segment depression, this is the P-QRS-T-wave complex at base line (first complex) and each minute thereafter. See Figure 1 also.

Table 7. Number of Times Exercise Was Terminated for Various Reasons in a Total of 376 Exercise Tests

Reason for Termination	Number of Times
Fatigue	79
Angina criterion	306
Falling blood pressure	5
ST > 3 mm	18
Hypertensive blood pressure	6
Subject request	172
Other	42

The reasons for termination in 376 exercise tests are presented in Table 7. Frequently, there was more than one reason for ending the test. Neither central nervous system dysfunction nor arrhythmia was ever a cause for termination.

Blinded Interpretation and Consensus Review of Electrocardiograms

The cardiologist investigator at each center who reviewed the unprocessed and computer-processed ECG records was blinded to name, date, and exposure history for test visits. The tracings were analyzed in a batch; all qualifying tests and tests of visits 2 through 4 were reviewed at one time. Visit 1, in which the reproducibility requirement was addressed, was read in an unblinded fashion. All of the exercise ECGs (raw tracings and computer-processed records) for each subject were reviewed by the principal cardiology investigators from at least two centers. These reviews were done at several consensus meetings that occurred during the course of the study. The following endpoints were reviewed at these working sessions:

1. Time of onset of an ischemic ST response, as determined by review of the computer-generated ST trend plots and processed ECGs, and confirmed by comparison with the raw data.
2. Duration of the ischemic ST change in the recovery phase.
3. Total number of leads exhibiting an ischemic ST response.
4. Coded maximum ST change and slope, during exercise and recovery, in 12 leads (including -AVR).

Since the computerized digital trend plot of ST 80 was used to define the time to ischemic ST, the cardiologists' main role in ECG overreading was to evaluate the trend plot for stability, to evaluate the ST-80 slope, and to define the type of ischemic ST. The raw data were compared to the digitized computer-processed data at each minute of exercise to confirm that the signal-processed data conformed to the original. The main concern was to verify that no major artifact had been included in the processed data. It was determined that the noise reduction was such that a more reliable estimate of ST-80 amplitude and slope could be made from the computer-processed waveforms. Once confirmed by review of the "raw" data, the processed waveforms were used thoughout the study to define the type of ischemic ST, to confirm the time to onset of ischemic ST to the nearest minute, and to code the maximal ST change and slope in each of the 12 leads.

The trend plots of ST-80 amplitude were then overread to refine the time to ischemic ST to the nearest 10 seconds. This time was agreed on by consensus in each case. The coded amplitude of the maximal ST 80 and the type of ischemic ST in each of the 12 ECG leads (including -AVR) were also reviewed at these meetings. The slope of the ST 80 was overread as follows: Using a 5× magnifying lens with an etched 1-mm scale at the focal plane of the magnifier, the base line of the 1-mm scale was aligned with the ST segment just prior to and at ST 80. The slope of ST 80 was defined as flat if the slope of this portion of the ST was equal to ± 0.2. It was called upsloping when the slope of this portion of the ST was greater than 0.2, and downsloping if less than -0.2. Consensus was reached in each case.

DOUBLE-BLIND CONDITIONS

The double-blind conditions of this study were maintained in a rigorous fashion. All personnel involved with the study were instructed to maintain these conditions, and were monitored by the investigators to assure compliance with this requirement. At each of the three centers, the exercise-cardiology personnel were kept blinded to the exposure conditions, but the exposure personnel were not. Communication regarding the exposure conditions between the two groups was restricted to issues of timing of collection of blood samples and duration of exposure. At no time were the chamber conditions discussed with the subject or with the exercise-cardiology personnel. At two centers (Johns Hopkins and St. Louis), exposure personnel sometimes withdrew blood samples from subjects, but did not communicate with them. On the qualifying day, visit 1, the subjects were exposed to 150 ppm CO in an unblinded fashion for one hour. This experience appeared to reduce the anxiety in the subjects; they rarely showed any curiosity regarding the operation of the chamber. Many of the subjects rested or slept during a portion of the exposure period.

The exposure personnel were instructed and monitored to assure consistent behavior patterns on all randomized days, to prevent their giving any subtle indication to the subjects or other personnel about the exposure conditions. The length of exposure on the air day was randomized in advance, but the end of the exposure was not announced until sufficient time had elapsed for analysis of the 40-minute blood sample and calculation of the exposure time. The chamber monitoring, blood-sample analysis, and exposure calculation were performed out of sight and hearing of the exercise-cardiology personnel. The exercise-cardiology team actively avoided information that could have voided the double-blind condi-

tions. The exercise-cardiology groups remained blinded through the data-analysis phase of the study. At the ECG consensus meetings, all the record information was covered. Moreover, there was no indication on the ECG records as to the exposure conditions; only subject name, date, and test time were indicated.

Maintenance of blinding was one of the critical observations monitored during quality assurance visits. There were no known breaches of the double-blind conditions at any of the three centers.

DATA COLLECTION AND MANAGEMENT

Staff from the Statistical and Data Management Center worked closely with the study investigators to design the data-collection forms. The forms were intended to serve as a complete record of each subject's eligibility for the study, medical history, laboratory history, CO exposures, exercise treadmill tests, and progress through the study from randomization to completion of the protocol. There was a standard header on every form that uniquely identified the form, the clinical center, the subject, and the date of the test visit. Forms were also provided for the collection of reference-standards data.

The data collection forms were self-coding, but detailed instructions were provided for completing every data item on each form. Included were descriptions and codes for ordinal and categorical items, formulae for calculated variables, instructions for rounding data values, requirements for units of measurement, and instructions for dealing with missing data and inappropriate questions.

Subjects who met the study inclusion criteria were randomized to the order of exposures after the qualifying visit. Randomization codes were issued by the staff of the Statistical and Data Management Center to the data coordinators at the clinical centers. Codes were the letters A through R; the exposure ordering represented by the letter codes was concealed from the clinical center staff involved in the exercise treadmill testing and from the cardiologists reading the ECGs.

Data forms were mailed to the Statistical and Data Management Center by the data coordinators at the clinical centers approximately weekly. Each subject's forms, as well as reference forms, were accompanied by a log form that enumerated the enclosed forms; completeness of form shipments was confirmed at the Statistical and Data Management Center.

Data were entered into a VAX 11/780 computer (Digital Equipment Corporation) running the UNIX operating system (Bell Laboratories and University of California, Berkeley) at Harvard Health Sciences Computing Facility, Boston, MA. The data-entry program performed range-checking on individual items and some cross-validation as data were entered. The data files that resulted from data entry corresponded to the data-collection forms and were processed (entered, verified, and queried) in weekly batches corresponding with the data submission schedule.

Within a very few days of receipt, the weekly data batches were entered into the computer. To ensure absolute accuracy in this data base, data listings were produced and visually checked against the data forms. Entry errors were then corrected in the data base. Logical errors and errors that showed up in cross-validation were queried at the clinical centers using a special query form, and these errors, too, were corrected in the data base. Data that passed the checking routine and data that passed after the query routine were advanced to a final data base for data analysis.

Once a month, the Statistical and Data Management Center prepared reports for the clinical centers that detailed the number of forms received, the status of the forms, and the number of missing forms.

The data base was maintained on-line during the entry and verification phase, and daily magnetic-tape backup copies of all disk files were made to insure against loss of data due to computer system failure or programmer error. To provide long-term backup of the study data base, additional magnetic-tape copies of the entire data base were made.

Analysis files were constructed from the data base and were maintained on-line on the VAX 11/780 computer, the IBM4341 (International Business Machines), or both, during data-analysis activity. Magnetic-tape copies of all analysis files were made. Data were analyzed periodically throughout the study, but the results were not revealed to the clinical investigators until testing was completed.

STATISTICAL ANALYSIS

The goal of the primary statistical analysis was to determine whether or not there is an effect on the subjects in the study when they are exposed to CO as compared to air, as measured by the time to the onset of ST endpoint (Type 1, 2, or 3 ischemic ST-segment changes in the ECG) and the time to angina. The measurements were obtained on three separate days. On each day, there was a preexposure exercise test to obtain base-line measurements and a second exercise test after exposure in the chamber. Depending on the day, subjects were exposed to air without elevated CO levels, or to CO levels designed to achieve approximately 2% COHb or approximately 4% COHb at the end of exercise. The measurements of both time to onset of angina and time to ST endpoint were compared to their respective base-line data to obtain the increase or decrease due to the exposure. Finally, the air exposure (no CO added) was used as a base-line measurement to get the four quantities for each subject: decrease in percent at 2% COHb and 4% COHb for the time to onset of angina and the time to ST endpoint (as specified in the protocol). A percent increase was recorded as a negative percent decrease.

The 2%- and 4%-exposure analyses are reported separately. Furthermore, separate analyses are also given for the three centers. To guard against outliers, trimmed means were used as summary statistics, with the two largest and two smallest

observations trimmed (Mosteller and Tukey 1977), as specified in the original protocol.

All significance calculations in the primary analysis used a one-sided 5% level, looking only for a significant decrease in time, based on the permutation distribution of the trimmed means (Lehmann 1959). The one-sided confidence interval was specified in the protocol because there was no reason to believe that CO would have a beneficial effect on the occurrence of ischemia. Since we are concerned with determining whether or not there is a significant decrease from zero, we considered all possible ways of assigning a plus or minus sign to every observation and calculated the resulting trimmed means. With m observations, there are thus 2^m points in the sample space, each point representing an onset of angina trimmed mean and an ST-segment-change trimmed mean. The significance of the observed statistic was then evaluated by considering all 2^m points as equally likely.

The above analysis strategy was planned prior to any data collection and is robust to outliers and model selection, yet it is efficient. Results of alternative analyses are presented in Appendix C.

QUALITY ASSURANCE PROCEDURES

Quality assurance procedures applied to this study were defined in the QA Plan developed and implemented during the initial stages of the study. The overall goal of the QA Plan was to assure that the study was carried out in a manner that produced data of high and well-documented quality, and that the conduct of the study was consistent among the centers and the resultant data were of equal reliability. An independent QA Officer was responsible for overseeing the implementation of the QA Plan and for monitoring compliance with the QA Plan as the study progressed.

As required by the QA Plan, the procedures used to conduct the study were defined in the Experimental Protocol, the standard operating procedures, and the Data Management Procedures Manual developed for and applied to the study. Each of these documents was developed by the investigators and HEI and was reviewed and approved by HEI, the investigators, and the QA Officer. The protocol defined the purpose and background of the study, the expected results, the significance of the expected results, and the overall organization of the study team. It provided an outline of the methodologies to be used and a description of the data to be collected. Standard operating procedures documented all routine and critical experimental procedures and techniques. They provided specific information on calibration and maintenance of instruments, quality control, sample handling procedures, and the specific methodologies used. The Data Management Procedures Manual included standardized forms for the collection and reporting of data. It provided specific direction on the use of the data collection forms, the flow of data between the investigators and the Statistical and Data Management Center, the entry of data into the computer, including verification and storage procedures, and the primary statistical methods to be used to analyze the data. Standard operating procedures, data management procedures, and data collection forms were prepared in document format, with each having a unique title and identification number.

Changes to the Experimental Protocol were documented as protocol amendments in order to assure that all study team members had the information needed to implement the changes, and that the changes were uniformly applied at each study center. The amendments detailed changes to be made, the reasons for making the changes, and the effective dates of the changes. Each amendment was approved by the principal investigators, the QA Officer, and the HEI project manager. The standard operating and data management procedures were revised, as needed, to correspond with the amended protocol. Inadvertent, or one-time-only, deviations from standard operating procedures and the protocol were documented in the study files at the test centers. An historical file of protocol amendments, standard operating procedures, data management procedures, and data collection forms was maintained to ensure that there was a complete record of all the procedures used.

Facilities inspections were conducted by the QA team at each study center to determine whether the physical facilities were adequate for effective completion of the study. Prestudy on-site inspections at each center were used to document formally the qualifications and experience of the personnel, their familiarity with the protocol and methods, and the adequacy of the testing facility. Laboratory inspections, conducted at approximately six-month intervals while the study was in progress, ensured that the quality and integrity of the data met the requirements defined by the QA Plan. These inspections included monitoring of the actual conduct of the study, data audits, review of data collection and management procedures, and discussion and review of the QA Plan with the investigators. The data audit compared the raw data and the reported results to verify the validity of the final report. A primary concern of these audits was the determination that clear data trails exist that demonstrate that the study was conducted as defined in the protocol, standard operating procedures, and Data Management Procedures Manual. A confidential inspection report was prepared for HEI's Executive Director at the conclusion of each audit. HEI's Executive Director gave each report to the project manager for the study; she transmitted it to the appropriate principal investigator. The report detailed any significant findings and requirements for corrective action.

Important deviations from the protocol are included in this report. A QA report for the study is included as Appendix R.

Table 8. Carboxyhemoglobin Levels in Venous Blood Determined by Gas Chromatography and by CO-Oximetry[a]

Method	Air Day			2%-COHb-Target Day			4%-COHb-Target Day		
	n	%COHb	SEM[b]	n	%COHb	SEM	n	%COHb	SEM
After Exercise Test 1 (Sample 2)									
GC	62	0.64	0.02	62	0.62	0.02	63	0.64	0.02
CO-Oximeter	63	1.24	0.05	62	1.24	0.05	63	1.23	0.04
After Exposure (Sample 5)									
GC target		—			2.2			4.4	
GC	59	0.70	0.02	56	2.38	0.05	59	4.66	0.09
CO-Oximeter	63	1.38	0.04	62	3.21	0.04	63	5.58	0.06
After Exercise Test 2 (Sample 6)[c]									
GC target		—			2.0			4.0	
GC	63	0.62	0.02	62	2.00	0.05	63	3.87	0.08
CO-Oximeter	63	1.16	0.05	62	2.65	0.04	63	4.69	0.05

[a] Measurements were made on samples taken at the end of exercise test 1, at the end of the exposure period, and at the end of exercise test 2.

[b] SEM = Standard error of the mean.

[c] Median values for %COHb in sample 6 are as follows. GC: air = 0.6, 2%-COHb target = 2.0, 4%-COHb target = 3.9; CO-Oximeter: air = 1.1, 2%-COHb target = 2.6, 4%-COHb target = 4.8.

RESULTS

SUBJECTS

Sixty-three male subjects, with a mean age of 62 years, who had stable angina pectoris and a positive exercise test with ST-segment changes on their ECGs suggestive of myocardial ischemia, completed all tests and met the protocol criteria (Tables 3 and 4). Each subject had at least one of three other specific indicators of coronary artery disease; 40 had angiographic evidence of at least a 70% narrowing in at least one major coronary artery, 26 had a documented prior myocardial infarction, and 27 had positive thallium stress tests. Additional information on these subjects and on the inclusion and exclusion criteria is provided in the Methods section (Tables 3 and 4).

CARBON MONOXIDE EXPOSURE

Exposure to CO in these subjects was varied individually to result in predetermined levels of COHb. Table 8 provides information on the mean COHb levels determined by GC and CO-oximetry (IL 282). As discussed in the Methods section, there is a 0.5% to 1.2% offset between GC and CO-Oximeter measurements. While the COHb levels measured by GC are considered more accurate, the CO-oximetry COHb values are presented for comparison with other studies. Table 8 shows that the combined mean COHb levels by GC at the end of the second exercise test were 2.00% ± 0.05%[2] and 3.87% ± 0.08% for the 2%- and 4%-COHb-target days, respectively. These values are close to the target levels of 2.0% and 4.0% at the end of exercise test 2, defined by the study protocol. The actual levels at the end of the CO exposure period, 2.38% and 4.66%, are slightly higher than the target levels of 2.2% and 4.4%. At all centers, the mean preexposure COHb levels were 0.6% to 0.7%. Table 9 shows that the mean COHb levels after CO exposures were higher at Johns Hopkins and Rancho Los Amigos than at St. Louis.

The desired CO exposure concentrations were calculated on the basis of CO-uptake rate constants determined during visit 1 for each subject. The mean values and ranges for the CO chamber concentrations are shown in Table 10. The individual CO exposure concentrations on the 2%-COHb-target day ranged from 42 to 202 ppm (mean of 117 ppm), and on the 4%-COHb-target day from 143 to 357 ppm (mean of 253 ppm). The mean chamber concentrations on the 2%-COHb-target day were 102 ppm at St. Louis, 116 ppm at Rancho Los Amigos, and 134 ppm at Johns Hopkins. On the 4%-COHb-target day, they were 237 ppm at St. Louis, 256 ppm at Rancho Los Amigos, and 267 ppm at Johns Hopkins. The COHb values at the end of exercise are lower than those at the end of exposure primarily due to respiratory loss of CO during the interval between the two measurements (see Appendix P).

[2] Throughout the Results section, means ± standard errors are presented.

Table 9a. Individual Center Results for Carboxyhemoglobin Levels in Venous Blood Samples Determined by Gas Chromatography[a]

	Air Day			2%-COHb-Target Day			4%-COHb-Target Day		
Center	n	%COHb	SEM[b]	n	%COHb	SEM	n	%COHb	SEM
After Exercise Test 1 (Sample 2)									
Johns Hopkins	22	0.60	0.04	22	0.60	0.03	22	0.65	0.04
Rancho Los Amigos	17	0.77	0.03	18	0.69	0.05	18	0.72	0.05
St. Louis	23	0.58	0.04	22	0.57	0.04	23	0.57	0.04
After Exposure (Sample 5)									
Target		—			2.2			4.4	
Johns Hopkins	21	0.66	0.05	20	2.56	0.08	21	4.91	0.13
Rancho Los Amigos	15	0.78	0.04	14	2.59	0.06	15	4.99	0.10
St. Louis	23	0.68	0.04	22	2.09	0.08	23	4.22	0.17
After Exercise Test 2 (Sample 6)[c]									
Target		—			2.0			4.0	
Johns Hopkins	22	0.58	0.05	22	2.25	0.06	22	4.00	0.12
Rancho Los Amigos	18	0.71	0.03	18	2.05	0.10	18	4.06	0.14
St. Louis	23	0.58	0.04	22	1.70	0.07	23	3.59	0.13

[a] Measurements were made in samples taken at the end of exercise test 1, at the end of the exposure period, and at the end of exercise test 2.

[b] SEM = Standard error of the mean.

[c] Median values for %COHb in sample 6 are as follows. Johns Hopkins: air = 0.60, 2%-COHb target = 2.30, 4%-COHb target = 3.85; Rancho Los Amigos: air = 0.70, 2%-COHb target = 2.00, 4%-COHb target = 4.20; St. Louis: air = 0.60, 2%-COHb target = 1.70, 4%-COHb target = 3.60.

Table 9b. Individual Center Results for Carboxyhemoglobin Levels in Venous Blood Samples Determined by CO-Oximeter[a]

	Air Day			2%-COHb-Target Day			4%-COHb-Target Day		
Center	n	%COHb	SEM[b]	n	%COHb	SEM	n	%COHb	SEM
After Exercise Test 1 (Sample 2)									
Johns Hopkins	22	1.13	0.07	22	1.18	0.07	22	1.12	0.05
Rancho Los Amigos	18	1.25	0.09	18	1.18	0.09	18	1.22	0.08
St. Louis	23	1.35	0.09	22	1.35	0.08	23	1.35	0.08
After Exposure (Sample 5)									
Johns Hopkins	22	1.25	0.06	22	3.33	0.06	22	5.76	0.08
Rancho Los Amigos	18	1.34	0.07	18	3.13	0.07	18	5.56	0.13
St. Louis	23	1.54	0.08	22	3.15	0.06	23	5.42	0.09
After Exercise Test 2 (Sample 6)									
Johns Hopkins	22	1.07	0.07	22	2.78	0.07	22	4.79	0.07
Rancho Los Amigos	18	1.13	0.08	18	2.58	0.07	18	4.76	0.10
St. Louis	23	1.26	0.09	22	2.56	0.05	23	4.55	0.10

[a] Measurements were made on samples taken at the end of exercise test 1, at the end of the exposure period, and at the end of exercise test 2.

[b] SEM = Standard error of the mean.

Table 10. Chamber Carbon Monoxide Concentrations[a]

Center	Air				2%-COHb Target				4%-COHb Target			
	n	Mean	SEM	Range	n	Mean	SEM	Range	n	Mean	SEM	Range
Combined	62	0.7	0.1	0-2	62	117.4	4.4	42-202	62	252.9	6.1	143-357
Johns Hopkins	22	0.1	0.1	0-2	22	133.9	6.7	84-202	22	267.1	9.6	177-357
Rancho Los Amigos	18	1.3	0.1	1-2	18	115.7	8.3	42-170	18	255.8	12.7	143-315
St. Louis	22	0.8	0.1	0-2	22	102.3	6.5	56-174	22	237.0	9.3	150-315

[a] Concentrations are given in ppm.

ELECTROCARDIOGRAPHIC ST-ENDPOINT ANALYSES

The results of the ST-endpoint analyses are summarized in Table 11a. For each subject, the percentage change between the pre- and postexposure exercise tests was calculated for each exposure day. Thus, each individual served as his own control on each day. The difference between the 2%-COHb-target- and air-exposure days was used for analysis of the effect of 2% COHb, and the difference between the 4%-COHb-target- and air-exposure days was used for the analysis of the effect of 4% COHb. A *positive* percentage indicates a *decrease* in time to ST endpoint due to CO exposure. It should be noted that the air exposure does not translate to zero COHb level, but rather gives the level of base-line (endogenous plus ambient) COHb, which averages 0.6% in this sample.

As presented in Table 11a, CO exposures that produced a mean level of 2% COHb resulted in a statistically significant decrease in the time to ST endpoint ($p = 0.01$). Of the 61 subjects in the 2% analysis, 42 experienced a relative decrease and 19 experienced a relative increase on the 2%-COHb-target day, compared to the air day. On the air day, there was an average increase of 5.2% in time to ST endpoint on the postexposure exercise test relative to the preexposure test. In contrast, after the 2%-COHb-target exposure, the time to ST endpoint decreased an average of 0.3%. Thus, the 2%-COHb-target exposure resulted in a net 5.1% (trimmed mean, mean = 5.1%) decrease (Table 11a and Table C.1 in Appendix C).[3]

At the higher CO exposure level, a statistically significant decrease in time to ST endpoint was also found ($p \leq 0.0001$). Of the 62 subjects in the 4% analyses, 49 experienced a relative decrease and 13 experienced a relative increase. Exposure to CO to attain a mean level of 4% COHb resulted in a 7.6% decrease in mean time to ST endpoint relative to the preexposure test. Thus, compared to the 5.2% postexposure increase in mean time to ST endpoint after the control air exposure, the 4%-COHb-target exposure resulted in a net 12.1% (trimmed mean, mean = 12.9%) decrease in time to ST endpoint (Table 11a, Table C.1).

[3] Note that because trimming is applied after all differences are calculated for each subject, the postexposure minus preexposure percentages reported for air- and 2%-COHb-target-exposure days do not sum to the mean decrease reported in Table 11a.

To assess a possible dose-response relationship, individual regressions were fitted to the three differences in time to ST endpoint, pre- versus postexposure, against the three actual COHb readings. When this was done, the average of the intercepts was 8.01% ± 2.48%, and the average of the slopes was −3.85% ± 0.63%/%COHb. This relationship is illustrated in Figure 10. Thus, in this range of COHb levels, there appears to be, roughly, a 3.9% decrease in time to ST endpoint for every increase of 1% in COHb. Figure 11 illustrates this dose-response relationship.

Six of the 63 subjects did not reach the ST endpoint in one or more of the 376 exercise tests (Table 12). One subject, 201, reached the ST endpoint in only one of the six exercise tests. Since the analyses are based on the differences between pre- and postexposure times, no information on ST endpoint is available on this subject, and thus he is not included in the ST analyses. One subject, 110, did not reach the ST endpoint on the postexposure test on the air day, and four subjects, 101, 105, 214, and 215, did not reach the ST endpoint on the preexposure exercise test on one of the days they were exposed to CO. In each of these instances, because we know that these subjects did not reach the ST endpoint by the end of exercise, the total duration of exercise was used instead of the time to ST endpoint in analyses. The effect of these substitutions is minimal. If they produce any effect, their influence on study results and the statistical analyses will be to underestimate any changes due to CO.

The mean heart rate-systolic blood pressure double product (beats per minute × mm Hg) at the time of ST endpoint is presented in Table 11b. On the air-exposure day, there was a mean 1.2% increase in the double product at the onset of ST endpoint on the postexposure test. On the 2%-COHb-target day, there was no significant change in the double product at the ST endpoint. However, on the 4%-COHb-target day, there was a 3.4% decrease in the double product on the postexposure exercise test, which represents a net 4.4% decrease ($p = 0.03$) compared to the air-exposure day. Thus, on the 4%-COHb-target day, the threshold ST change occurred both earlier in the exercise test and at a lower heart rate-blood pressure double product.

Table 11a. Effect of Carbon Monoxide on Time to ST Endpoint (Combined Data)

Exposure Day	Sample Size	COHb Levels at End of Exercise Pre- and Postexposure			Time to ST Endpoint Pre- and Postexposure[a] (seconds)			Change in Time to ST Endpoint Post- vs. Preexposure (seconds)		% Decrease Between Air and CO Days			
			Mean %COHb[b]	SEM		Trimmed Mean	SEM	Trimmed Mean	SEM	Trimmed Mean %[c]	p-Value[d]	90% Confidence Interval	95% Confidence Interval
Air	62	Pre	0.64	0.02	Pre	560.0	26.6	16.0	11.6				
		Post	0.62	0.02	Post	575.9	26.6						
2%-COHb target	61	Pre	0.62	0.02	Pre	574.1	26.8	−16.3	13.0	5.1	0.01	1.46, 8.74	0.77, 9.43
		Post	2.00	0.05	Post	557.8	25.4						
4%-COHb target	62	Pre	0.64	0.02	Pre	562.9	27.6	−52.7	12.7	12.1	≤0.0001	9.0, 15.3	8.4, 15.9
		Post	3.87	0.08	Post	510.1	25.9						

[a] Median time to ST endpoint: air, pre = 540, post = 570; 2%-COHb target, pre = 560, post = 524; 4%-COHb target, pre = 540, post = 500.

[b] CO measured by GC.

[c] Median percent decrease: air vs. 2%-COHb target = 6.5; air vs. 4%-COHb target = 13.2. For analysis of nontrimmed means, see Appendix C.

[d] One-sided p-values, as described in the Methods section.

Table 11b. Effect of Carbon Monoxide on Heart Rate-Systolic Blood Pressure Double Product at the Onset of ST Endpoint (Combined Data)

Exposure Day	Sample Size	Double Product[a] at ST Endpoint in Pre- and Postexposure Exercise Tests			Difference Between Pre- and Postexposure Exercise Tests		% Decrease Between Air and CO Days		
			Mean	SEM	Mean	SEM	Mean %[b]	SEM	p-Value[c]
Air	61	Pre	16,134	563	214	320			
	61	Post	16,375	649					
2%-COHb target	60	Pre	16,558	620	−151	289	1.0	2.2	0.65
	61	Post	16,300	597					
4%-COHb target	58	Pre	15,986	575	−632	256	4.4	2.0	0.03
	62	Post	15,361	528					

[a] Beats per minute × mm Hg.

[b] Nontrimmed means.

[c] Two-sided p-values.

Table 12. Time to Respective Endpoints for Subjects Who Had Missing Data

Subject	Exposure	Time (seconds)					
		Exercise Test 1 (Preexposure)			Exercise Test 2 (Postexposure)		
		ST[a]	Angina[b]	Max[c]	ST	Angina	Max
101	4%-COHb target	—	1,020	1,039	960	885	1,020
105	4%-COHb target	—	330	603	480	225	537
110	Air	900	902	990	—	665	1,028
201	Air	—	605	630	—	727	755
	2%-COHb target	—	590	655	—	635	673
	4%-COHb target	540	365	660	—	380	600
214	2%-COHb target	—	452	697	810	499	986
215	4%-COHb target	—	1,073	1,113	930	1,005	960

[a] ST = time to the onset of ST endpoint.

[b] Angina = time to onset of Level 1 angina.

[c] Max = total duration of exercise.

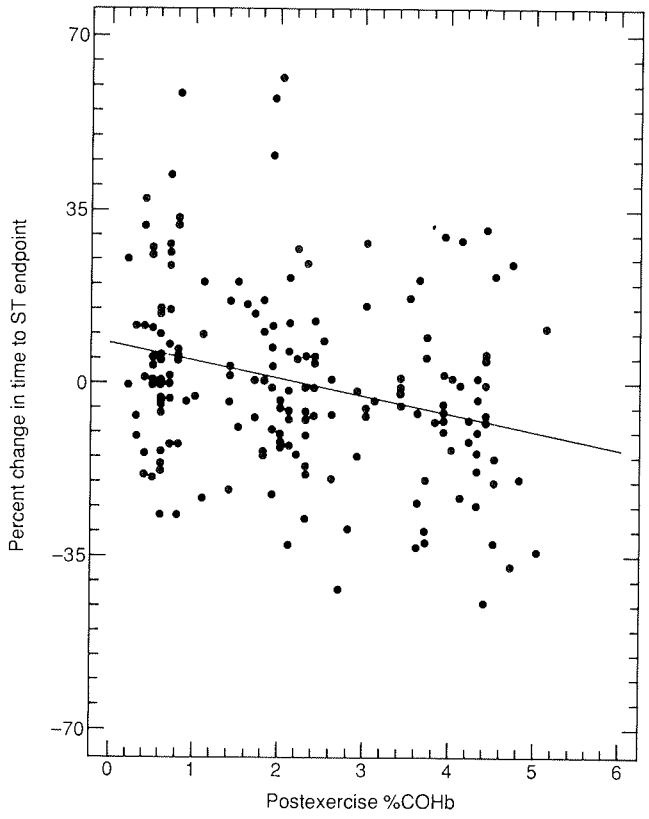

Figure 10. Regression of the percent change in time to ST endpoint between the pre- and postexposure exercise tests [(postexposure – preexposure)/preexposure] and the measured blood COHb levels at the end of exercise for the 63 subjects combined. The line represents the average of individual regressions.

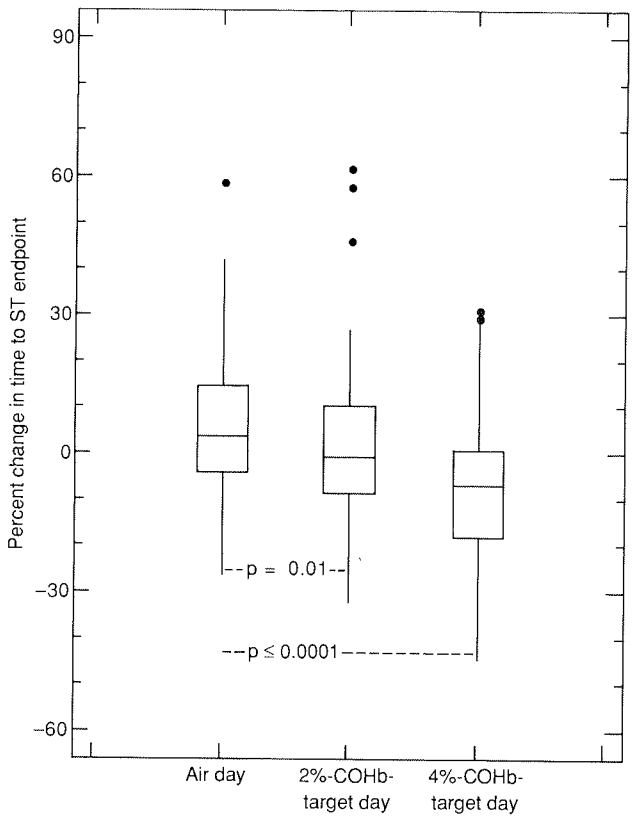

Figure 11. Box-and-whisker plots of percent change in time to ST endpoint between the pre- and postexposure exercise tests [(postexposure – preexposure)/preexposure] on the air day, 2%-COHb-target day, and 4%-COHb-target day. The bar across the box is the median, the ends of the box are the quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals outside that range.

Table 13a presents data on the maximum ST amplitude during exercise in the selected ECG lead, and Table 13b presents data on the ST severity score, which is the sum of the maximum changes in all leads with ST-segment changes equal to or greater than 0.5 mm. There was an 11% increase in the maximum amplitude of ST changes on the 2%-COHb-target-exposure day, compared to the air day ($p = 0.002$). On the 4%-COHb-target-exposure day, there was a 17% increase in the maximum ST amplitude, compared to the air day ($p \leq 0.0001$). The summed ST score showed a 21% increase on the 2%-COHb-target-exposure day ($p = 0.004$) and a 23% increase on the 4%-COHb-target-exposure day, compared to the air day ($p = 0.001$). The duration of ST-segment changes was measured (Table 13c) but no changes in duration were found.

ANGINA ANALYSES

Figure 12 illustrates the significant correlation between change in time to ST endpoint and change in time to angina (Spearman rank correlation coefficient = 0.49, $p \leq 0.0001$; Pearson correlation coefficient = 0.49, $p \leq 0.0001$). The results for time to onset of angina are presented in Table 14a and in Figure 13. There was a statistically significant decrease of 4.2% in time to onset of angina on the 2%-COHb-target day, compared to the air day ($p = 0.03$). Of the 62 subjects, 23 experienced a relative increase in time to onset of angina, and 38 experienced a relative decrease (one subject had no change). On the 4%-COHb-target day, there was also a statistically significant decrease in time to angina, with a mean decrease of 7.1% compared to the air day ($p = 0.002$). Of the 63 subjects, 45 experienced a relative decrease in time to onset of angina and 18 experienced a relative increase. There were no significant differences, however, between the air, 2%-COHb-target, and 4%-COHb-target days with respect to heart rate-blood pressure product at the onset of angina (Table 14b).

There was only one subject (306) with missing angina data; he did not experience angina on the preexposure exercise test on the 2%-COHb-target day. The total duration of time on the treadmill was again used as the data point, as was done with the missing ST data described above. The effect of estimating this value, as in the ST analyses, is minimal, and, if anything, makes the above p-values conservative.

We also examined, as we did for the ST endpoint, the dose-response relationship between angina and COHb. The individual regressions resulted in an average intercept of –1.00%

Table 13a. Effect of Carbon Monoxide on Maximum ST Amplitude

Exposure Day	Sample Size		Maximum ST on Pre- and Postexposure Exercise Tests (mm)		Differences Between Pre- and Postexposure Exercise Tests (mm)		% Increase Between Air and CO Days		
			Mean	SEM	Mean	SEM	Mean %	SEM	p-Value[a]
Air	62	Pre	1.86	0.09					
	62	Post	1.66	0.09	− 0.20	0.05			
2%-COHb target	61	Pre	1.85	0.11					
	61	Post	1.82	0.09	− 0.02	0.05	11.4	3.5	0.002
4%-COHb target	61	Pre	1.85	0.10					
	62	Post	1.88	0.09	+ 0.03	0.05	17.2	4.1	≤ 0.0001

[a] Two-sided p-values.

Table 13b. Effect of Carbon Monoxide on Summed ST Score

Exposure Day	Sample Size		ST Score[a] in Pre- and Postexposure Exercise Tests		Difference Between Pre- and Postexposure Exercise Tests		% Increase Between Air and CO Days		
			Mean	SEM	Mean	SEM	Mean %	SEM	p-Value[b]
Air	63	Pre	7.4	0.4	−1.3	0.3			
	62	Post	6.2	0.4					
2%-COHb target	61	Pre	7.3	0.5					
	61	Post	7.0	0.4	−0.2	0.2	20.8	6.9	0.004
4%-COHb target	62	Pre	7.2	0.5					
	63	Post	7.0	0.4	−0.2	0.2	23.3	6.9	0.001

[a] ST score is the sum of the maximum changes in all leads with ST-segment changes ≥ 0.5 mm.

[b] Two-sided p-values.

Table 13c. Effect of Carbon Monoxide on Duration of ST-Segment Changes

Exposure Day	Sample Size		ST Duration in Pre- and Postexposure Exercise Tests (seconds)		Difference Between Pre- and Postexposure Exercise Tests (seconds)		% Increase Between Air and CO Days		
			Mean	SEM	Mean	SEM	Mean %	SEM	p-Value[a]
Air	61	Pre	118	20	21	12			
	61	Post	99	15					
2%-COHb target	61	Pre	118	19					
	60	Post	107	15	−10	12	61	61	0.32
4%-COHb target	58	Pre	130	22					
	62	Post	113	17	−12	11	− 7	32	0.84

[a] Two-sided p-values.

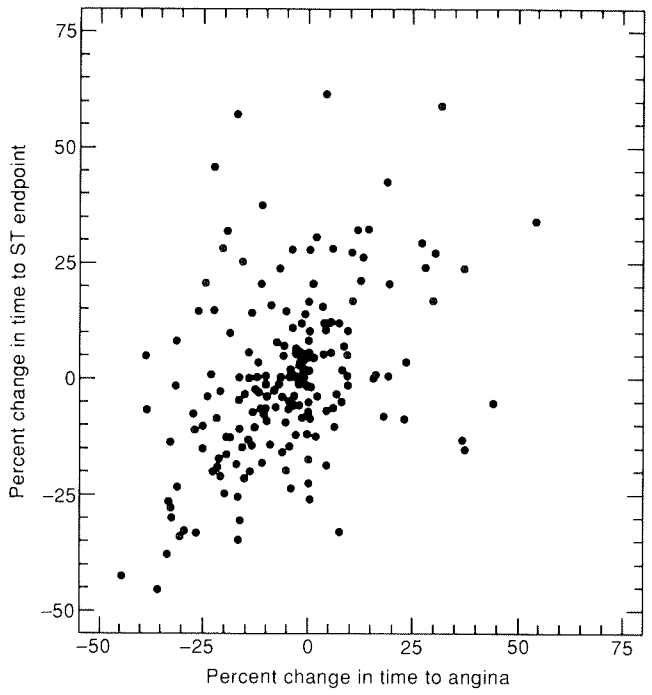

Figure 12. Relationship between percent change in time to ST endpoint [(postexposure - preexposure)/preexposure] and the percent change in time to angina [(postexposure - preexposure)/preexposure].

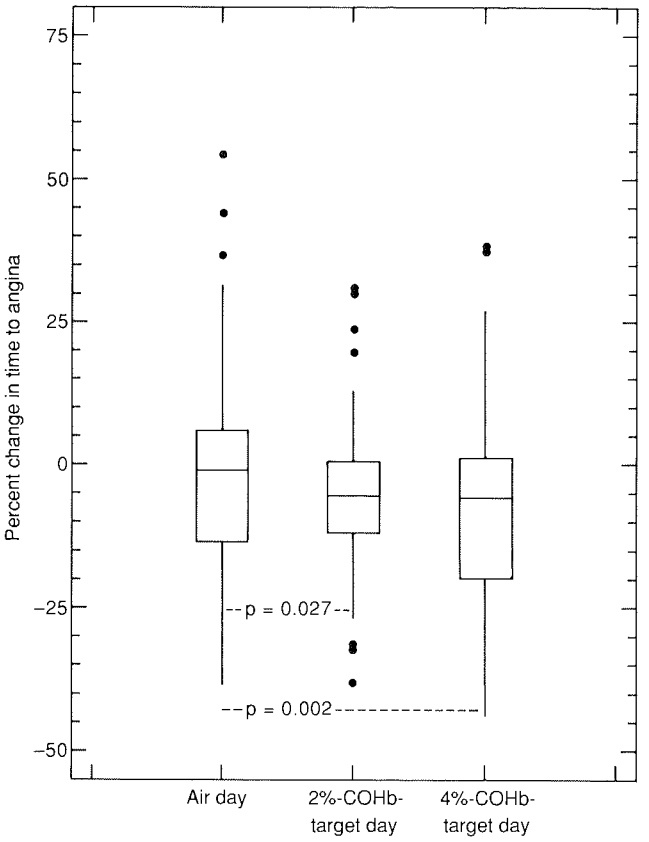

Figure 13. Box-and-whisker plots of percent change in time to angina between the pre- and postexposure exercise tests [(postexposure - preexposure)/preexposure] on the air day, the 2%-COHb-target day, and the 4%-COHb-target day. The bar across the box is the median, the ends of the box are the quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals outside that range.

± 2.11% and an average slope of –1.89% ± 0.81%/%COHb. The average decrease in time to angina pectoris appears to be roughly 1.9% for every 1% increase in COHb, over the range of COHb studied. This relationship is illustrated in Figure 14.

TOTAL EXERCISE DURATION

The effect of CO exposure on total exercise duration is presented in Table 15a. On the air day, the mean total exercise duration was 692 ± 27 seconds on the preexposure test, and was 680 ± 26 seconds on the postexposure test (a 1% decrease). On the 2%-COHb-target day, there was a 1.7% ± 1.6% decrease ($p = 0.29$) in the total exercise duration, compared to the air day. After exposure on the 4%-COHb-target day, there was a 6.2% decrease ($p \leq 0.0001$) in the total exercise duration, compared to the air day. There were no statistically significant differences between test days, however, with regard to the heart rate-systolic blood pressure double product at maximum exercise (Table 15b).

COVARIATE ANALYSES

The primary analyses evaluated the effect of COHb levels on the time to the ST and angina endpoints. Data were also collected on other factors that could influence these results. The factors generally fall into the categories of study-design variables, anthropometric characteristics of the subjects, factors related to severity of an individual's coronary artery disease, factors that could affect cardiovascular performance or pulmonary function, variables related to the study centers, and the actual COHb levels. As described below, the actual COHb level was the most significant covariate.

The order in which the exposures were assigned to each subject was randomized to guard against a learning effect. The average times to reach ST endpoint for the three days' preexposure exercise tests were 546 ± 8.3 seconds, 569 ± 8.5 seconds, and 580 ± 8.4 seconds. The learning trend is apparent ($p = 0.02$). For the time to angina, the three averages were: 498 ± 9.1 seconds, 528 ± 9.2 seconds, and 532 ± 9.1 seconds ($p = 0.02$). For the analyses that examined the effect of CO, the raw values were not used, but rather the difference between the pre- and postexposure values were used. The question, then, is whether the order in which the exposures were assigned had an effect on these differences. To answer this question, analyses of covariance were performed on both the time to ST endpoint and the time to angina for the differences in seconds. In these analyses the order in which the exposures were assigned, together with the actual COHb percentage, was tested. In none of the analyses was the order of exposures significant.

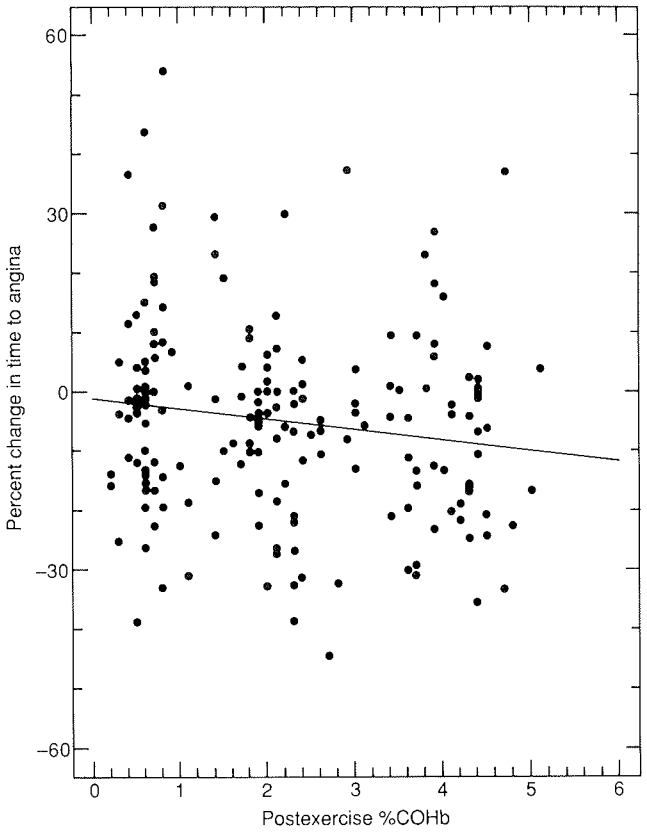

Figure 14. Regression of the percent change in time to angina between the pre- and postexposure exercise tests [(postexposure – preexposure)/preexposure] and the measured blood COHb levels at the end of exercise.

Regressions were performed to determine whether or not the differences found in both the ST endpoint and the angina analyses could be explained by other measured covariates. Four sets (decrease in percentages in time to ST endpoint and time-to-angina for both the 2%-COHb-target data and the 4%-COHb-target data) of simple regressions were performed (each involving one covariate). In each case, to guard against possible effects of nonlinearity and outliers, we performed a rank regression with normal scores for all variables. In each set of regressions, the individual covariates were the subject's age, smoking history, history of hypertension, prior myocardial infarction, occupation, use of beta-blockers, use of other medications, height, weight, the maximal time spent on the treadmill at visit 1, the average time to angina on three preexposure exercise tests, QRS duration, sodium level, potassium level, chloride level, FEV_1, percent of predicted FEV_1, percent of predicted FVC, the study center, and the actual COHb level by GC. The actual COHb level was significant for the analyses of time to ST endpoint (for 2%-COHb-target analysis, $p = 0.014$; for 4%-COHb-target analysis, $p = 0.014$), but was not significant for the angina analyses (for 2%-COHb-target analysis, $p = 0.86$; for 4%-COHb-target analysis, $p = 0.87$). The only other significant ($p \leq 0.05$) covariate was weight for the time-to-angina analysis at the 4%-COHb level. With 80 (4×20) regression coefficients, we would expect an average of four to be significant at 5% by pure chance, compared to the three that were found.

The fact that the time spent on the treadmill on the first exercise test of visit 1 was not a significant covariate indicates that the *percent change* due to CO is not correlated with the severity of limitation of exercise capacity. This conclusion is

Table 14a. Effect of Carbon Monoxide on Time to Angina (Combined Data)

		COHb Levels at End of Exercise Pre- and Postexposure			Time to Angina Pre- and Postexposure[a] (seconds)			Change in Time to Angina Post- vs. Preexposure (seconds)		% Decrease Between Air and CO Days			
Exposure Day	Sample Size		Mean %COHb[b]	SEM		Trimmed Mean	SEM	Trimmed Mean	SEM	Trimmed Mean %[c]	p-Value[d]	90% Confidence Interval	95% Confidence Interval
Air	63	Pre	0.64	0.02	Pre	519.0	26.7	-17.4	10.9				
		Post	0.62	0.02	Post	501.6	24.6						
2%-COHb target	62	Pre	0.62	0.02	Pre	525.2	26.2	-42.8	10.6	4.2	0.027	0.66, 7.94	0.4, 8.74
		Post	2.00	0.05	Post	482.4	22.0						
4%-COHb target	63	Pre	0.64	0.02	Pre	515.0	26.5	-49.6	10.9	7.1	0.002	3.06, 10.94	5.18, 14.46
		Post	3.87	0.08	Post	465.4	24.1						

[a] Median time to angina: air, pre = 520, post = 489; 2%-COHb target, pre = 482, post = 460; 4%-COHb target, pre = 480, post = 440.

[b] CO measured by GC.

[c] Median percent decrease: air vs. 2%-COHb target = 4.2; air vs. 4%-COHb target = 9.0. For analysis of nontrimmed means, see Appendix C.

[d] One-sided p-values, as described in the Methods section.

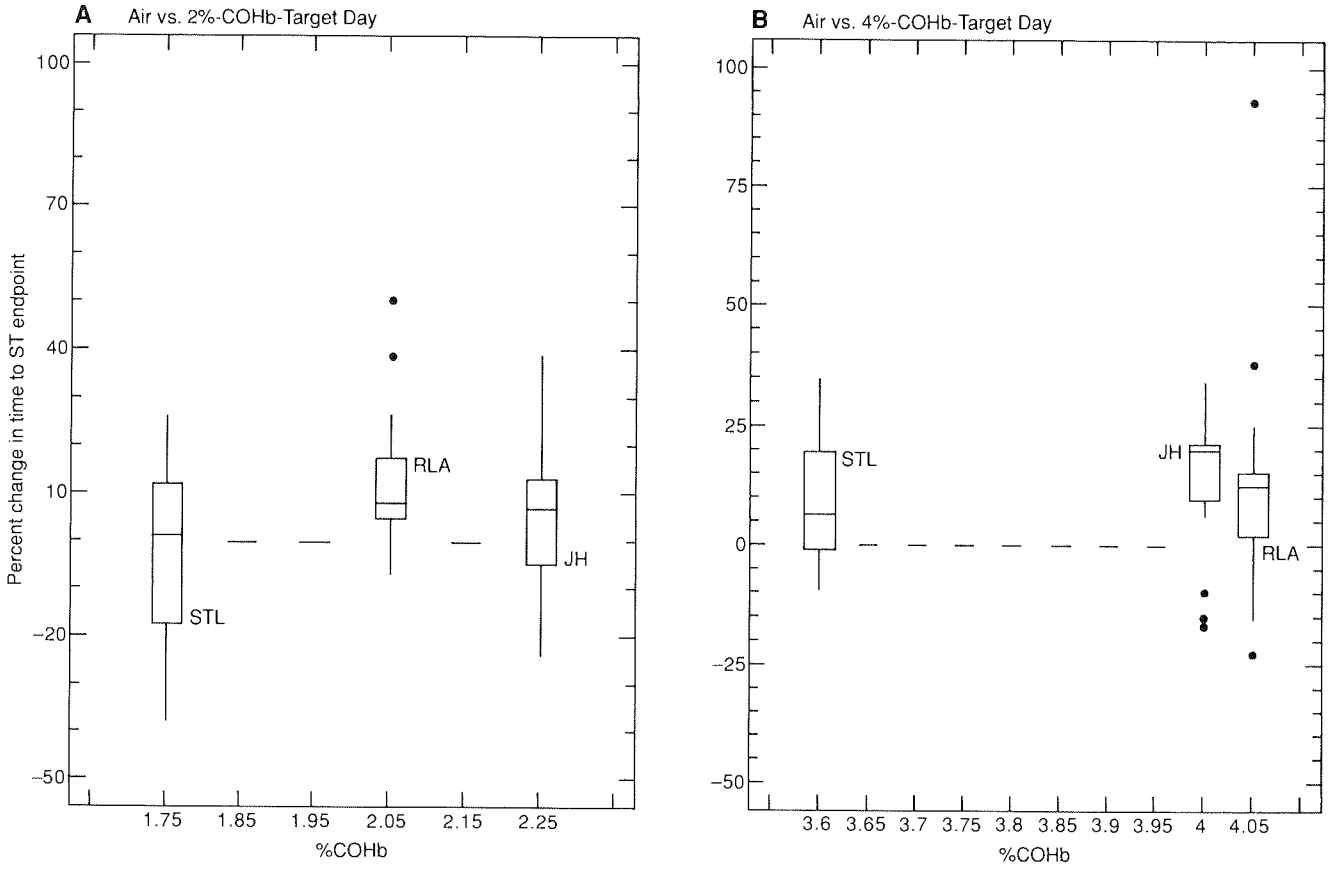

Figure 15. Box-and-whisker plots of individual differences in percentages of time to ST endpoint on air day compared to CO days at the three centers, showing the mean COHb level at the end of the exercise test after CO exposure at each center. The bar across the box is the median, the ends of the box are quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals beyond that range. The dashed line is at a difference of 0. A: results for the air vs. 2%-COHb-target day. B: results for the air vs. 4%-COHb-target day.

Table 14b. Effect of Carbon Monoxide on Heart Rate-Systolic Blood Pressure Double Product at the Onset of Angina (Combined Data)

Exposure Day	Sample Size		Double Product[a] at Angina in Pre- and Postexposure Exercise Tests		Difference Between Pre- and Postexposure Exercise Tests		% Decrease Between Air and CO Days		
			Mean	SEM	Mean	SEM	Mean %[b]	SEM	p-Value[c]
Air	63	Pre	15,989	583					
	63	Post	15,225	572	-764	228			
2%-COHb target	61	Pre	15,822	596	-444	248	-2.0	1.8	0.26
	62	Post	15,365	571					
4%-COHb target	63	Pre	15,540	512	-562	190	-1.1	1.7	0.50
	63	Post	14,978	493					

[a] Beats per minnte × mm Hg.

[b] Nontrimmed means.

[c] Two-sided p-values.

Table 15a. Effect of Carbon Monoxide on Total Exercise Duration

Exposure Day	Sample Size		Total Exercise Time in Pre- and Postexposure Tests (seconds)		Change in Time (seconds)		% Decrease Between Air and CO Days	
			Mean	SEM	Mean	SEM	Mean %	p-Value[a]
Air	63	Pre	692	27	-12.1	8.2	—	—
		Post	680	26				
2%-COHb target	62	Pre	700	26	-27.0	9.7	1.7	0.29
		Post	673	24				
4%-COHb target	63	Pre	693	27	-53.0	9.0	6.2	≤ 0.0001
		Post	640	26				

[a] Two-sided p-values.

Table 15b. Effect of Carbon Monoxide on Heart Rate-Systolic Blood Pressure Double Product at Peak Exercise

Exposure Day	Sample Size		Double Product[a] at Peak Exercise in Pre- and Postexposure Tests		Difference in Double Product (Pre- Minus Postexposure Mean Difference)		% Decrease Between Air and CO Days	
			Mean	SEM	Mean	SEM	Mean %	p-Value[b]
Air	63	Pre	18,486	649	-285	265	—	—
	63	Post	18,201	748				
2%-COHb target	62	Pre	18,700	718	61	232	-3.23	0.08
	62	Post	18,761	678				
4%-COHb target	63	Pre	18,761	637	-569	243	0.23	0.89
	63	Post	17,686	590				

[a] Beats per minute × mm Hg.

[b] Two-sided p-values.

also supported by the fact that the average time to angina in the preexposure exercise tests of visits 2, 3, and 4 was not a significant covariate. Thus, subjects with varying degrees of cardiac disease have similar percent effects of CO on their exercise capacity.

INDIVIDUAL CENTER ANALYSES

Table 16 presents data on ST endpoint by center. The 2%-COHb-target-exposure level, compared to the air day, produced a statistically significant effect on time to ST endpoint at Johns Hopkins and Rancho Los Amigos, but not at St. Louis. The 4%-COHb-target-exposure level produced a statistically significant decrease in time to ST endpoint at all three centers, with a range of 9.0% to 17.5%. Table 16 shows that the effect on ST endpoint was greater after the 4%-COHb-target exposure than after the 2%-COHb-target exposure at two centers, Johns Hopkins and St. Louis, but not at Rancho Los Amigos.

The box-and-whisker plots shown in Figure 15 depict this analysis. These plots show the overlap among the centers and incorporate the differing average COHb levels at the different centers, thus the slight increase in reading from left to right. Analysis of covariance was first performed with the raw differences in seconds as responses and the measured COHb levels as covariates. Next, center indicator variables were introduced as individual covariates. This analysis was repeated for the percent difference as responses. In neither analysis did the center prove significant; only the COHb level was significant.

Table 17 presents results of the angina analyses at the three centers. The effect on angina of the 2%-COHb-target exposure is statistically significant at two centers, Johns Hopkins and Rancho Los Amigos. The 4%-COHb-target exposure also had a significant effect on angina at only two centers, Rancho Los Amigos and St. Louis. The box-and-whisker plots shown in Figure 16 depict these analyses. As with the ST analyses, these plots show the overlaps among the centers and incorporate the differing average COHb levels at the different centers. As with the ST endpoint, analysis of covariance was performed using the raw differences, and then the percent differences, as responses. In neither analysis did the center prove significant; again, only the COHb level was significant.

Table 16. Effect of Carbon Monoxide on Time to ST Endpoint at the Three Centers

Exposure Day	Sample Size	COHb Levels at End of Exercise Pre- and Postexposure	Mean COHb[b]	SEM	Time to ST Endpoint Pre- and Postexposure[a] (seconds)	Mean	SEM	Change in Time to ST Endpoint Post- vs. Preexposure (seconds)	Mean	SEM	% Decrease Between Air and CO Days	Trimmed Mean %[c]	p-Value[d]	90% Confidence Interval	95% Confidence Interval
Johns Hopkins University															
Air	22	Pre	0.60	0.04	Pre	596.0	48.9		7.1	21.8					
		Post	0.58	0.05	Post	603.1	50.7								
2%-COHb target	22	Pre	0.60	0.03	Pre	601.8	47.7		-29.6	29.3		5.9	0.042	0.27, 11.73	- 0.1, 12.84
		Post	2.25	0.06	Post	572.2	47.7								
4%-COHb target	22	Pre	0.65	0.04	Pre	610.1	48.0		-88.7	25.7		17.5	≤ 0.0001	11.67, 22.02	10.36, 22.93
		Post	4.00	0.12	Post	521.4	46.7								
Rancho Los Amigos Medical Center															
Air	17	Pre	0.77	0.03	Pre	580.0	45.7		51.8	24.8					
		Post	0.71	0.03	Post	631.8	42.8								
2%-COHb target	17	Pre	0.69	0.05	Pre	606.8	39.6		- 5.4	17.3		10.8	0.0003	6.08, 17.24	5.18, 18.91
		Post	2.05	0.10	Post	601.2	40.7								
4%-COHb target	17	Pre	0.72	0.05	Pre	588.4	54.8		-20.8	26.7		10.5	0.008	3.78, 17.84	2.29, 19.62
		Post	4.06	0.14	Post	567.6	49.1								
St. Louis University															
Air	23	Pre	0.58	0.04	Pre	510.6	42.3		- 2.0	13.8					
		Post	0.58	0.04	Post	508.6	40.0								
2%-COHb target	22	Pre	0.57	0.04	Pre	521.2	48.0		-11.3	16.8		-1.1	> 0.5	- 8.44, 5.33	-10.03, 6.53
		Post	1.70	0.07	Post	509.9	41.1								
4%-COHb target	23	Pre	0.57	0.04	Pre	498.8	41.1		-41.9	11.0		9.0	0.025	4.35, 13.64	3.47, 14.63
		Post	3.59	0.13	Post	456.9	38.6								

[a] Median time to ST endpoint: Johns Hopkins (JH): air, pre = 570.0, post = 555.0; 2%-COHb target, pre = 570.0, post = 522.0; 4%-COHb target, pre = 611.5, post = 480.0. Rancho Los Amigos (RLA): air, pre = 600.0, post = 630.0; 2%-COHb target, pre = 600.0, post = 590.0; 4%-COHb target, pre = 550.0, post = 540.0. St. Louis (STL): air, pre = 480.0, post = 480.0; 2%-COHb target, pre = 532.5, post = 480.0; 4%-COHb target, pre = 490.0, post = 450.0.

[b] CO measured by GC.

[c] Median percent decrease: JH: air vs. 2%-COHb target = 7.7; air vs. 4%-COHb target = 20.3. RLA: air vs. 2%-COHb target = 8.5; air vs. 4%-COHb target = 12.7. STL: air vs. 2%-COHb target = 1.0; air vs. 4%-COHb target = 7.6. For analysis of nontrimmed means, see Appendix B.

[d] One-sided p-values, as described in the Methods section.

DISCUSSION

BIOLOGICAL EFFECTS

This study demonstrates, with the use of objective ECG measurements for the first time, the influence of low levels of COHb on the development of ischemia in subjects with coronary artery disease. Carbon monoxide exposure caused a reduction in the time to ischemic ST-segment changes during exercise (Table 11a). Both the earlier attainment of this level of ischemia and the increase in the maximal change in ST segments during exercise indicate that low levels of COHb limit exercise tolerance in this group of individuals. This limitation is further demonstrated at the 3.9%-COHb level, at which the duration of the symptom-limited exercise test was reduced.

The subjective endpoint of time to onset of angina was also reduced at both the 2.0%- and 3.9%-COHb levels. As expected, both the ECG and angina endpoints are closely correlated ($R = 0.49$, $p \leq 0.0001$; Figure 12) and, together, strongly suggest that these low-COHb levels produce an effect of CO on the myocardium in subjects with coronary-blood-flow limitation. It is an important finding of this study that small increments in %COHb (for example, for the lower level of CO exposure at which the increase in %COHb was 2.0% – 0.6% = 1.4%, Table 8) were associated with ischemic changes represented by a relatively larger decrease (5.1%, Table 11a) in the time to ST endpoint during the progressive exercise. Furthermore, there is an important and significant exposure-response relationship between COHb levels and the decrease in time to ST endpoint (Figure 10). There was a 3.9% decrease in time to ST endpoint for every 1% increase in

COHb. All of these experimental results indicate that low levels of CO exposure affect cardiac function during exercise by facilitating the development of ischemia. The most likely mechanism responsible for these changes is a reduction in the oxygen-carrying capacity of the blood, but more complex effects of CO on myocardial function cannot be excluded.

Starling and coworkers (1984) performed experiments that were similar in design to this protocol: subjects with coronary artery disease exercised twice each day on two days separated by less than one week. The study design permitted analysis of the results of repeated exercise tests between days and between tests on the same day. Virtually all of the cardiovascular and exercise parameters reported by Starling had reduced variability when measured on the same day, relative to tests performed on different days. The data in our study also show greater day-to-day variability than within-day variability for the cardiovascular parameters. Starling and coworkers reported that the time to onset of angina and time to 0.1-mV ST-segment change was longer in the second test on the same day, although the heart rate-blood pressure double product was unchanged at these time points in the exercise test. The results of Starling and associates are similar to the increase in time to ST endpoint observed in the present study on exercise testing after air exposure.

The heart rate-systolic blood pressure double product provides a clinical index of the work of the heart and myocardial oxygen consumption (Jorgenson et al. 1973), since heart rate and blood pressure are two of the major determinants of myocardial oxygen consumption (Sonnenblick et al. 1968). If low levels of COHb significantly reduce oxygen delivery to the myocardium, then the development of ischemia at a lower double product or level of myocardial oxygen consumption

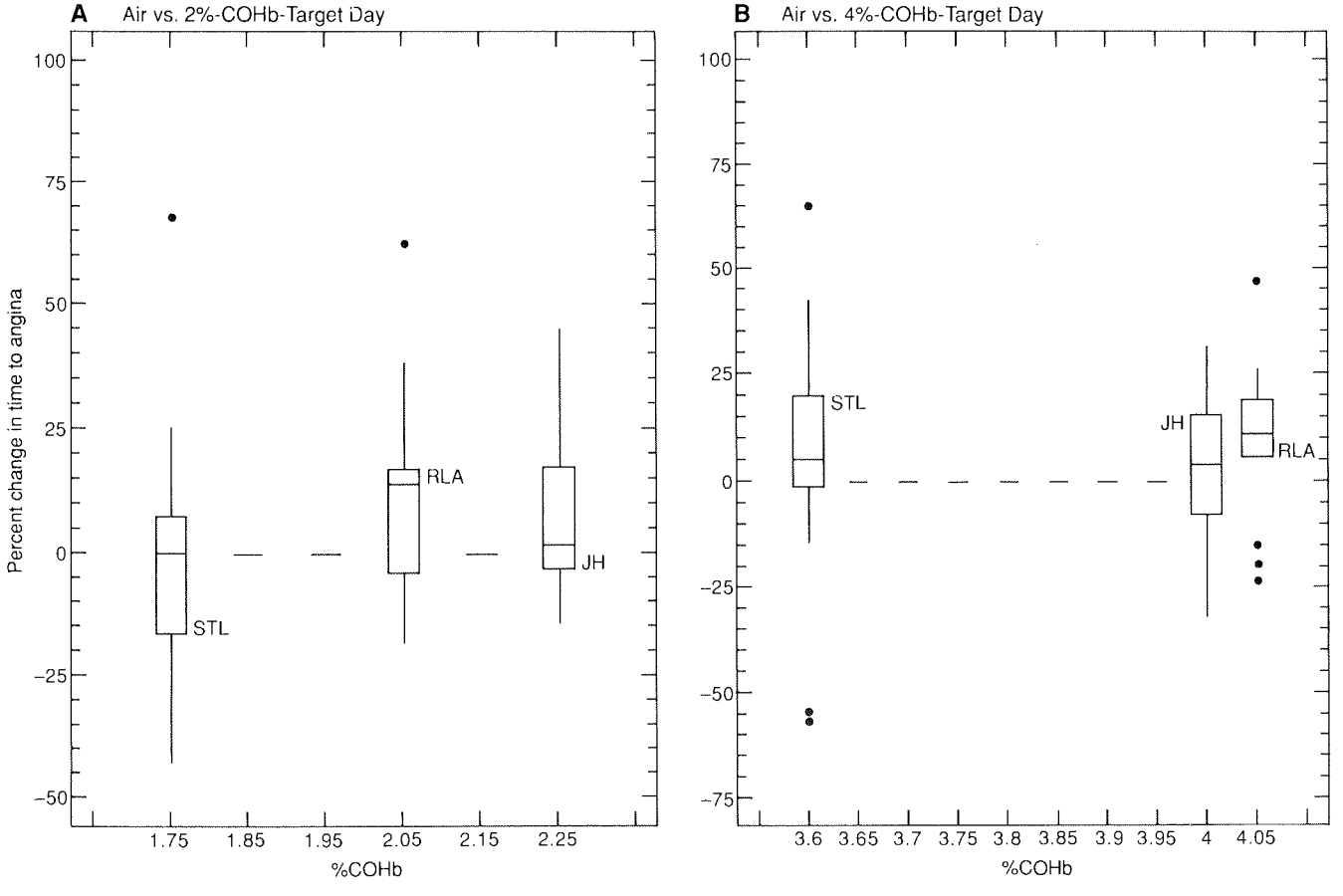

Figure 16. Box-and-whisker plots of individual differences in percentages of time to angina on air compared to CO days at the three centers, showing the mean COHb level at the end of the exercise test after CO exposure at each center. The bar across the box is the median, the ends of the box are quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals beyond that range. The dashed line is at a difference of 0. A: results for the air vs. 2%-COHb-target day. B: results for the air vs. 4%-COHb-target day.

Table 17. Effect of Carbon Monoxide on Time to Angina at the Three Centers

Exposure Day	Sample Size	COHb Levels at End of Exercise Pre- and Postexposure	Mean %COHb[b]	SEM	Time to Angina Pre- and Postexposure[a] (seconds)	Mean	SEM	Change in Time to Angina Post- vs. Preexposure (seconds)	Mean	SEM	% Decrease Between Air and CO Days: Trimmed Mean %[c]	p-Value[d]	90% Confidence Interval	95% Confidence Interval
Johns Hopkins University														
Air	22	Pre	0.60	0.04	Pre	562.2	57.5	-46.1	16.9					
		Post	0.58	0.05	Post	516.0	51.2							
2%-COHb target	22	Pre	0.60	0.03	Pre	547.7	53.9	-76.2	19.6		5.2	0.014	0.48, 9.93	- 0.42, 11.03
		Post	2.25	0.06	Post	471.4	46.1							
4%-COHb target	22	Pre	0.65	0.04	Pre	557.1	51.7	-62.2	18.9		4.6	0.09	-0.32, 10.44	- 2.53, 11.53
		Post	4.00	0.12	Post	494.9	49.4							
Rancho Los Amigos Medical Center														
Air	18	Pre	0.77	0.03	Pre	541.5	43.0	14.7	22.7					
		Post	0.71	0.03	Post	556.2	41.7							
2%-COHb target	18	Pre	0.69	0.05	Pre	555.9	40.6	-35.7	15.7		9.4	0.01	3.18, 16.82	1.78, 18.21
		Post	2.05	0.10	Post	520.2	36.3							
4%-COHb target	18	Pre	0.72	0.05	Pre	522.9	48.7	-33.0	21.1		10.4	0.002	2.18, 17.44	0.48, 18.73
		Post	4.06	0.14	Post	489.9	47.6							
St. Louis University														
Air	23	Pre	0.58	0.04	Pre	460.0	33.3	-15.0	16.4					
		Post	0.58	0.04	Post	445.1	31.4							
2%-COHb target	22	Pre	0.57	0.04	Pre	477.6	37.9	-15.2	16.6		-3.2	> 0.5	-9.44, 3.44	-10.83, 4.93
		Post	1.70	0.07	Post	462.4	29.5							
4%-COHb target	23	Pre	0.57	0.04	Pre	468.4	37.0	-50.5	17.4		7.7	0.045	0.28, 14.23	- 1.51, 15.54
		Post	3.59	0.13	Post	418.0	26.8							

[a] Median time to angina: JH: air, pre = 535.0, post = 492.5; 2%-COHb target, pre = 512.5, post = 430.0; 4%-COHb target, pre = 562.0, post = 452.5. RLA: air, pre = 545.5, post = 478.5; 2%-COHb target, pre = 500.0, post = 491.5; 4%-COHb target, pre = 466.0, post = 439.0. STL: air, pre = 450.0, post = 480.0; 2%-COHb target, pre = 448.5, post = 452.5; 4%-COHb target, pre = 470.0, post = 435.0.

[b] CO measured by GC.

[c] Median percent decrease: JH: air vs. 2%-COHb target = 2.5; air vs. 4%-COHb target = 4.3. RLA: air vs. 2%-COHb target = 13.4; air vs. 4%-COHb target = 13.1. STL: air vs. 2%-COHb target = 0.1; air vs. 4%-COHb target = 5.3. For analysis of nontrimmed means, see Appendix B.

[d] One-sided p-values, as described in the Methods section.

should be expected. The results for the heart rate-systolic blood pressure double product at the onset of ST endpoint show this trend (Table 11b). On the 4%-COHb-target-exposure day, there was a statistically significant reduction in double product on the postexposure test, although on the 2%-COHb-target-exposure day, there was a decrease in the double product; this change was not statistically significant. The earlier onset of ischemic ST-segment changes at a lower double product (level of myocardial oxygen consumption) is consistent with the expected inability of these subjects to increase blood flow to meet myocardial oxygen demands. In these subjects, when blood flow reaches its limit, the reduction in arterial oxygen content by CO results in earlier development of ischemia.

Electrocardiographic evidence of ischemia occurred at lower levels of exercise with CO exposure than with ambient air exposure. It is implied that at a constant level of treadmill exercise, and therefore a constant myocardial workload, exposure to CO would result in greater myocardial ischemia (larger changes in ST segment). This concept is partially supported by the analysis of end-of-exercise data. For example, in the 4%-COHb experiments, there was a significantly greater maximal change in ST segments observed at the end of exercise (Table 13a). This occurred at a lower workload (Table 15a) and at a significantly lower double product (Table 11b). These findings further demonstrate augmentation of myocardial ischemia by increases in %COHb.

The primary mechanism responsible for the ability of CO to decrease oxygen delivery to the myocardium is the direct reduction in the oxygen-carrying capacity of the blood. This occurs even at very low partial pressures of CO because of the high affinity of hemoglobin for CO, relative to oxygen (Douglas et al. 1912). Several other factors may influence myocardial

oxygenation and function. The combination of CO with hemoglobin can influence the available binding sites for oxygen, increasing the overall affinity of hemoglobin for oxygen (that is, a leftward shift in the oxygen-dissociation curve). The magnitude of this shift can be calculated from the change in P_{O_2} required to half-saturate available hemoglobin. In humans, this value is approximately 0.35 torr O_2/1% COHb (Roughton and Darling 1944). In the present study, this would mean 0.5-torr and 1.1-torr shifts in the oxygen-dissociation curve for the 2.0%- and 3.9%-COHb levels, respectively. In addition to reduced oxygen delivery, there is the potential for reduced tissue uptake of oxygen in the presence of CO. The uptake of oxygen in cardiac muscle is dependent upon the availability of myoglobin and the ability to transfer oxygen from myoglobin to cytochrome oxidase. However, it is less likely that cytochrome oxidase is affected by the low levels of COHb achieved in this study, since Chance and coworkers (1970) have reported a negligible effect of CO on this part of the respiratory chain. The additional contributions by these other factors on oxygen availability and cardiac function are unknown and require further investigation.

COMPARISON WITH OTHER STUDIES

Comparison of the results of this investigation with previously published reports that evaluated the influence of 2%-COHb-target levels on exercise performance in subjects with coronary artery disease must be approached cautiously because of the substantial differences in study design. For example, the exercise tests varied in the type of exercise and rate of incremental workload; the subject populations were different as a result of differing selection and screening criteria; the COHb measurements were performed using different techniques; and the exposure procedures varied. The HEI multicenter study has several major advantages: the number of subjects studied was large, the presence of coronary artery disease was carefully documented, the analysis of COHb levels was rigorously performed, and objective ECG criteria were used to evaluate exercise-induced myocardial ischemia.

Table 18 and Figure 17 compare the HEI study with other studies that evaluated the effect of CO on time to onset of angina in subjects with coronary artery disease, while Table 19 describes the characteristics of the subjects in these studies. Caution should be used in interpreting the percent decrease in time to angina reported by different investigators (Table 18) because the exercise stress-test protocols differed. The subjects in the present study exercised approximately twice as long because a gradual incremental workload was employed for the exercise stress test. However, insufficient detail is available from most of the other studies to fully substantiate this assumption.

Aronow and Isbell (1973) reported a 15% decrease in time to angina at COHb levels of 2.7% (IL 182 CO-Oximeter), measured at the end of exposure. More recently, Aronow (1981) reported a 12% decrease in the time to angina at 2% COHb (IL 282 CO-Oximeter), measured at the end of exposure. Anderson and coworkers (1973) reported data that, in our analysis, showed a 21% decrease in time to onset of angina after CO exposures that resulted in 2.9% COHb and 4.5% COHb (measured by spectrophotometry). Our analysis of their data by a one-tailed T-test demonstrates the time to angina to be significantly decreased. However, we cannot show any dose-response effect. Kleinman and Whittenberger's (1985) results showed a 6.9% decrease in time to angina at 3.0% COHb (IL 282 CO-Oximeter), measured at the end of exposure, but Sheps and coworkers (1987) did not show a significant effect on the time to angina at COHb levels of 4.1% that were measured (IL 282 CO-Oximeter) at the end of exposure.

It is difficult to compare COHb levels in different studies because of the variability in the spectrophotometric measurements of COHb, especially when different instruments were used. The lower exposure level in the HEI study (3.2% COHb by IL 282 CO-Oximeter at the end of exposure, 2.4% COHb by GC) is higher than the 2.7% (CO-Oximeter) level in the Aronow and Isbell study and the 2.0% (CO-Oximeter) level in the Aronow study, assuming that instruments in different

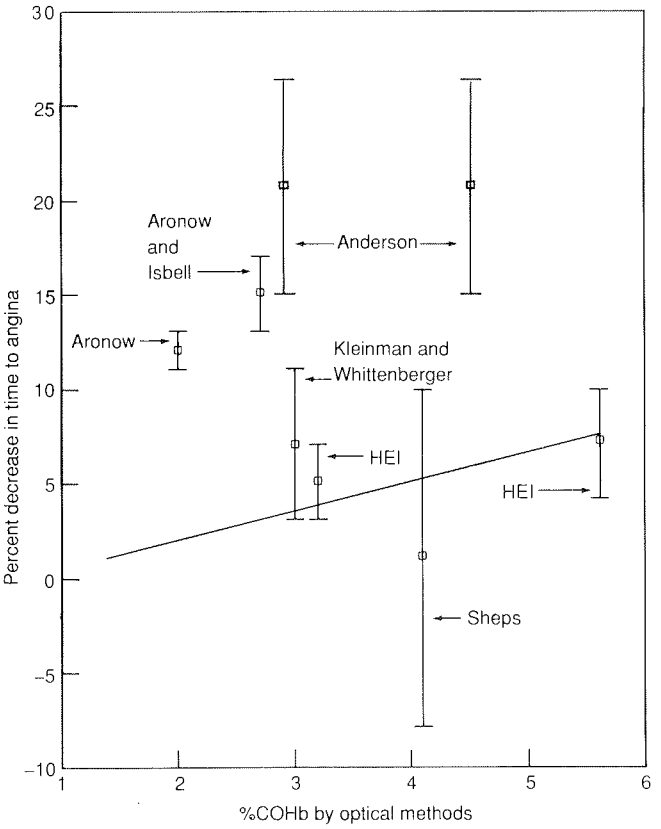

Figure 17. Comparison of effect of CO exposure on time to angina in several studies (see Tables 18 and 19 for more detailed information). The regression line is based on data from the HEI study. Bars indicate standard errors of the mean that we calculated. Because of major protocol differences among these studies and the imprecision in optical measurements of COHb (see text), these comparisons must be interpreted extremely cautiously. Note that in this figure only, we refer to end-of-exposure %COHb levels rather than end-of-exercise levels.

laboratories give similar readings. It produced a 4.2% (trimmed mean; mean = 5.0%) decrease in the time to onset of angina. While less than in the Aronow studies, this effect is statistically significant (one-sided p = 0.027). The higher exposure level in the HEI study (5.6% by CO-oximetry at the end of exposure, 4.7% by GC) produced a 7.1% (trimmed mean; mean = 6.6%) decrease (one-sided p = 0.002) in the time to the onset of angina, which was less than the effect in the Aronow studies at much lower COHb levels. The Anderson study demonstrated a greater percent change in time to angina at both COHb levels that were evaluated, each one lower than the corresponding level in the HEI study (2.9 vs. 3.2 %COHb and 4.5 vs. 5.6 %COHb), but did not find a dose-response relation as we did. In the report by Kleinman and Whittenberger, a 6.9% decrease in time to angina was found at a COHb level (measured by CO-Oximeter) that appears to be similar to the lower level in the HEI study. The Sheps study produced COHb levels that were intermediate between the two HEI levels, but these investigators did not find a significant change in the time to angina.

Table 18. Comparison of HEI Multicenter Study with Other Studies Evaluating the Effect of Carbon Monoxide on the Time to Onset of Angina in Subjects with Coronary Artery Disease

Investigators	No. of Subjects	Exposures	%COHb[a] (mean ± SD) Spectrophotometry	%COHb[a] (mean ± SD) Gas Chromatography	Effect of CO on Time to Angina[b] % Decrease CO Day vs. Air Day	Effect of CO on Time to Angina[b] SEM
Aronow and Isbell 1973	10	Air/2 hr[c]	0.8 ± 0.2 [d]	—	—	
		50 ppm CO/2 hr[c]	2.7 ± 0.2 [d]	—	15	2
Aronow 1981	15	Air/1 hr[c]	1.0 ± 0.1 [e]	—	—	
		50 ppm CO/1 hr[c]	2.0 ± 0.2 [e]	—	12	1
Anderson et al. 1973	10	Air/4 hr[c]	1.3 ± 0.4 [f]	—	—	
		50 ppm CO/4 hr[c]	2.9 ± 0.7 [f]	—	21	6
		100 ppm CO/4 hr[c]	4.5 ± 0.8 [f]	—	21	6
Kleinman and Whittenberger 1985	26	Air/1 hr[c]	1.4 ± 0.5 [d] (1.4 ± 0.5)[e]	—	—	
		100 ppm CO/1 h[c]	3.0 ± 0.5 [e] (2.8 ± 0.5)[e]	—	7	4
Sheps et al. 1987	23[h]	Air/about 1 hr[i]	1.7 ± 0.1 [e]	—	—	
		100 ppm CO/h[i] about 1 hr	4.1 ± 0.1 [e] (3.6 ± 0.1)[e]	—	1[g]	9
HEI multicenter study	63	0-2 ppm CO[i] mean 0.69/ 50-70 min	1.4 ± 0.4 [e] (1.2 ± 0.4)[e]	0.70 ± 0.19 (0.62 ± 0.19)	—	
		42-202 ppm CO[i] mean 117/ 50-70 min	3.2 ± 0.3 [e] (2.6 ± 0.3)[e]	2.38 ± 0.40 (2.00 ± 0.41)	5	2
		143-357 ppm CO[i] mean 253/ 50-70 min	5.6 ± 0.5 [e] (4.7 ± 0.4)[e]	4.66 ± 0.62 (3.87 ± 0.62)	7	3

[a] %COHb at end of exposure. We calculated SD for Kleinman and Whittenberger data. Numbers in parentheses are %COHb at the end of the exercise test after the exposure period.

[b] The design of the HEI study was the same as the two Aronow studies in that it had a pre- and postexposure exercise test each day. For those studies, we calculated the pairwise percent change between the tests on each day and then, pairwise, subtracted the result on the CO day from the result on the air day. For the Kleinman and Sheps data, we calculated the pairwise percent change between the air and CO days. For the Anderson data, we calculated the pairwise percent changes between the randomized air day and the CO days (as did Anderson). We used the average of the two nonrandomized air days for the two subjects for whom data from the randomized air day were not available. For comparison with the other studies, the percentages listed in this table for the HEI study are means (see Appendix C) rather than trimmed means (Table 14). We calculated SEMs for all studies.

[c] Mask exposure.

[d] IL 182 CO-Oximeter.

[e] IL 282 CO-Oximeter.

[f] Spectrophotometric methods (Buchwald 1969).

[g] Not statistically significant by one-tailed T-test.

[h] Thirty subjects in study, but angina analyzed in only 23 of them.

[i] Chamber exposure.

One important difference among the various studies is in the number of subjects that were evaluated. The number of subjects in the HEI study enabled detection of small effects that would not have been found with a smaller cohort. Each of the individual centers in the HEI study enrolled approximately as many subjects as did the Sheps study, and analysis of the data reported by one of them (St. Louis) did not show a reduction in time to angina at the 2%-COHb-target exposure. That center also had the lowest mean COHb levels (by GC) when compared to the other centers (Table 9). Also, the CO effects on angina were not significant at the 5% confidence level at Johns Hopkins on the 4%-COHb-target-exposure day. Based on COHb levels by IL 282 CO-oximetry, the COHb levels employed in the Sheps study appear to be between the two HEI exposure levels. Yet the actual exposure conditions in the Sheps study are similar to those employed for the 2%-COHb-target level in the HEI study. In fact, the mean CO level of 117 ppm for the 2%-COHb target in the HEI study is higher than the exposure level of 100 ppm in the Sheps study, and the duration of exposure was approximately one hour in both.

The COHb levels reported in most of the previous investigations were measured at the end of exposure, rather than at the time of angina. Decreases in COHb levels may have occurred, depending on the time interval to the beginning of the exercise stress test. The duration and severity of exercise would also affect CO loss caused by exercise-induced hyperventilation. Thus, the ability of the COHb levels measured at the end

Table 19. Comparison of Subjects in Studies of the Effect of Carbon Monoxide Exposure on Occurrence of Angina During Exercise

	Subject Characteristics					
Study	No. of Subjects	Gender	Medication	Smoking History	Description of Disease	Age (years)
Aronow and Isbell 1973	10	male	Not described	No current smokers	Classic exertional angina, CAD with > 50% stenosis of 1 or more major vessels	40-55 (mean = 49)
Aronow 1981	15	14 male 1 female	Not described	No current smokers	Stable angina pectoris with angiographically demonstrated CAD; 8 had prior MI	50.0 ± 7.2
Anderson et al. 1973	10	male	1 subject took digitalis; drug therapy basis for exclusion	5 smokers (refrained for 12 hours prior to exposure)	Stable angina pectoris, positive exercise test (ST changes); reproducible angina on treadmill	(mean = 49.9)
Kleinman and Whittenberger 1985	26	male	14 on beta-blockers, 19 on nitrates	No current smokers	Ischemic heart disease, stable exertional angina pectoris	49-66 (mean = 59)
Sheps et al. 1987	30 (23 with angina)	25 male 5 female	26 subjects on medication; 19 on beta-blockers; 11 on Ca-channel-blockers; 1 on long-acting nitrates	No current smokers	Ischemia during exercise (ST changes or abnormal ejection fraction response) and 1 or more of the following: (1) angiographically proven CAD; (2) prior MI; (3) typical angina	36-75 (mean = 58.2)
HEI Multicenter Study	63	male	38 on beta-blockers; 36 on nitrates; 40 on calcium antagonists	No current smokers	Stable exertional angina and positive exercise test (ST changes) plus 1 or more of the following: (1) ≥ 70% lesion by angiography in 1 or more major vessels; (2) prior MI; (3) positive exercise thallium test	41-75 (mean = 62.1)

of exposure to reflect accurately the conditions at the time of exercise-induced ischemia is clearly influenced by these additional factors related to study design. In the HEI study, both postexposure and postexercise COHb levels are reported (Tables 8 and 9), and one can see significant decreases in COHb levels between the two samples.

Another important difference among the six studies is whether or not subjects were maintained on antianginal medications. Therapeutic approaches and the efficacy of treatment have evolved during the time period spanning these studies. Currently employed therapy may be more effective in preventing ischemia, and studies using these antianginal regimens may be important for the development of regulatory standards. In the Kleinman and Whittenberger, Sheps, and HEI studies, subjects remained on medications, as indicated in Table 19. The rationale for the HEI study was that the subjects should be studied under conditions that approximated the usual level of health care, so that the effects would be representative of those that would occur outside of the experimental laboratory. In the Anderson study, drug therapy, such as propranolol and quinidine, was a basis for exclusion from the study, perhaps indicating a milder severity of disease since their subjects did not require antianginal medication or could tolerate withdrawal from it. Unfortunately, therapy was not discussed in the Aronow or Aronow and Isbell reports.

While it is difficult to compare the subject populations by using data from these published reports, the subjects in the HEI study were selected by strictly defined criteria. The purpose of these entry criteria was to assure that all subjects had well-documented coronary artery disease and could provide reproducible experimental data about the ST and angina endpoints, while still being representative of the general population of individuals with coronary artery disease. Besides stable exertional angina and a positive exercise treadmill test, all subjects were required to have one or more of three additional indicators of ischemic myocardial disease: angiographic evidence of 70% or more obstruction of at least one coronary artery, documented myocardial infarction, or a positive thallium stress test. Finally, the Anderson study included smokers who refrained from smoking for 12 hours prior to exposure, whereas the other studies excluded current smokers. The effects of chronic exposure to CO in cigarette smokers with coronary artery disease are unknown, and inclusion of smokers in that study makes comparison with other studies difficult.

The type of exercise employed to induce angina pectoris differed among these studies. In the Anderson and HEI studies, subjects exercised on a treadmill, whereas a bicycle ergometer was used in the other four studies. It is difficult to evaluate the specific exercise protocols because of the lack of detail provided in the published reports. However, inferences can be drawn, based upon the performance of the subjects in each study. Under room air conditions, the modified Naughton exercise protocol used in the HEI study resulted in an average duration of exercise prior to the onset of angina of more than 500 seconds, compared to 325 seconds in the Anderson study, 312 seconds in the Sheps study, 226 seconds in the Aronow and Isbell study, 323 seconds in the Aronow study, and 390 seconds in the Kleinman and Whittenberger study. Some of these protocols must have employed rapid incremental exercise workloads or studied subjects with severe coronary artery disease who were very sensitive to exercise. Since none of the studies measured oxygen consumption, it is not possible to determine the actual level of exercise at which angina occurred. Because the exercise protocols and subjects' exercise capabilities varied among the six studies, comparisons of the percent reduction in time to angina appear to be more useful than comparisons of change in actual time to angina. This is supported by a finding in the HEI study of similar percent changes among subjects with different exercise capabilities.

The time to onset of ECG ST-segment changes, which are thought to be indicative of myocardial ischemia, is a more objective indicator than angina. We found a 5.1% decrease (one-sided $p = 0.01$) at the 2%-COHb-target-exposure level, and a 12.1% decrease (one-sided $p \leq 0.0001$) at the 4%-COHb-target-exposure level in the time to the onset of ST changes. We also observed an 11% and 17% increase in the magnitude of the maximal ST amplitude at the end of exercise after the 2%- and 4%-COHb-target exposures, respectively, but found no effect on the duration of ST-segment changes. Although the other studies did not carefully quantify ST-segment changes, several include anecdotal information on this parameter. Aronow and Isbell reported that ST-segment depression occurred earlier after CO exposure, compared to normal air, but stated that ST-segment depression was not rigorously quantified. In addition, an increase from 1.30 to 1.45 mm (not statistically significant) in maximal ST-segment depression was reported after CO exposure. Aronow also reported that ST-segment depression equal to or greater than 1 mm occurred earlier with CO exposure, after less exercise, and at a lower heart rate-blood pressure product. Anderson stated that "generally, ST-segment depression appeared earlier and was deeper after one or both concentrations of CO, compared with air." Kleinman and Whittenberger reported that seven (of 26) subjects "showed small depressions of the ST segment of their ECGs at the point of angina on both test days, and one subject showed ST-segment depression only on the clean air day." The average ST-segment depression was only 0.4 mm, however, and the differences were not statistically significant. These data do not suggest an effect on ST-segment depression; however, there is insufficient documentation of coronary artery disease in the subjects studied by Kleinman and Whittenberger. Sheps also did not find an effect of CO exposure on the time to 1-mm ST-segment depression, the maximal ST depression, or the heart rate-blood pressure double product at the time of ST-segment depression.

In summary, of the six studies discussed here, five dem-

onstrated statistically significant effects of low levels of COHb on time to angina. For a variety of reasons discussed in this section, including the small numbers of subjects and differences in subject populations, it is not surprising that one study did not show an effect. None of the other studies provide substantive analysis of ST segments, which showed highly significant effects in this study.

IMPLICATIONS OF THE FINDINGS

EFFECTS OF CARBON MONOXIDE ON HEALTH AND QUALITY OF LIFE: SIGNIFICANCE OF RESULTS

The clinical significance of these findings must be interpreted with respect to the population studied, the levels of exercise achieved, and the physiological effects of the actual CO exposure. The subjects selected for the HEI study all had evidence of atherosclerotic coronary artery obstruction, which limits coronary blood flow and reduces myocardial function. The increased cardiac output and myocardial oxygen demand required by exercise cannot be met beyond a specific threshold level in these individuals with coronary artery disease. The results of this study show that CO exposures producing 2%-COHb-target levels produce detectable changes in the ischemic threshold, as assessed by a reduction in the time to ST endpoint and the early development of angina pectoris. However, the relationship between the severity of these changes in exercise function and subsequent alterations in an individual's quality of life is more complicated and requires careful consideration.

The magnitude of the change in exercise performance produced by 2%-COHb-target levels in this study are similar to those considered clinically significant when evaluating the efficacy of antiischemic therapeutic interventions (Thadani et al. 1982; Petra et al. 1983; Parker and Fung 1984; Boden et al. 1985; Parker et al. 1987). While the production of ischemia during exercise is not necessarily related to major cardiac events, such as an increased risk of myocardial infarction or an acceleration of mortality, it is generally agreed that myocardial ischemia is detrimental. Intermittent brief periods of ischemia have a cumulative effect and may cause myocardial necrosis (Geft et al. 1982). In addition, myocardial ischemia can predispose an individual to ventricular arrhythmias (Bolli et al. 1986), lower the fibrillation threshold (Moore and Spear 1975), and may be related to sudden death (Morady et al. 1987).

The relationship between exercise capacity, as assessed on a treadmill or bicycle ergometer, and the ability to perform work or recreational activities is complicated. Unfortunately, there are no well-controlled, objective studies available to correlate these activities precisely with different levels of treadmill exercise. Thus, one is forced to approximate these relationships. In addition, although exercise level is best quantified by direct assessment of oxygen consumption ($\dot{V}_{O_2}$), it was felt that the changes in experimental methods that would have been required to measure $\dot{V}_{O_2}$ during exercise might have affected the primary cardiac endpoints. Thus, direct measurement of $\dot{V}_{O_2}$ was not performed, but a modified Naughton protocol was selected that utilizes a gradual stepwise increase in workload that correlates with $\dot{V}_{O_2}$. However, because there are individual differences in $\dot{V}_{O_2}$ during treadmill exercise, the precise $\dot{V}_{O_2}$ or METs[4] utilized at each level in the modified Naughton protocol can only be estimated. Despite these reservations, it is useful to consider the approximate exercise level at which ischemic changes occurred in this study population. Electrocardiographic evidence of ischemia developed in the average subject at approximately five to six METs. Thus, ischemia might be expected to occur in this "at-risk" population when individuals performing at light to moderate levels of activity were exposed to CO that produced COHb levels as low as 2%. These exercise levels might be reproduced by climbing one to two flights of stairs or walking on level, firm ground at a slow to moderate pace (two to three mph) for a distance of one-half to one mile. Thus, the subjects recruited for this study are not sedentary, and would be likely to perform different levels of recreational or work activity, but could not engage in heavy work. In those individuals with angina pectoris, exertional activity would be self-limited by the development of chest pain, whereas those with silent ischemia would not experience such a warning signal.

Physicians treating patients with coronary artery disease try to limit physical activity below a known ischemic threshold, usually defined by the results of exercise stress testing. Knowledge of the effect of CO on objective evidence of ischemia during exercise testing (such as time to ST changes) would therefore have an impact on the allowable levels of activity recommended by physicians, and thus on the patient's quality of life, independent of symptoms of angina pectoris. Thus, low levels of COHb produce health effects that can have significant consequences on the functional capacity of an individual performing activities of daily life. These effects occur with light to moderate levels of exercise that are routinely performed by most individuals.

Another important finding of this study was the fact that there was a dose-response relationship between COHb and ischemia without evidence of a measurable threshold effect. Examination of the results that compared the effects of increasing COHb from base-line levels (0.6%) to 2% and 3.9% COHb showed that each increment produced further changes in objective ECG measures of ischemia. This finding implies that even small increments in COHb could adversely affect myocardial function and produce ischemia. Thus, increases in COHb in individuals with high base-line COHb levels, such as cigarette smokers, might produce deleterious health effects. Further study in such exposed population groups is necessary to investigate this possibility.

[4] One MET equals 3.5 ml of O_2 consumed per kilogram body weight per minute, and is roughly equivalent to the $\dot{V}_{O_2}$ during resting basal conditions.

DEMOGRAPHIC PERSPECTIVE

An important question concerns the applicability of these findings to a general population of individuals with coronary artery disease. While this study was restricted to male nonsmokers with stable angina pectoris and reproducible exercise stress tests, clearly the biological effects of CO exposure are unlikely to be limited to this specific population group. Active tobacco smoking was an exclusion criterion for this study. Thus, it does not provide direct information on whether or not smokers with chronically elevated blood COHb levels respond with similar decreases in exercise capacity when incrementally subjected to higher CO levels or, less likely, whether or not they develop tolerance with chronic CO exposure. Although men were chosen to minimize the possibility of false-positive exercise tests, there is no evidence that women with coronary artery disease are less sensitive to CO. In addition, even those individuals with coronary artery disease who do not experience angina during exercise do develop typical ECG evidence of ischemia. Thus, the results of this investigation should be applicable to most individuals who develop ECG changes during exercise treadmill testing.

The fact that all subjects continued to take their usual antiischemic medications also makes them representative of an ambulatory population receiving optimal medical care for their coronary artery disease. There is no evidence that medications might affect these results, and an analysis showed no differences between subjects who either were or were not receiving beta-adrenergic blockers. It is also possible that some medications were protective, and that a less well-managed group might have been more sensitive to the effects of CO exposure. However, it was beyond the scope of this study to address the important questions of possible interactions between antianginal drugs and the responses to CO exposure.

If they are compared with another large, stable-angina, multicenter study population (2,982 patients) derived from the Coronary Artery Surgery Study (CASS) registry (Weiner et al. 1987), one finds that subjects in the HEI study are similar in terms of their base-line characteristics: age, gender, angina severity, incidence of prior myocardial infarctions, drug therapy, extent of coronary artery disease, and exercise performance. In addition, it should be noted that these characteristics are also similar to those of groups of individuals with silent ischemia who have ST-segment changes without angina during exercise treadmill testing, as well as those who develop angina without ST changes. Thus, the primary results of the study appear to be applicable to a more general population of subjects with coronary artery disease and stable angina pectoris.

There are 6.7 million individuals in the United States with ischemic heart disease, as defined by the U.S. Department of Health and Human Services (1986) in the National Heart Interview Survey of 1985. Overall, they comprise 2.9% of the United States population. This includes individuals known to have silent ischemia as well as those with angina pectoris. The presence of cardiac disease is in part a function of the age distribution of the population in the United States. For example, the frequency of individuals with ischemic heart disease is 61.8/1,000 among those ages 45 to 64 years, and 138.5/1,000 among those 65 years or older. Because survival in the United States has improved and the population distribution is shifting toward the elderly, the fraction of individuals with age-related diseases, such as ischemic heart disease, is continuing to increase.

RELEVANCE OF EXPOSURE CONDITIONS

The CO-exposure protocol in this study was designed to produce specific target levels of COHb in subjects at the end of exercise. The lower target level, 2.0% COHb by GC, is at the upper end of the range of values expected to occur after eight hours of exposure to CO at levels meeting the current NAAQS (an average of 9 ppm over eight hours) (U.S. Environmental Protection Agency 1984). It is also within the range expected from a one-hour exposure at 35 ppm (the level of the one-hour standard) if the subject is exercising and increases his ventilatory and CO loading rates. Thus, the COHb levels attained on the 2%-COHb-target-exposure day are relevant to the levels set by the NAAQS and are comparable to the range of levels in the studies summarized in Table 18.

Although COHb levels of about 2% are comparable to those achieved under exposure conditions that meet present standards, the actual exposure levels in the HEI study were higher than those defined by the one-hour standard of 35 ppm. In this study, the exposure levels ranged from 42 to 202 ppm for the 2%-COHb target, and from 143 to 357 ppm for the 4%-COHb target. In other studies summarized in Table 18, the exposure levels were 50 or 100 ppm and exposure times were one, two, or four hours.

Wallace and Ziegenfus (1985) summarized CO data from 36 outdoor monitors, and COHb levels in 1,528 nonsmokers, in 20 U.S. cities with populations greater than 100,000. The data were collected between 1976 and 1980 as part of the second National Health and Nutrition Examination Survey (NHANES). While some cities had several monitors, the subjects were not geographically localized in relation to specific CO monitors. Most COHb measurements were made by spectrophotometry, but there were reported to be no differences between those measurements and GC measurements on a subset of 200 blood samples.

The highest eight-hour mean COHb level in the 20 cities studied was 1.6%, found in residents of the District of Columbia, where the two CO monitors had mean levels of 1.8 and 3.5 ppm for the eight-hour period preceding blood sampling for COHb levels. In contrast, the lowest mean COHb level, 0.55%, was found in Des Moines, IA, where the mean CO level was 3.8 ppm. Thus, the average atmospheric CO levels at the monitoring stations in these cities did not correlate well with the measured COHb levels.

It is also interesting to examine data from individuals with the highest actual COHb levels (Wallace and Ziegenfus 1985). In four of the 20 cities, individuals with COHb levels in the top 5% had mean levels of greater than or equal to 2.0% COHb: District of Columbia (4.6%), San Diego (3.0%), Los Angeles (2.77%), and New York City (Bronx) (2.4%). We do not know whether or not the source of CO in those individuals is ambient air; nor can we necessarily equate these COHb levels with our GC values. However, even with an offset of 1%, the COHb levels are in the range that produces adverse effects in individuals with coronary artery disease.

Although many individuals had COHb levels greater than 2% in the 20 cities (Wallace and Ziegenfus 1985), only one of the 36 monitoring stations reported a mean level above the eight-hour average standard of 9 ppm. This occurred at one of the six Manhattan sites, where the CO level was recorded as 13.2 ppm. Two other Manhattan sites had the next highest levels of the 36 sites, at 5.9 and 5.2 ppm. Yet the COHb levels of the Manhattan residents were relatively low (1.04% mean) when compared to the COHb levels of residents of other cities, such as Washington, DC (1.6% COHb). Thus, the CO monitors did not provide an accurate method to predict COHb levels because they may not have been located near the relevant sources of CO. Furthermore, individual behavior patterns and activity levels may affect COHb levels. Given the limitations of COHb measurement at low concentrations, and the inability to relate ambient CO levels in large cities to COHb levels, the true prevalence of biologically significant COHb elevations in different urban populations is uncertain. Air-quality standards to limit "background" CO concentrations (as measured at monitoring stations) are unlikely to prevent localized exposures and are unlikely to prevent all individuals at risk from experiencing elevated COHb.

Ayres and coworkers (1979) measured COHb levels in more than 1,000 New York City residents. These individuals included people occupationally exposed to high levels of CO, such as policemen, bridge workers, and tunnel workers. Even among nonsmokers, COHb levels were higher than those reported by Wallace and Ziegenfus (1985). For example, the mean COHb level in nonsmoking policemen working in congested precincts in New York City was 3.4%. Among nonsmoking hospital patients, whose exposure to ambient CO should be relatively low, average COHb levels were 1.56%, compared to the 1.04% reported by Wallace and Ziegenfus (1985) for Manhattan residents. Some of the differences between the studies could be due to differences in the way COHb was measured, or to differences in the study population.

While the exposure levels in the HEI study, even for the 2%-COHb target, are above the one-hour standard of 35 ppm, they may not represent unusual exposure conditions. The 42- to 202-ppm CO-exposure levels that produced the 2%-COHb target fell within the range of CO-exposure levels to which parking-garage employees (80 ppm; Ramsey 1967), inspectors at border stations (5 to 120 ppm; Cohen et al. 1971), and vehicle-inspection-station employees (10 to 150 ppm; Hofreuter et al. 1962) are exposed. Anderson and coworkers (1973), citing the 1972 Air Quality Criteria for Carbon Monoxide by the NATO Committee on the Challenges of Modern Society, reported the following levels under various traffic conditions: 40 ppm was the maximal eight-hour average in the most polluted 5% of off-street central urban areas; 115 ppm was the average concentration inside automobiles in heavy traffic in such urban areas; and 80 ppm was the average concentration in automobiles on expressways. Thus, the mean exposure level of 117 ppm for the 2%-COHb target in the HEI study was not an uncommon level of exposure in the past. Aronow and coworkers (1972) reported results that suggest higher exposure levels; they observed a mean increase in arterial COHb levels from 1.1% to 5.1% (IL 182 CO-Oximeter) in 10 subjects after they spent 90 minutes in heavy freeway traffic. These levels were similar to the postexposure COHb level (5.6% by CO-Oximeter) on the 4%-COHb-target day in the HEI study. Thus, even on the 4%-COHb-target day, the blood COHb levels in the HEI study may be relevant to traffic-related exposures. Hopefully, these 4%-COHb-target levels are rarer today because of present vehicle-emission standards.

Smoking is the other major contributing factor to elevated COHb levels. In the study by Ayres and coworkers (1979), policemen from congested areas who did not smoke had COHb levels of 3.14%, whereas policemen from the same areas who did smoke had COHb levels of 8.11%. The NHANES II report describes average COHb levels of 4.53% in smokers and 0.88% in nonsmokers (Radford 1983). The 2%- and 4%-COHb levels at the end of exercise attained in the HEI study represent levels commonly observed among United States adults who live in urban environments. Smokers may be a particularly sensitive group with respect to CO exposure because of their elevated levels and increased risk for the development of coronary artery disease. Thus, there are important questions concerning the health effects of CO exposure in smokers and whether or not these individuals develop physiologic adaptations to chronic CO exposure.

In summary, the CO-exposure conditions and the resulting COHb levels in the HEI study appear to be within a realistic range for a fraction of the adult, nonsmoking, United States population that is heavily exposed to traffic or other local sources of CO. Unfortunately, such local exposures may be difficult to control with ambient air-quality standards. In addition, since very low levels affect myocardial function in subjects with coronary artery disease, one must also question whether or not CO exposures affect the function of other organ systems in individuals with generalized vascular disease.

SUMMARY AND CONCLUSIONS

We report results showing the effects of low-COHb levels on myocardial ischemia in 63 subjects with documented coronary artery disease. The lower dose of CO to subjects (mean 2% COHb) is consistent with the COHb levels that result from ambient exposure patterns that meet the eight-hour 9-ppm standard. Although the actual exposure levels are all above the one-hour 35-ppm standard, some short-term localized exposures might involve CO levels that approach the range of the lower experimental exposures.

At mean COHb levels of about 2% (by GC), subjects had a 5% reduction (trimmed mean) in the time to onset of ischemic ST-segment changes in the ECG, and a 4% reduction (trimmed mean) in time to onset of angina pectoris, compared to the control day. The 90% confidence intervals were 1.5% and 8.7% for ST, and 0.7% and 7.9% for angina, for the 2%-COHb analysis. At mean COHb levels of about 4% (by GC), subjects showed a 12% decrease (trimmed mean) in time to ST endpoint and a 7% decrease (trimmed mean) in time to angina, compared to the control day. The 90% confidence intervals were 9.0% and 15.3% for ST, and 3.1% and 10.9% for angina, for the 4%-COHb analysis.

In addition, a significant exposure-response relationship was found for the individual differences in the time to ST endpoint, for the pre- versus postexposure exercise tests, at the three COHb levels ($p \leq 0.0001$). For this range of COHb levels (0.2% to 5.1%), a 3.85 $\pm$ 0.63% decrease in time to ST endpoint occurred for every 1% increase in COHb.

These findings show that myocardial ischemia, as measured by both ECG changes and angina, develops at an earlier stage of exercise after CO exposure than after exposure to air alone.

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APPENDIX A. Prediction Equations for Forced Vital Capacity and Forced Expiratory Volume in One Second for Nonblack Male Subjects 25 Years or Older.

$FVC = (age \times -0.029) + (height \times 0.065) - 5.459$

$FEV_1 = (age \times -0.027) + (height \times 0.052) - 4.203$

Note: All results are in liters, body temperature, and pressure, saturated with water vapor. All heights are in centimeters. Equations are taken from Knudson and coworkers (1976).

APPENDIX B. Data on Individual Subjects

Data on the 63 subjects in the main analysis are listed in Table B.1. Data on six additional subjects, who completed all test visits but were not included in the main analysis because they did not meet protocol requirements, are listed in Table B.2.

KEY TO ABBREVIATIONS IN TABLES

ID = subject identification number; Johns Hopkins = 101-125; Rancho Los Amigos = 201-243; St. Louis = 301-339.
AGE = age in years (rounded to the nearest year).
WKG = weight in kilograms.
HCM = height in centimeters.
FEV_1 = forced expiratory volume in 1 second in liters.
CCSC = Canadian Cardiovascular Society class.
COAVG0 = average chamber CO concentration in ppm on air-exposure day.
COAVG2 = average chamber CO concentration in ppm on 2%-COHb-target-exposure day.
COAVG4 = average chamber CO concentration in ppm on 4%-COHb-target-exposure day.
GC01 = sample 2 (taken at end of first exercise test) %COHb by GC on air-exposure day.
GC02 = sample 6 (taken at end of second exercise test) %COHb by GC on air-exposure day.
GC21 = sample 2 %COHb by GC on 2%-COHb-target-exposure day.
GC22 = sample 6 %COHb by GC on 2%-COHb-target-exposure day.
GC41 = sample 2 %COHb by GC on 4%-COHb-target-exposure day.
GC42 = sample 6 %COHb by GC on 4%-COHb-target-exposure day.
CX01 = sample 2 (taken at end of first exercise test) %COHb by CO-Oximeter on air-exposure day.
CX02 = sample 6 (taken at end of second exercise test) %COHb by CO-Oximeter on air-exposure day.
CX21 = sample 2 %COHb by CO-Oximeter on 2%-COHb-target-exposure day.
CX22 = sample 6 %COHb by CO-Oximeter on 2%-COHb-target-exposure day.
CX41 = sample 2 %COHb by CO-Oximeter on 4%-COHb-target-exposure day.
CX42 = sample 6 %COHb by CO-Oximeter on 4%-COHb-target-exposure day.
ISTB1 = time to ST endpoint on the first exercise test on the qualifying visit (visit 1).
ISTB2 = time to ST endpoint on the second exercise test on the qualifying visit (visit 1).

IST01 = time to ST endpoint on the first exercise test on the air-exposure day.
IST02 = time to ST endpoint on the second exercise test on the air-exposure day.
IST21 = time to ST endpoint on the first exercise test on the 2%-COHb-target-exposure day.
IST22 = time to ST endpoint on the second exercise test on the 2%-COHb-target-exposure day.
IST41 = time to ST endpoint on the first exercise test on the 4%-COHb-target-exposure day.
IST42 = time to ST endpoint on the second exercise test on the 4%-COHb-target-exposure day.
DIST2 = percent decrease in time to ST on 2%-COHb-target day compared to air-exposure day.
DIST4 = percent decrease in time to ST on 4%-COHb-target day compared to air-exposure day.
IDPB1 = double product at ST endpoint on the first exercise test on the qualifying visit (visit 1).
IDPB2 = double product at ST endpoint on the second exercise test on the qualifying visit (visit 1).
IDP01 = double product at ST endpoint on the first exercise test on the air-exposure day.
IDP02 = double product at ST endpoint on the second exercise test on the air-exposure day.
IDP21 = double product at ST endpoint on the first exercise test on the 2%-COHb-target-exposure day.
IDP22 = double product at ST endpoint on the second exercise test on the 2%-COHb-target-exposure day.
IDP41 = double product at ST endpoint on the first exercise test on the 4%-COHb-target-exposure day.
IDP42 = double product at ST endpoint on the second exercise test on the 4%-COHb-target-exposure day.
DIDP2 = percent decrease in double product at ST endpoint on 2%-COHb-target day, compared to air-exposure day.
DIDP4 = percent decrease in double product at ST endpoint on 4%-COHb-target day, compared to air-exposure day.
ANGB1 = time to angina on the first exercise test on the qualifying visit (visit 1).
ANGB2 = time to angina on the second exercise test on the qualifying visit (visit 1).
ANG01 = time to angina on the first exercise test on the air-exposure day.
ANG02 = time to angina on the second exercise test on the air-exposure day.
ANG21 = time to angina on the first exercise test on the 2%-COHb-target-exposure day.
ANG22 = time to angina on the second exercise test on the 2%-COHb-target-exposure day.
ANG41 = time to angina on the first exercise test on the 4%-COHb-target-exposure day.
ANG42 = time to angina on the second exercise test on the 4%-COHb-target-exposure day.
DANG2 = percent decrease in time to angina on 2%-COHb-target-exposure day, compared to air-exposure day.
DANG4 = percent decrease in time to angina on 4%-COHb-target-exposure day, compared to air-exposure day.
ADP01 = double product at angina on the first exercise test on the air-exposure day.
ADP02 = double product at angina on the second exercise test on the air-exposure day.
ADP21 = double product at angina on the first exercise test on the 2%-COHb-target-exposure day.
ADP22 = double product at angina on the second exercise test on the 2%-COHb-target-exposure day.
ADP41 = double product at angina on the first exercise test on the 4%-COHb-target-exposure day.
ADP42 = double product at angina on the second exercise test on the 4%-COHb-target-exposure day.
DADP2 = percent decrease in double product at angina on 2%-COHb-target day, compared to air-exposure day.
DADP4 = percent decrease in double product at angina on 4%-COHb-target day, compared to air-exposure day.

Table B.1. Data on 63 Subjects in Main Analysis

ID	AGE	WKG	HCM	FEV_1	CCSC	COAVG0	COAVG2	COAVG4
101	60	83.2	190.5	3.23	2	0	141	280
102	66	70.0	167.6	3.47	2	0	185	235
103	65	72.3	175.3	1.86	2	2	159	303
104	55	104.0	177.8	2.47	2	0	119	241
105	62	79.5	182.8	3.01	2	0	202	320
106	70	80.5	172.7	1.69	2	0	195	343
107	51	86.4	172.7	2.10	3	0	127	217
108	72	89.6	168.9	2.81	2	0	174	357
109	58	77.2	167.5	3.28	2	0	115	217
110	61	70.0	170.0	3.36	2	0	130	264
111	71	74.5	193.8	2.61	2	0	129	268
112	41	72.0	167.5	—[a]	2	0	84	177
113	70	90.5	180.3	2.91	2	0	135	271
114	57	91.8	182.5	1.98	2	0	123	207
115	55	80.0	170.2	2.57	2	0	91	240
116	63	91.8	172.5	2.08	2	0	116	253
117	64	75.0	182.9	2.47	2	0	137	251
118	76	71.4	171.5	2.47	3	0	102	286
121	74	93.2	188.0	2.74	2	0	116	257
122	54	75.0	174.0	2.88	2	0	110	264
124	58	84.1	171.2	2.63	2	0	116	331
125	73	84.1	167.5	2.53	2	0	140	295
201	56	99.1	186.7	3.22	2	1	42	170
205	65	89.1	177.8	2.88	2	2	61	143
207	65	70.9	162.6	1.71	2	1	57	163
213	63	75.5	170.2	2.89	2	2	144	289
214	57	77.7	170.2	3.77	2	1	112	245
215	47	85.5	185.0	3.29	2	1	129	303
218	72	81.8	182.9	3.39	2	1	89	222
221	56	81.7	177.8	—	2	1	142	300
222	52	76.4	180.0	3.86	2	1	117	299
227	75	74.4	172.7	2.64	2	1	126	266
229	66	71.7	177.8	2.71	2	2	170	302
234	62	82.0	165.0	2.71	2	1	95	224
235	68	78.0	173.0	3.41	2	1	112	245
236	75	75.0	173.0	2.87	2	2	128	242
237	60	—	—	—	2	2	130	263
239	73	125.0	167.6	2.61	2	1	161	315
241	63	71.4	168.0	3.39	2	1	121	307
243	64	—	180.3	3.17	2	1	147	307
301	67	88.6	180.3	2.79	2	2	63	219
303	56	90.9	176.3	3.22	2	1	89	173
304	65	78.2	167.6	2.25	2	1	81	172
306	61	110.0	182.9	2.98	2	0	68	210
310	63	83.2	177.8	2.47	2	1	134	252
311	59	71.8	162.6	2.77	2	1	56	150
317	42	85.5	182.9	2.95	2	1	67	197
318	53	81.8	185.4	3.56	2	—	123	252
323	63	97.0	182.5	2.88	2	1	114	274
324	65	79.5	185.4	2.63	2	1	174	315
325	60	91.8	188.0	3.38	3	1	110	279
326	57	75.5	180.3	3.07	2	1	82	238
327	62	79.1	172.7	2.81	2	0	—	217
328	70	83.6	182.5	3.17	2	1	102	231
330	73	85.9	168.7	2.23	2	1	119	283
332	63	80.5	176.5	2.19	2	0	76	188
333	55	86.4	175.0	4.06	2	1	118	222
334	52	87.7	170.2	1.98	2	0	89	236
335	58	77.3	170.2	2.81	2	1	152	299
336	68	72.7	182.9	2.85	2	1	84	210
337	70	91.8	177.8	2.43	2	0	110	262
338	72	95.9	182.9	3.20	2	1	141	294
339	45	86.4	177.8	3.53	2	1	98	277

[a] — = data not available. See main text for discussion.

Table B.1. Continued

ID	GC01	GC02	GC21	GC22	GC41	GC42	CX01	CX02	CX21	CX22	CX41	CX42
101	0.5	0.5	0.7	2.3	0.5	3.0	0.9	1.0	1.3	2.6	0.9	4.6
102	0.6	0.4	0.5	2.6	0.7	5.1	1.0	0.8	0.8	3.2	0.9	4.9
103	0.8	1.1	0.7	2.8	0.7	4.7	1.5	1.5	1.4	3.6	1.2	5.2
104	0.4	0.3	0.5	2.1	0.7	4.4	0.8	0.9	0.8	2.6	0.9	4.9
105	0.6	0.7	0.7	2.9	0.9	4.8	0.7	0.8	1.2	3.4	1.2	5.4
106	1.1	1.1	0.6	2.3	0.5	4.4	1.4	1.2	0.6	2.4	0.9	4.8
107	0.6	0.6	0.6	2.3	1.1	3.7	0.7	0.7	0.7	2.5	1.3	4.2
108	0.5	0.5	0.6	2.2	0.5	3.7	1.0	1.0	1.1	2.7	1.0	4.5
109	0.5	0.5	0.5	2.4	0.5	3.8	0.9	0.8	1.1	2.8	1.4	5.0
110	0.8	0.6	0.5	2.3	0.6	3.9	1.2	1.3	1.1	2.7	1.3	4.8
111	0.7	0.7	0.5	2.1	0.5	4.0	1.3	0.9	1.1	2.6	0.9	4.6
112	0.5	0.4	0.5	1.9	0.5	3.0	1.7	1.8	1.7	2.8	1.6	4.3
113	0.4	0.4	0.5	2.4	0.7	3.8	1.1	1.1	1.4	3.1	1.1	4.5
114	0.5	0.6	0.8	2.3	0.8	3.6	1.3	1.4	1.3	2.8	1.2	4.6
115	0.8	0.8	0.8	1.9	0.9	3.7	1.8	1.6	1.8	2.8	1.6	5.0
116	0.7	0.6	0.7	2.1	0.7	3.6	1.3	1.1	1.1	2.4	1.0	4.3
117	0.7	0.7	0.7	2.3	0.7	4.3	1.0	1.2	1.0	2.7	1.0	4.7
118	0.5	0.6	0.7	2.3	0.4	3.7	1.0	0.7	1.5	2.6	0.9	4.5
121	0.2	0.2	0.2	1.8	0.5	4.4	0.8	0.5	1.0	2.6	0.7	5.4
122	0.5	0.4	0.6	2.1	0.5	3.5	1.1	1.0	1.3	3.1	1.1	4.9
124	0.6	0.5	0.7	1.8	0.8	4.5	1.5	1.5	1.7	2.6	1.5	5.3
125	0.6	0.6	0.7	2.4	0.6	4.3	0.8	0.7	1.0	2.6	1.0	4.9
201	1.0	1.0	0.7	1.3	0.6	3.2	2.0	1.8	1.9	2.3	1.7	4.5
205	0.7	0.8	0.3	1.4	0.3	2.7	1.4	1.5	1.3	2.3	1.5	4.1
207	0.6	0.6	1.0	2.0	0.6	3.4	1.4	1.4	1.8	2.8	1.5	4.4
213	0.7	0.6	0.5	2.0	0.7	4.3	1.0	0.8	0.9	2.8	1.0	4.9
214	0.8	0.7	0.5	1.8	0.8	3.4	1.4	1.2	1.3	2.4	1.6	4.4
215	0.9	0.8	0.9	1.9	1.3	4.5	1.5	1.5	1.4	2.8	1.4	5.5
218	0.8	0.8	0.8	1.6	0.8	3.9	2.1	1.7	1.7	2.8	2.0	5.0
221	0.8	0.8	0.6	2.3	0.6	4.5	1.0	1.0	0.9	2.6	0.8	5.0
222	0.6	0.7	0.7	2.2	0.6	4.4	1.3	1.3	1.3	2.7	0.9	5.3
227	—	0.5	0.6	2.4	0.7	4.4	0.7	0.6	0.8	2.4	0.7	4.5
229	0.8	0.7	0.5	2.0	0.8	3.9	0.7	0.9	0.5	2.2	0.9	4.3
234	0.8	0.6	1.0	2.1	0.8	3.9	1.3	1.1	1.3	2.4	1.2	4.4
235	0.5	0.5	0.9	2.1	0.6	4.2	0.7	0.7	1.0	2.3	0.9	4.6
236	0.7	0.8	0.5	1.8	0.8	4.1	0.8	1.0	0.8	2.0	1.1	4.6
237	0.7	0.6	0.4	1.9	0.6	4.2	1.3	1.1	1.0	2.7	1.2	4.9
239	0.9	0.8	0.8	2.5	0.8	5.0	1.3	1.0	0.9	2.9	1.2	5.5
241	0.9	0.8	1.0	2.6	0.8	4.7	1.3	1.0	1.4	2.8	1.1	4.9
243	0.9	0.7	0.7	3.0	0.7	4.4	1.3	0.8	1.1	3.3	1.3	4.8
301	0.4	0.2	0.4	1.0	0.3	2.4	1.2	1.2	1.7	2.4	1.3	3.7
303	0.5	0.5	0.4	1.1	0.3	2.3	1.7	1.8	1.2	2.5	2.1	3.9
304	0.7	0.6	0.5	1.5	0.3	2.6	1.7	1.4	1.2	2.5	1.5	4.0
306	0.8	0.6	0.6	1.4	0.7	3.4	2.2	1.6	1.8	2.2	1.3	4.0
310	0.5	0.4	0.5	1.4	0.5	3.1	0.8	0.8	1.0	2.5	1.1	4.1
311	0.7	0.7	0.9	1.5	0.8	3.0	1.4	1.0	1.9	2.3	2.2	4.1
317	0.4	0.6	0.4	1.4	0.5	2.9	1.8	2.1	2.2	2.9	1.5	4.4
318	0.3	0.3	0.6	2.1	0.4	4.3	0.7	0.7	0.9	2.6	0.9	4.8
323	1.0	0.9	0.5	1.7	0.8	4.3	1.9	1.8	1.2	2.4	1.2	4.9
324	0.3	0.4	0.3	1.8	0.5	4.4	0.6	0.6	0.6	2.2	0.8	4.8
325	0.6	0.6	0.6	2.1	0.5	4.1	1.5	1.5	1.5	3.1	1.3	5.3
326	0.7	0.7	0.6	2.0	0.6	3.9	1.5	1.4	1.6	2.6	1.1	4.9
327	0.9	0.8	—	—	0.8	3.7	2.1	1.8	—	—	2.2	5.5
328	0.5	0.8	0.4	1.7	0.7	3.4	1.2	1.5	1.3	2.6	1.4	4.8
330	0.6	0.7	0.7	2.2	0.9	4.5	1.2	1.1	1.3	2.7	1.2	4.9
332	0.7	0.7	1.0	1.9	0.6	3.6	1.5	1.4	1.7	2.7	1.9	4.8
333	0.4	0.3	0.7	2.0	0.7	3.4	0.8	0.9	1.1	2.6	1.4	4.2
334	0.5	0.6	0.8	1.9	0.6	3.4	1.1	0.8	1.5	2.7	1.3	4.1
335	0.5	0.5	0.3	1.7	0.5	4.0	0.7	0.7	0.7	2.2	0.8	4.6
336	0.6	0.6	0.6	1.9	0.6	4.1	1.4	1.1	1.5	2.8	1.4	4.9
337	0.7	0.6	0.8	1.7	0.5	3.6	1.4	1.1	1.3	2.4	1.1	4.6
338	0.5	0.5	0.5	2.0	0.4	4.3	1.0	1.0	0.9	2.6	0.9	4.9
339	0.6	0.7	0.5	1.4	0.6	3.9	1.6	1.6	1.6	2.8	1.2	4.4

Table B.1. Continued

ID	ISTB1	ISTB2	IST01	IST02	IST21	IST22	IST41	IST42	DIST2	DIST4
101	870	820	1,005	1,000	1,010	830	1,039	960	17.324	7.106
102	120	250	190	250	220	220	200	220	31.579	21.579
103	739	700	800	610	860	600	840	520	6.483	14.345
104	590	540	660	590	720	480	660	360	22.727	34.848
105	460	420	540	540	540	524	603	480	2.963	20.398
106	360	320	300	360	340	420	330	300	−3.529	29.091
107	240	240	300	220	360	290	360	240	−7.222	6.667
108	420	480	520	420	460	390	520	360	−4.013	11.538
109	240	525	390	490	340	380	440	400	13.876	34.732
110	840	840	900	1,028	900	840	900	840	20.889	20.889
111	350	360	460	460	510	470	480	410	7.843	14.583
112	700	850	700	960	720	1,130	510	650	−19.802	9.692
113	690	600	700	570	700	650	770	700	−11.429	−9.481
114	720	640	907	780	750	540	820	540	13.998	20.144
115	400	550	480	420	470	520	460	480	−23.138	−16.848
116	600	600	630	720	700	740	710	660	8.571	21.328
117	530	550	520	525	490	450	530	430	9.125	19.829
118	600	680	650	740	420	440	780	620	9.084	34.359
121	460	430	360	450	460	390	230	240	40.217	20.652
122	1,010	990	1,020	1,030	1,050	980	870	1,010	7.647	−15.112
124	520	640	600	600	620	680	750	590	−9.677	21.333
125	430	495	480	505	600	625	620	460	1.042	31.015
201	520	430	—	—	—	—	—	—	—	—
205	690	525	780	570	615	480	540	310	−4.972	15.670
207	490	460	440	420	460	400	420	420	8.498	−4.545
213	720	600	750	720	800	690	780	660	9.750	11.385
214	670	645	640	790	697	810	920	900	7.225	25.611
215	820	930	910	970	900	960	1,113	930	−0.073	23.035
218	250	300	360	480	520	600	420	540	17.949	4.762
221	650	710	620	650	600	590	440	530	6.505	−15.616
222	480	910	710	900	700	730	600	780	22.475	−3.239
227	635	660	540	600	580	600	630	620	7.663	12.698
229	780	840	870	840	910	870	870	820	0.947	2.299
234	540	470	420	440	350	390	470	430	−6.667	13.273
235	720	670	660	660	680	590	690	600	13.235	13.043
236	380	380	380	400	400	400	360	460	5.263	−22.515
237	510	590	600	630	600	540	570	520	15.000	13.772
239	260	320	240	380	400	430	370	240	50.833	93.468
241	410	450	440	580	560	520	260	320	38.961	8.741
243	410	510	500	710	540	620	550	570	27.185	38.364
301	471	542	662	660	649	630	720	707	2.625	1.503
303	644	693	708	731	700	767	627	556	−6.323	14.572
304	420	449	424	348	300	360	420	335	−37.925	2.314
306	445	508	617	580	748	717	660	641	−1.852	−3.118
310	240	300	280	240	310	360	220	210	−30.415	−9.740
311	390	400	480	420	540	490	480	450	−3.241	−6.250
317	700	690	720	600	650	670	700	590	−19.744	−0.952
318	400	440	430	480	430	420	540	480	13.953	22.739
323	360	620	500	480	370	420	440	420	−17.514	0.545
324	580	740	690	770	890	760	630	580	26.201	19.531
325	270	300	300	300	290	350	290	220	−20.690	24.138
326	400	500	550	630	540	510	530	533	20.101	13.979
327	300	400	410	540	—	—	490	530	—	23.544
328	420	360	700	540	720	470	615	410	11.865	10.476
330	145	145	145	185	170	215	100	120	1.116	7.586
332	480	420	390	420	525	540	480	440	4.835	16.026
333	705	753	840	785	875	780	780	760	4.310	−3.983
334	799	750	785	780	690	679	720	681	0.957	4.780
335	200	424	400	420	390	390	360	360	5.000	5.000
336	440	540	410	390	390	300	320	315	18.199	−3.315
337	290	290	264	290	270	250	360	270	17.256	34.848
338	150	150	220	280	180	290	180	180	−33.838	27.273
339	780	780	820	830	840	850	810	720	0.029	12.331

Table B.1. Continued

ID	IDPB1	IDPB2	IDP01	IDP02	IDP21	IDP22	IDP41	IDP42	DIDP2	DIDP4
101	21,877	20,574	23,842	20,960	20,328	19,920	—	18,392	-10.081	—
102	12,948	12,876	11,319	9,936	11,454	11,952	—	11,544	-27.610	-25.250
103	16,800	15,908	20,384	15,840	18,144	15,936	—	17,264	-2.116	1.020
104	11,570	10,980	12,015	9,152	10,672	9,350	10,005	7,584	-11.441	0.369
105	17,222	17,440	14,356	14,356	14,016	13,968	—	14,688	0.342	—
106	17,670	16,878	13,616	15,470	15,486	15,416	16,704	16,340	14.068	15.795
107	16,896	15,792	13,090	14,442	13,572	13,104	12,240	12,118	13.777	11.325
108	11,592	10,140	11,316	10,270	12,464	11,016	11,316	10,010	2.374	2.298
109	17,460	—	15,675	19,190	17,945	16,740	—	16,150	11.150	1.405
110	16,585	15,500	15,520	—	15,965	13,650	—	11,180	—	—
111	16,380	18,600	18,600	16,240	23,560	19,965	18,290	18,880	2.571	-15.914
112	24,940	25,420	21,645	28,200	21,255	25,200	23,310	24,360	11.724	25.780
113	27,470	23,370	25,740	21,960	27,090	25,625	24,960	23,180	-9.277	-7.554
114	17,860	16,465	15,345	15,810	15,015	13,120	—	12,300	3.876	11.099
115	16,000	16,320	14,570	16,625	16,660	16,490	15,190	16,320	15.125	6.665
116	24,510	22,860	20,900	23,595	18,330	22,935	24,510	23,180	-12.228	18.321
117	11,760	12,015	12,600	13,440	11,620	13,175	14,850	12,900	-6.715	19.798
118	25,410	26,445	27,930	29,400	27,060	25,410	—	21,875	9.692	25.273
121	12,740	13,175	13,600	12,450	12,600	12,600	—	13,175	-11.577	-8.452
122	20,060	18,880	17,760	26,230	21,250	21,240	17,670	18,645	47.739	42.174
124	12,600	16,320	13,630	13,020	13,195	13,485	—	11,000	1.506	22.999
125	17,160	19,800	16,200	17,440	19,080	21,060	—	16,275	-5.727	4.188
201	22,344	18,645	—	—	—	—	—	—	—	—
205	17,440	15,600	—	14,157	16,050	14,400	13,860	12,183	—	—
207	12,264	13,585	12,212	11,524	10,368	10,962	11,658	12,354	-11.363	-11.604
213	13,680	14,124	14,300	14,472	14,560	14,985	—	13,764	-3.250	3.418
214	8,300	9,108	9,200	8,526	—	9,894	—	10,710	—	-24.854
215	14,336	13,680	—	12,992	14,022	14,986	—	13,420	-13.239	—
218	17,549	16,536	17,331	14,148	18,240	15,429	16,800	16,060	-2.955	-13.961
221	28,250	29,748	21,186	26,445	23,940	23,128	16,856	21,450	28.215	-2.431
222	11,400	15,554	12,580	14,948	13,770	12,320	11,040	14,700	29.354	-14.329
227	17,700	18,150	16,748	17,228	18,290	17,374	17,538	17,690	7.874	1.999
229	25,344	23,400	23,056	22,078	25,632	25,988	25,110	21,280	-5.631	11.011
234	14,700	14,112	14,342	12,376	13,824	13,528	14,162	13,104	-11.567	-6.237
235	28,800	23,852	21,082	22,176	23,674	19,520	22,078	20,320	22.736	13.152
236	15,092	16,416	14,356	16,170	13,936	15,762	15,484	16,724	-0.467	4.628
237	17,952	19,600	15,252	19,440	17,816	15,494	15,616	17,136	40.492	17.725
239	10,248	9,744	9,794	10,080	10,208	10,384	—	9,912	6.083	6.603
241	11,328	11,408	12,800	13,720	12,348	12,350	12,512	11,700	7.171	13.677
243	14,784	15,244	14,204	16,650	16,906	16,016	—	15,194	17.069	4.835
301	15,480	16,296	—	15,744	16,856	15,400	—	15,352	11.864	—
303	19,040	19,500	16,352	19,680	17,250	17,666	18,170	17,280	17.941	25.250
304	11,532	12,416	12,152	12,416	12,864	13,668	13,328	12,730	-4.078	6.659
306	18,060	16,800	16,016	15,092	10,812	13,578	—	14,840	-48.896	-15.970
310	9,394	9,300	8,280	8,400	10,140	9,728	—	8,280	-8.437	-12.500
311	13,950	13,950	13,870	13,286	14,476	13,536	16,095	12,848	2.283	15.963
317	14,980	15,008	14,388	13,568	14,300	16,380	15,594	14,874	-20.245	-1.082
318	11,532	11,960	10,476	10,528	10,890	10,800	12,154	11,780	1.323	3.574
323	16,960	22,860	18,334	22,000	13,720	20,240	16,000	15,958	-27.526	20.258
324	14,100	14,850	14,592	14,200	15,194	18,180	—	14,550	-13.572	6.369
325	15,088	14,352	13,468	15,456	14,040	14,940	13,764	10,416	8.351	39.085
326	11,880	12,360	11,400	12,036	11,660	11,400	—	11,000	6.564	7.843
327	13,724	17,400	16,072	16,324	—	—	—	17,304	—	-11.256
328	14,700	14,550	21,384	18,880	19,296	16,872	16,284	14,628	0.852	-1.540
330	23,552	23,312	18,816	17,696	19,200	18,600	22,784	17,612	-2.827	16.748
332	20,172	19,680	16,274	14,040	20,592	17,908	17,600	17,854	-0.693	-15.171
333	26,880	27,170	27,416	31,518	33,598	29,682	—	29,400	18.860	-0.033
334	11,570	12,880	13,200	12,240	10,920	12,780	—	12,460	-19.890	2.749
335	14,630	15,088	14,476	13,580	15,840	14,630	13,140	13,870	1.449	-11.745
336	19,188	18,720	13,720	15,708	16,590	13,580	—	12,610	1.626	-8.427
337	13,312	14,144	15,552	15,540	17,056	13,400	—	12,800	29.352	25.612
338	19,520	20,172	16,800	19,968	16,800	22,606	17,760	18,876	-15.702	12.573
339	21,600	19,458	21,754	19,832	21,024	24,864	19,388	20,002	-27.100	-12.002

Table B.1. Continued

ID	ANGB1	ANGB2	ANG01	ANG02	ANG21	ANG22	ANG41	ANG42	DANG2	DANG4
101	840	803	983	957	1,005	790	1,020	885	18.748	10.590
102	263	328	260	290	256	239	255	265	18.179	7.617
103	630	538	709	489	593	400	630	419	1.517	2.462
104	574	462	655	490	710	520	645	415	1.570	10.468
105	393	370	240	200	245	225	330	255	-8.503	6.061
106	200	251	295	298	315	293	310	305	8.001	2.630
107	210	225	238	239	320	250	327	230	22.295	30.084
108	530	482	475	495	510	430	525	440	19.897	20.401
109	420	305	520	588	380	400	378	465	7.814	-9.939
110	945	630	902	665	660	405	610	645	12.361	-32.013
111	285	320	294	350	330	240	345	298	46.320	32.671
112	650	670	715	635	960	795	630	605	5.999	-7.221
113	645	554	675	600	690	610	705	707	0.483	-11.395
114	690	625	765	655	700	470	725	505	18.478	15.966
115	540	576	540	525	515	495	590	645	1.106	-12.100
116	390	471	530	502	515	500	534	508	-2.370	-0.414
117	270	280	300	300	280	280	362	300	0.000	17.127
118	489	585	562	485	465	465	610	525	-13.701	0.233
121	450	420	285	240	330	315	250	250	-11.244	-15.789
122	1,140	1,130	1,245	1,190	1,140	1,050	1,170	1,170	3.477	-4.418
124	710	800	760	750	705	770	855	675	-10.536	19.737
125	380	365	420	410	425	430	450	375	-3.557	14.286
201	490	530	605	727	590	635	365	380	12.538	16.056
205	647	530	660	442	448	380	453	251	-17.852	11.561
207	579	533	495	445	485	493	375	410	-11.750	-19.434
213	537	608	547	463	718	482	652	489	17.513	9.644
214	456	592	549	701	452	499	693	757	17.288	18.451
215	526	669	924	1,001	883	830	1,073	1,005	14.336	14.671
218	249	316	252	388	280	255	296	375	62.897	27.279
221	443	654	554	537	515	503	568	428	-0.738	21.579
222	824	919	713	785	893	838	786	800	16.257	8.317
227	549	628	535	556	548	541	555	516	5.203	10.952
229	907	706	871	767	752	724	621	670	-8.217	-19.831
234	409	412	440	456	400	429	407	480	-3.614	-14.300
235	551	591	657	632	728	592	676	547	14.876	15.278
236	499	468	544	466	478	429	478	380	-4.087	6.164
237	373	394	300	315	475	450	410	320	10.263	26.951
239	340	412	320	420	530	490	360	300	38.797	47.917
241	290	300	367	419	465	415	190	260	24.922	-22.673
243	250	340	414	491	366	379	454	450	15.047	19.480
301	290	315	420	361	393	344	520	356	-1.579	17.491
303	756	781	801	705	772	628	770	562	6.668	15.028
304	445	495	480	480	390	465	470	445	-19.231	5.319
306	410	480	420	604	820	622	780	615	67.956	64.963
310	240	300	205	280	315	408	240	225	7.062	42.835
311	260	300	315	315	351	316	360	352	9.972	2.222
317	240	418	640	515	498	614	343	470	-42.824	-56.557
318	349	353	450	472	455	455	574	480	4.889	21.265
323	390	510	450	480	501	497	480	490	7.465	4.583
324	470	570	590	580	650	590	560	500	7.536	9.019
325	310	310	278	275	280	315	240	230	-13.579	3.088
326	530	470	527	408	450	450	495	380	-22.581	0.652
327	345	450	505	490	—	—	442	435	—	-1.387
328	354	270	720	580	719	557	706	486	3.087	11.717
330	240	240	210	222	200	260	190	204	-24.286	-1.654
332	575	493	506	506	644	632	600	532	1.863	11.333
333	414	468	522	502	580	616	497	500	-10.038	-4.435
334	640	660	524	604	447	401	440	420	25.558	19.813
335	262	203	398	244	428	375	333	386	-26.310	-54.609
336	390	437	380	317	350	350	400	390	-16.579	-14.079
337	364	302	304	305	306	319	362	290	-3.919	20.218
338	180	180	256	257	245	255	203	194	-3.691	4.824
339	660	630	680	735	714	705	769	671	9.349	20.832

Table B.1. Continued

ID	ADPB1	ADPB2	ADP01	ADP02	ADP21	ADP22	ADP41	ADP42	DADP2	DADP4
101	19,754	18,696	23,114	19,680	20,328	18,093	19,276	17,365	-3.862	-4.943
102	15,604	13,200	13,110	12,784	11,454	11,952	13,950	11,988	-6.834	11.578
103	15,326	14,062	16,400	13,024	12,006	12,848	16,296	13,248	-27.599	-1.881
104	11,310	10,736	12,060	9,288	10,672	9,288	10,266	7,520	-10.017	3.763
105	16,380	14,790	11,658	11,424	11,730	11,122	12,920	11,776	3.176	6.847
106	16,928	14,924	12,876	13,608	14,608	13,172	15,438	16,340	15.515	-0.158
107	16,896	15,792	12,474	14,442	12,636	12,300	12,240	12,118	18.436	16.774
108	12,006	10,140	10,744	10,530	12,464	11,016	11,316	10,530	9.626	4.954
109	20,384	18,228	19,775	21,060	18,810	16,625	15,345	16,490	18.114	-0.964
110	17,250	12,880	12,285	9,125	12,615	10,010	12,000	11,760	-5.072	-23.722
111	17,400	18,870	15,950	14,580	17,360	15,900	17,440	16,740	-0.179	-4.576
112	23,520	22,000	21,534	22,464	22,800	20,865	23,400	23,165	12.806	5.323
113	26,058	21,294	25,026	21,960	27,090	24,400	23,232	22,936	-2.321	-10.977
114	14,960	16,465	14,960	13,120	13,920	12,160	13,485	12,300	0.344	-3.512
115	17,160	16,665	15,840	18,200	17,340	17,170	16,335	19,250	15.879	-2.946
116	18,576	20,592	20,492	17,622	15,860	16,800	19,800	19,440	-19.932	-12.187
117	11,592	9,828	9,360	11,780	10,050	10,626	12,090	11,060	20.123	34.374
118	20,710	23,205	24,800	20,900	28,575	26,840	19,980	20,880	-9.654	-20.230
121	12,740	13,175	12,900	10,780	11,900	11,745	13,600	13,175	-15.132	-13.309
122	21,760	22,925	25,800	27,090	21,285	22,100	25,460	24,700	1.171	7.985
124	15,190	18,375	15,965	15,750	13,950	14,355	13,920	11,875	-4.250	13.344
125	17,160	16,160	15,360	16,480	14,260	15,500	15,520	15,655	-1.404	6.422
201	21,780	21,125	28,690	30,135	26,325	28,371	23,125	20,520	-2.735	16.301
205	16,960	16,536	15,520	12,696	13,300	14,100	13,200	11,960	-24.211	-8.802
207	14,210	14,300	10,710	12,240	12,282	12,768	11,658	12,354	10.329	8.316
213	12,180	14,124	12,870	12,177	12,720	13,000	12,600	11,446	-7.586	3.774
214	10,622	8,342	8,930	8,330	7,998	8,281	9,312	10,098	-10.257	-15.160
215	9,991	10,506	14,112	12,992	13,673	15,904	12,915	10,283	-24.253	12.443
218	17,549	14,688	16,170	13,416	16,200	10,028	15,656	13,332	21.067	-2.187
221	19,136	25,288	22,422	20,176	17,670	19,000	22,504	18,952	-17.544	5.767
222	14,852	16,324	14,440	13,020	15,840	13,680	14,664	15,200	3.803	-13.489
227	16,872	18,150	16,748	16,128	17,328	16,206	17,538	16,352	2.773	3.061
229	30,300	19,720	23,936	19,926	20,496	23,048	17,976	18,720	-29.204	-20.892
234	13,616	14,112	14,484	13,160	15,038	15,132	12,600	14,112	-9.766	-21.141
235	24,384	22,272	21,082	21,840	24,300	19,520	21,580	20,088	23.266	10.509
236	16,720	16,872	16,352	15,960	15,120	15,904	16,060	17,248	-7.582	-9.795
237	14,640	17,250	13,680	13,080	15,180	15,750	12,656	13,806	-8.141	-13.473
239	10,266	10,976	10,602	11,328	11,640	11,252	10,208	10,324	10.181	5.711
241	11,136	10,620	12,800	11,520	11,844	11,520	12,512	11,700	-7.264	-3.510
243	12,672	12,848	14,850	14,112	14,544	11,700	13,936	12,936	14.585	2.206
301	12,782	12,792	13,284	12,636	13,284	11,680	14,104	12,000	7.197	10.040
303	19,840	19,500	17,550	18,720	18,876	15,840	21,760	17,628	22.751	25.656
304	11,532	12,544	12,524	12,800	14,204	14,338	14,000	13,936	1.260	2.661
306	17,340	18,618	15,040	15,092	—	14,600	16,350	14,560	—	11.294
310	9,394	9,300	8,968	9,516	10,140	11,180	8,280	9,072	-4.146	-3.455
311	13,350	13,650	12,460	12,600	11,826	10,586	13,172	11,900	11.609	10.780
317	9,612	11,200	13,992	12,090	12,152	14,690	10,152	12,726	-34.479	-38.948
318	11,532	11,036	10,670	10,528	10,890	10,800	12,154	11,780	-0.504	1.746
323	17,600	20,416	18,792	22,000	17,628	21,712	16,640	18,094	-6.097	8.333
324	12,556	12,600	13,800	12,780	11,960	14,378	14,208	13,350	-27.609	-1.352
325	14,104	14,352	12,580	12,144	14,040	14,220	14,352	12,144	-4.748	11.919
326	12,960	13,260	11,424	9,790	12,000	10,340	9,672	10,148	-0.470	-19.225
327	16,728	16,800	17,304	15,200	—	—	15,484	13,912	—	-2.007
328	13,440	13,616	21,384	18,056	19,296	19,040	17,544	15,984	-14.236	-6.671
330	22,680	24,700	22,072	17,696	19,200	19,500	23,852	20,540	-21.389	-5.940
332	23,040	21,248	18,150	16,974	25,024	20,000	19,650	18,944	13.597	-2.886
333	22,680	23,500	25,020	24,180	25,298	24,742	22,176	23,140	-1.160	-7.704
334	10,790	11,730	9,796	11,340	9,856	11,340	10,780	12,480	0.705	-0.008
335	14,852	13,248	14,476	10,720	15,840	14,630	13,140	13,870	-18.308	-31.502
336	17,538	16,352	17,160	14,454	16,590	14,280	14,980	16,060	-1.845	-22.979
337	14,204	14,144	16,016	15,540	17,056	14,600	15,552	13,600	11.428	9.579
338	19,520	20,172	16,800	18,056	19,188	19,520	18,796	18,876	5.746	7.051
339	18,144	15,410	17,152	18,354	17,554	20,592	18,216	19,126	-10.299	2.012

Table B.2. Data on Six Subjects Who Completed Four Visits But Were Excluded from Main Analysis

ID	AGE	WKG	HCM	FEV_1	CCSC	COAVG0	COAVG2	COAVG4
202	55	74.5	175.3	2.92	2	2	76	157
203	67	86.4	188.0	3.76	2	2	79	172
208	55	101.8	188.0	3.04	2	2	92	271
210	61	70.5	171.4	3.00	2	1	129	310
211	57	100.0	175.3	2.84	2	1	114	284
309	68	80.9	172.7	2.48	2	1	55	196

ID	GC01	GC02	GC21	GC22	GC41	GC42	CX01	CX02	CX21	CX22	CX41	CX42
202	0.6	0.4	0.4	1.4	0.5	2.9	1.0	1.0	0.8	2.0	1.0	4.3
203	0.5	0.6	0.5	1.9	0.5	2.9	1.3	1.3	1.1	2.6	1.4	4.2
208	0.9	0.9	0.7	1.6	0.6	4.4	1.6	1.4	1.8	2.4	1.6	5.0
210	0.4	0.4	0.5	1.9	0.8	4.3	1.2	1.3	1.4	2.8	1.4	5.2
211	0.7	0.7	1.0	2.8	0.7	5.5	1.0	0.9	1.4	3.0	1.2	5.4
309	0.6	0.4	0.5	1.6	1.0	3.5	1.5	1.1	2.1	2.1	1.8	4.5

ID	ISTB1	ISTB2	IST01	IST02	IST21	IST22	IST41	IST42	DIST2	DIST4
202	1,020	1,080	—[a]	—	—	—	—	—	—	—
203	900	720	—	—	—	—	—	—	—	—
208	790	639	—	—	—	—	—	—	—	—
210	960	960	840	900	990	840	900	780	22.2944	20.4762
211	466	550	—	—	—	—	—	—	—	—
309	430	420	—	—	—	—	—	—	—	—

ID	IDPB1	IDPB2	IDP01	IDP02	IDP21	IDP22	IDP41	IDP42	DIDP2	DIDP4
202	32,926	30,176	—	—	—	—	—	—	—	—
203	—	—	—	—	—	—	—	—	—	—
208	—	—	—	—	—	—	—	—	—	—
210	14,742	15,860	15,048	19,096	14,388	14,720	—	12,654	51.2961	—
211	—	—	—	—	—	—	—	—	—	—
309	9,798	11,840	—	—	—	—	—	—	—	—

ID	ANGB1	ANGB2	ANG01	ANG02	ANG21	ANG22	ANG41	ANG42	DANG2	DANG4
202	1,017	975	904	1,065	760	844	—	965	6.7571	—
203	540	480	671	619	630	630	655	649	−7.7496	−6.834
208	359	258	524	552	706	695	485	382	6.9016	26.581
210	720	840	780	660	900	660	969	780	11.2821	4.120
211	295	408	369	479	341	353	322	241	26.2912	54.966
309	180	280	286	240	332	241	236	240	11.3257	−17.779

ID	ADPB1	ADPB2	ADP01	ADP02	ADP21	ADP22	ADP41	ADP42	DADP2	DADP4
202	29,605	30,176	28,917	30,690	21,679	28,461	—	31,845	−25.152	—
203	19,092	19,435	16,912	22,352	20,048	22,506	21,122	20,252	19.906	36.2854
208	—	11,590	12,502	12,768	14,763	17,930	17,632	16,060	−19.325	11.0433
210	10,282	14,384	11,448	11,948	13,608	12,000	17,136	13,570	16.184	25.1776
211	10,664	12,784	10,440	13,300	9,628	10,920	9,632	10,556	13.975	17.8016
309	8,704	10,656	7,812	8,710	9,108	10,064	7,280	7,680	0.999	6.0006

[a] — = data not available. See main text for discussion.

APPENDIX C. Alternative Analyses

NONTRIMMED-MEAN ANALYSES

The original protocol for the study laid out plans for the primary analyses of the percent effect of CO on time to ST endpoint and time to angina at two target COHb levels. These plans included the use of trimmed means to guard against outliers. The results of these analyses are presented in the Results section. In this appendix, we present the results of the analyses of percent changes in time to ST endpoint and time to angina that do not use trimmed means. The results for the 63 subjects who completed all test visits and met the protocol requirements are shown in Tables C.1 and C.2. These are the subjects on whom the results described in the Results section of the report are based. Results are also presented for 69 subjects, the 63 subjects who are in the main analysis, plus six subjects who completed the test visits but did not meet all the protocol requirements. Those results are presented in Tables C.3 and C.4. In all four tables we also present analyses of the change in time between air and CO days, as well as percent change in time to angina and to ST endpoint.

Comparing the results of Tables C.1 and C.2 with Tables 11 and 14 of the Results section enables a comparison of the results of the trimmed-mean analyses with the analyses of the percent change in time to ST endpoint and to angina. With respect to time to ST endpoint, the differences are small. For example, for the combined data, after the 2%-COHb-target exposure, both trimmed and untrimmed means show a 5.1% decrease; at the 4%-COHb-target exposure, the trimmed-mean decrease is 12.1% and the nontrimmed-mean decrease is 12.9%. Whether or not means are trimmed, 2%-COHb-target exposure did not have an effect at St. Louis, but effects are significant, at $p \leq 0.05$, in all other cases.

With respect to angina, again the picture is similar whether the traditional analyses or the trimmed-means analyses of percent change are used. For the combined data, the mean change in time to angina after the 2%-COHb-target exposure is 5.0%, and the trimmed mean is 4.2%; the mean change in time to angina after the 4%-COHb-target exposure is 6.6%, and the trimmed-mean change is 7.1%. The trimmed-mean analyses give statistically significant effects ($p \leq 0.05$) in all but two instances (4%-COHb target at Johns Hopkins; 2%-COHb target at St. Louis), and the traditional analyses give significant effects except for three instances (the two above and 4%-COHb target at St. Louis).

Comparing Tables C.1 and C.2 with Tables C.3 and C.4 shows the difference in results when six subjects who did not meet the protocol requirements are included in the analyses. With respect to the analyses of percent change in time to ST endpoint, most of the subjects who did not meet protocol requirements provided no ST data. Thus, it is not surprising that the results are similar; the ST analyses of 69 subjects compared to 63 subjects actually add only two subjects at the 2%-COHb-target exposure and one at the 4%-COHb target. However, in the angina analyses, seven are added at the 2%-COHb-target level and five at the 4%-COHb-target level of exposure. Nonetheless, the results are similar, and the differences are statistically significant in the same instances.

All four tables also present the results of analyses of change in time to angina and to ST endpoints between air- and CO-exposure days. These results are similar to the analyses of percent change, as is seen by comparing the p-values under the last two headings in Tables C.1, C.2, C.3, and C.4. In the ST and angina analyses of 63 subjects (Tables C.1 and C.2), there is a statistically significant effect in all the same instances, whether difference in time or percent difference in time is used to compare results on air- and CO-exposure days. In the analysis of 69 subjects (Tables C.3 and C.4), there is, again, a statistically significant effect in all the same instances, for both the ST and angina analyses, when comparing time difference to percent time difference between days.

RELATION BETWEEN TIME TO ST ENDPOINT OR ANGINA AND CARBON MONOXIDE EXPOSURE MEASURED AS THE PRODUCT OF CONCENTRATION AND TIME

Although the body of this report emphasizes changes in COHb concentration, the dose of CO received by an experimental subject also may be quantified by the conventional toxicologic approach, that is, as the product of average CO concentration and exposure time ($C \times T$). The purpose of this analysis is to demonstrate that results from $C \times T$ measurements do indeed corroborate the results derived from COHb measurements.

The following pages show graphs (Figures C.1 and C.2) of time to ST endpoint and time to angina as functions of CO dose in ppm $\times$ minutes. These graphs are analogous to Figures 8 and 12 in the Results section of the report, which plot responses in terms of %COHb. In both cases, although there is considerable scatter, the best-fit regression lines have significantly negative slopes (for ST, $p \leq 0.0001$; for angina, $p = 0.007$). That is, time to ST endpoint or angina has a tendency to decrease with increasing ppm $\times$ minutes as well as increasing %COHb, not attributable to chance. Also, in both cases, the effect of center is nonsignificant; that is, the three laboratories do not differ consistently in terms of their subjects' dose-response behavior.

RANDOMIZED ANALYSIS ALLOWING FOR BLOCKING

The order in which the exposures were administered to each subject was tested and found not to be significant. As a further examination of the effect of the order of exposure, randomization tests were performed to account for the possible induced correlation.

Table C.1. Effect of Carbon Monoxide on Time to ST Endpoint[a]

Exposure Comparison	Change in Time to ST Comparison Between Air and CO Days (seconds)			% Decrease in Time to ST Between Air and CO Days		
	Mean	p-Value[b]	95% Confidence Interval	Mean %	p-Value[b]	95% Confidence Interval
Combined Data						
Air vs. 2%-COHb target	30.4	0.003	9.20, 51.40	5.1	0.01	0.68, 9.46
Air vs. 4%-COHb target	68.7	≤0.0001	47.06, 90.14	12.9	≤0.0001	8.42, 17.28
Johns Hopkins University						
Air vs. 2%-COHb target	36.7	0.04	−3.71, 76.91	6.1	0.04	−0.78, 13.02
Air vs. 4%-COHb target	95.8	≤0.0001	53.94, 139.66	16.0	≤0.0001	9.50, 22.56
Rancho Los Amigos Medical Center						
Air vs. 2%-COHb target	57.2	0.001	23.52, 90.68	12.9	0.001	5.17, 20.69
Air vs. 4%-COHb target	72.5	0.004	21.70, 123.30	13.5	0.02	
St. Louis University						
Air vs. 2%-COHb target	3.3	0.42	−32.44, 39.04	−2.0	>0.5	−5.55, 1.45
Air vs. 4%-COHb target	39.9	0.0007	17.37, 62.43	9.3	0.0007	4.07, 14.53

[a] Same 63 subjects as main analysis, nontrimmed means.

[b] One-sided p-values.

Table C.2. Effect of Carbon Monoxide on Time to Angina[a]

Exposure Comparison	Change in Time to Angina Comparison Between Air and CO Days (seconds)			% Decrease in Time to Angina Between Air and CO Days		
	Mean	p-Value[b]	95% Confidence Interval	Mean %	p-Value[b]	95% Confidence Interval
Combined Data						
Air vs. 2%-COHb target	25.4	0.018	1.65, 48.95	5.0	0.02	0.10, 9.80
Air vs. 4%-COHb target	32.2	0.006	6.88, 57.52	6.6	0.006	1.52, 11.74
Johns Hopkins University						
Air vs. 2%-COHb target	30.1	0.024	0.26, 59.95	6.2	0.02	0.03, 12.37
Air vs. 4%-COHb target	16.1	0.22	−25.69, 57.69	4.4	0.10	−2.53, 11.35
Rancho Los Amigos Medical Center						
Air vs. 2%-COHb target	50.3	0.014	5.99, 94.61	11.3	0.01	1.87, 20.77
Air vs. 4%-COHb target	47.7	0.014	5.87, 89.33	9.9	0.02	0.67, 19.11
St. Louis University						
Air vs. 2%-COHb target	0.23	0.50	−50.20, 50.60	− 1.5	>0.5	−11.30, 8.28
Air vs. 4%-COHb target	35.5	0.08	−15.00, 86.00	6.2	0.13	−4.81, 17.19

[a] Same 63 subjects as main analysis, nontrimmed means.

[b] One-sided p-values.

Table C.3. Effect of Carbon Monoxide on Time to ST Endpoint in 69 Subjects[a]

		COHb Levels at End of Exercise Pre- and Postexposure			Time to ST Endpoint Pre- and Postexposure (seconds)		Change in Time To ST Endpoint Post- vs. Preexposure (seconds)		Decrease in Time Between Air and CO Days (seconds)			% Decrease Between Air and CO Days		
Exposure Day	Sample Size		Mean %COHb[b]	SEM	Mean	SEM	Mean	SEM	Mean	p-Value[c]	95% Confidence Interval	Mean %	p-Value[c]	95% Confidence Interval
Combined Data														
Air	63	Pre	0.63	0.02	564.4	26.5	16.7	11.5						
		Post	0.61	0.02	581.1	26.6								
2%-COHb target	63	Pre	0.62	0.02	577.0	27.1	−15.7	13.0	32.3	0.002	11.01, 53.59	5.0	0.01	0.71, 9.37
		Post	2.00	0.05	561.3	25.0								
4%-COHb target	63	Pre	0.64	0.02	568.2	27.7	−53.8	12.5	70.5	≤0.0001	49.01, 91.99	13.0	≤0.0001	8.61, 17.33
		Post	3.88	0.08	514.4	25.9								
Johns Hopkins University														
Air	22	Pre	0.60	0.04	596.0	48.9	7.1	21.8						
		Post	0.58	0.05	603.1	50.7								
2%-COHb target	22	Pre	0.60	0.03	601.8	47.7	−29.6	29.3	36.7	0.04	−3.71, 76.91	6.1	0.04	−0.78, 13.02
		Post	2.25	0.06	572.2	47.7								
4%-COHb target	22	Pre	0.65	0.04	610.1	48.0	−88.7	25.7	95.8	≤0.0001	53.94, 137.66	6.0	≤0.0001	9.50, 22.56
		Post	3.99	0.12	521.4	46.7								
Rancho Los Amigos Medical Center														
Air	18	Pre	0.74	0.04	594.4	45.4	52.2	23.4						
		Post	0.68	0.03	646.7	43.0								
2%-COHb target	18	Pre	0.68	0.05	627.9	42.9	−13.4	18.2	65.7	0.0007	29.62, 101.78	13.4	0.0007	6.08, 20.82
		Post	2.08	0.09	614.4	40.6								
4%-COHb target	18	Pre	0.73	0.05	605.7	54.5	−26.3	25.8	78.5	0.002	29.40, 127.60	13.9	0.014	1.70, 26.16
		Post	4.12	0.13	579.4	47.7								
St. Louis University														
Air	23	Pre	0.58	0.04	510.7	42.3	−2.0	13.8						
		Post	0.58	0.04	508.7	40.0								
2%-COHb target	23	Pre	0.59	0.04	513.4	46.5	−4.1	17.6	2.1	0.45	−32.00, 36.20	−2.6	0.25	−10.20, 5.08
		Post	1.71	0.07	509.3	39.3								
4%-COHb target	23	Pre	0.57	0.04	498.8	41.1	−41.9	11.0	39.9	0.0007	17.37, 62.43	9.3	0.0007	4.07, 14.53
		Post	3.59	0.13	456.9	38.6								

[a] Including subjects excluded from main analysis.
[b] CO measured by GC.
[c] One-sided p-values, as described in the Methods section.

Table C.4. Effect of Carbon Monoxide on Time to Angina in 69 Subjects[a]

Exposure Day	Sample Size	COHb Levels at End of Exercise Pre- and Postexposure			Time to Angina Pre- and Postexposure (seconds)		Change in Time To Angina Post- vs. Preexposure (seconds)		Decrease in Time Between Air and CO Days (seconds)			% Decrease Between Air and CO Days		
			Mean %COHb[b]	SEM	Mean	SEM	Mean	SEM	Mean	p-Value[c]	95% Confidence Interval	Mean %	p-Value[c]	95% Confidence Interval
Combined Data														
Air	69	Pre	0.64	0.02	Pre 525.1	25.7	−14.7	10.6						
		Post	0.61	0.02	Post 510.4	24.4								
2%-COHb target	69	Pre	0.62	0.02	Pre 531.2	24.9	−42.6	10.2	27.9	0.005	6.21, 49.59	5.3	0.009	0.93, 9.75
		Post	1.99	0.05	Post 488.6	21.3								
4%-COHb target	68	Pre	0.64	0.02	Pre 516.3	26.0	−51.5	10.4	34.2	0.003	10.02, 58.38	7.0	0.0035	1.99, 12.09
		Post	3.87	0.08	Post 472.1	24.2								
Johns Hopkins University														
Air	22	Pre	0.60	0.04	Pre 562.2	57.5	−46.1	16.9						
		Post	0.58	0.05	Post 516.1	51.2								
2%-COHb target	22	Pre	0.60	0.03	Pre 547.7	53.9	−76.2	19.6	30.1	0.024	0.26, 59.95	6.2	0.025	0.03, 12.37
		Post	2.25	0.06	Post 471.5	46.1								
4%-COHb target	22	Pre	0.65	0.04	Pre 557.1	51.7	−62.2	18.9	16.1	0.22	−25.69, 57.69	4.4	0.1	−2.53, 11.35
		Post	3.99	0.12	Post 498.9	49.4								
Rancho Los Amigos Medical Center														
Air	23	Pre	0.74	0.03	Pre 565.0	39.5		17.0	20.4					
		Post	0.69	0.03	Post 582.0	39.6								
2%-COHb target	23	Pre	0.67	0.05	Pre 580.1	37.8	−34.7	16.4	51.6	0.004	15.49, 87.92	10.8	0.0038	3.20, 18.30
		Post	2.02	0.09	Post 545.5	34.0								
4%-COHb target	22	Pre	0.70	0.04	Pre 538.3	46.1	−44.2	18.9	54.7	0.004	16.65, 92.55	11.7	0.005	2.81, 20.54
		Post	4.05	0.14	Post 514.6	46.4								
St. Louis University														
Air	24	Pre	0.58	0.04	Pre 452.8	32.7	−16.3	15.7						
		Post	0.57	0.04	Post 436.5	31.2								
2%-COHb target	24	Pre	0.58	0.04	Pre 469.3	35.3	−19.4	15.6	3.2	0.45	−43.11, 49.51	− 0.6	0.44	−9.63, 8.37
		Post	1.7	0.06	Post 449.8	28.7								
4%-COHb target	24	Pre	0.59	0.04	Pre 458.8	36.7	−48.2	16.8	32.0	0.09	−16.93, 80.93	5.2	0.16	−5.51, 15.91
		Post	3.59	0.13	Post 410.5	26.7								

[a] Including subjects dropped from main analysis.
[b] CO measured by GC.
[c] One-sided p-values, as described in the Methods section.

As explained in Appendix D, subjects were stratified by whether or not they had had a prior myocardial infarction. Then, within strata, the order of exposure was assigned, at random, in balanced blocks of the six possible orders. The randomization p-values reported in Table C.5 are a result of pairing subjects within blocks. The pairing within each block was determined by when the "other" exposure was administered. So, for example, for the 2%-COHb-target analysis, the two within a block who had the 4%-COHb-target exposure on the first experimental visit were paired, the two who had the 4%-COHb-target exposure on the second experimental visit were paired, and finally, the two who had the 4%-COHb-target exposure on the third experimental visit were paired. If the pairing could not be done because of a block not being filled, the observation was not paired. This pairing based on when the "other" exposure was administered yields an unbiased test, even if the order—or the blocking—is important.

In the randomization, the two responses "exposure day minus air day" for each pair of subjects were both multiplied by +1, or by −1, with equal probability.

These analyses were only performed for the centers where the samples were relatively small, and where the effect of blocking should be felt the most. An examination of Table C.5, in comparison with Tables 16, 17, C.1, and C.2, shows that except for the time-to-angina p-value for the air versus 2%-COHb-target exposure, the three analyses yield very comparable results. The smallest and largest p-values in Table C.5 are proportionally different by the same amounts from the corresponding p-values in Tables 16, 17, C.1, and C.2.

Table C.5. Effect of Carbon Monoxide on Time to ST Endpoint and Time to Angina: Block-Randomized p-Values

Analysis	Time-to-ST p-Value	Time-to-Angina p-Value
Johns Hopkins University		
Air vs. 2%-COHb target	0.042	0.08
Air vs. 4%-COHb target	0.0005	0.14
Rancho Los Amigos Medical Center		
Air vs. 2%-COHb target	0.001[a]	0.004
Air vs. 4%-COHb target	0.01	0.02
St. Louis University		
Air vs. 2%-COHb target	>0.5	>0.5
Air vs. 4%-COHb target	0.0049	0.045

[a] Most extreme attainable in this analysis.

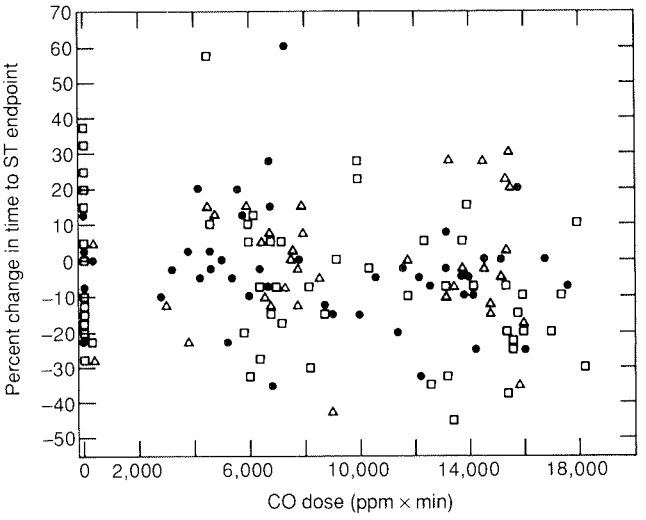


Figure C.1. Plot of percent change in time to ST endpoint between the pre- and postexposure exercise tests [(postexercise − preexercise)/preexercise] vs. CO dose (ppm CO × minutes of exposure). Five observations had missing values; 36 observations were hidden. □ = Johns Hopkins; • = St. Louis; and Δ = Rancho Los Amigos.

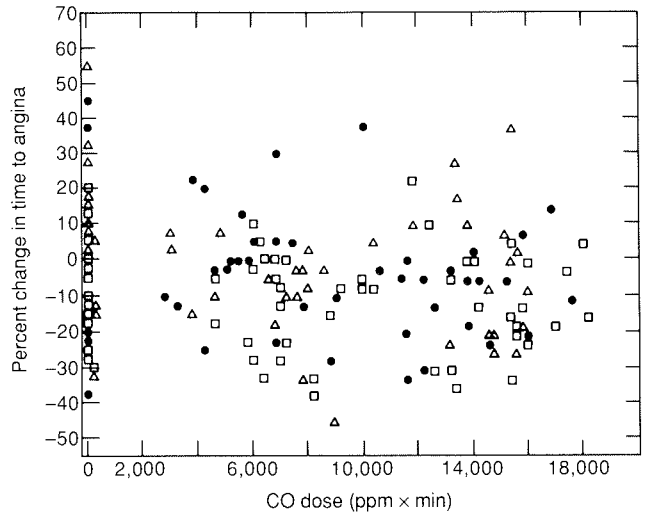


Figure C.2. Plot of percent change in time to angina between the pre- and postexposure exercise tests [(postexercise − preexercise)/preexercise] vs. CO dose (ppm CO × minutes of exposure). Two observations had missing values; 41 observations were hidden. □ = Johns Hopkins; • = St. Louis; and Δ = Rancho Los Amigos.

APPENDIX D. Randomization to Exposure

The randomization plan called for stratification by institution, and by whether or not the subjects had a previous myocardial infarction (MI). Within each of these six strata (three institutions times two previous MI statuses), the subjects were

balanced in groups of six so that each of the six possible orders of exposure (air, 2%-COHb target, 4%-COHb target) was represented (see Table D.1). Consequently, each center received two randomization lists, one for MI subjects and one for non-MI subjects. An example of a randomization list is shown in Table D.2. Randomized order was used to guard against a "learning" phenomenon. The results of the orders of exposure are reported in the Methods section of the report.

The order of exposure was kept blind to everyone who did not have an initial need to know until after the ECG readings had been made.

The randomization plan was not perfectly obeyed. The Statistical and Data Management Center sent out the randomization lists to the three test centers but, due to a misunderstanding, two of the centers used the sample randomization list contained in the protocol document. Before the error was caught and corrected, Johns Hopkins had registered five subjects with no prior MI and three subjects with MI, and Rancho Los Amigos had registered four patients with no prior MI and seven patients with MI. However, neither the randomization nor the balancing was compromised. The effect of the error was that if someone knew that the wrong randomization list was being used, then the blinding would have been threatened. Since the same people knew which randomization list was being used throughout the study, this error did not add any problems.

Table D.1. Six Possible Exposure Orderings in Three Visits

Ordering	Visit 2	Visit 3	Visit 4
1	Air	2%-COHb target	4%-COHb target
2	Air	4%-COHb target	2%-COHb target
3	2%-COHb target	Air	4%-COHb target
4	2%-COHb target	4%-COHb target	Air
5	4%-COHb target	Air	2%-COHb target
6	4%-COHb target	2%-COHb target	Air

Table D.2. Example of Randomization List

Ordering	Visit 2	Visit 3	Visit 4
A	Air	4%-COHb target	2%-COHb target
B	2%-COHb target	Air	4%-COHb target
C	Air	2%-COHb target	4%-COHb target
D	4%-COHb target	Air	2%-COHb target
E	2%-COHb target	4%-COHb target	Air
F	4%-COHb target	2%-COHb target	Air
G	2%-COHb target	4%-COHb target	Air
H	Air	2%-COHb target	4%-COHb target
I	4%-COHb target	Air	2%-COHb target
J	4%-COHb target	2%-COHb target	Air
K	Air	4%-COHb target	2%-COHb target
L	2%-COHb target	Air	4%-COHb target
M	4%-COHb target	2%-COHb target	Air
N	2%-COHb target	4%-COHb target	Air
O	2%-COHb target	Air	4%-COHb target
P	Air	4%-COHb target	2%-COHb target
Q	Air	2%-COHb target	4%-COHb target
R	4%-COHb target	Air	2%-COHb target

APPENDIX E. Validation of Calculated Carbon-Monoxide-Carrying Capacity of Hemoglobin

The conventional expression of the body burden of CO that results from intrinsic and extrinsic sources is the percent of the hemoglobin-combining capacity for CO that is combined with CO (%COHb). This measure is used rather than the partial pressure of CO because of the high affinity of hemoglobin for CO, and the resulting reduction in oxygen-carrying capacity at very low partial pressures of CO. The physiological effects of CO have generally all been related to the level of %COHb after exposure. As with any measurement that is expressed as a percentage, it is important that the 100% level be quantified. The use of %COHb, with analysis of blood CO by GC, requires measurement or estimation of the total CO-carrying capacity of hemoglobin. The GC analysis measures ml of CO per dl of blood, which can be converted to %COHb only when the capacity (100%) is known. In this study, the capacity was routinely determined by measuring the hemoglobin concentration of the blood sample in question and multiplying the hemoglobin concentration by the theoretical combining capacity of 1.389 ml/g of hemoglobin. This theoretical value was verified on a regular basis throughout this study by actually measuring the CO-combining capacity of individual samples. This procedure provided several important technical functions: (1) verification of the GC technique; (2) verification of the cyanmethemoglobin technique routinely used for measurement of hemoglobin; and (3) provision of a means of obtaining an absolute standard for discrepancies that occurred between techniques and laboratories for the measurement of hemoglobin. This appendix reports the methods used to standardize the hemoglobin measurement at the St. Louis Reference Laboratory.

On a monthly basis, the CO-combining capacity of one or two samples was measured. The samples used were freshly drawn venous blood samples, blood standards that were developed in the laboratory (Appendix J), or samples from the blood bank one to three days after collection.

CALCULATION OF CARBON MONOXIDE CAPACITY

The measurement of hemoglobin content by the cyanmethemoglobin technique (reagents and standards from Sigma, St. Louis, MO) was carried out in triplicate. All pipettes were routinely standardized and new standards checked against old. The American Society of Pathologists (Eilers 1967) adopted a molecular weight of 64,500 for hemoglobin A in the tetrameric form. The stoichiometric ratio of CO that will combine with hemoglobin is 1:1. Since one mole of CO contains 22,400 cc and one mole of monomeric hemoglobin weighs 16,125 g, the theoretical combining capacity of hemoglobin is 1,389 cc/g at STPD conditions. Therefore, if the hemoglobin concentration is known, the CO-combining capacity can be calculated by multiplying the hemoglobin in grams by 1.389.

It has been shown that the formation of cyanmethemoglobin can be slowed by the presence of CO on the hemoglobin molecule. Experiments were carried out to show the time course of the development of maximal absorbance samples containing 100% COHb (Figures E.1 and E.2). It required from four to six hours for the reaction to go to completion when the %COHb was 90% (Figure E.2). Therefore, all samples measured in these capacity checks were pipetted, covered, placed in the dark, and allowed to react overnight. The measurement of total hemoglobin remained constant from four to 12 hours (Figure E.2).

The samples in which the combining capacity was to be checked were placed in a 50-ml syringe and equilibrated with 99% CO for 15 minutes (repeated once) at room temperature. The gas phase was then ejected and replaced with a gas mixture containing 1,700 ppm CO in nitrogen for five minutes. This level of CO was sufficiently high, according to the Haldane relationship (Douglas et al. 1912), to maintain saturation, but low enough that the amount of dissolved CO became undetectable. Therefore, the measurement of the CO content of these saturated samples did not have to be corrected for dissolved gas. Checks were carried out, with great care taken to measure the dissolved gas in blood samples and samples of artificial plasma with these techniques. The levels of dissolved CO were similar to those reported by Dahms and Horvath (1974). The GC analysis of these saturated samples yielded values of ml of CO per 100 ml of blood at STPD conditions.

Comparisons were then made between the calculated CO-carrying capacity of hemoglobin from the cyanmethemoglobin analysis and the measured CO-carrying capacity from the GC. The results for 37 comparisons, over three years, are shown in Table E.1. The hemoglobin values ranged from 7.2 g/dl to 18.9 g/dl, with the agreement ranging from 118% to 93% of the GC capacity. It can be seen that the agreement between techniques is very good: the average ratio of the calculated values to the measured capacity was 103% over the entire time period. The values obtained over the past year have averaged 100% (perfect agreement), which is probably due to consistency in carrying out this procedure.

It would be expected that there would be a slight difference in these two techniques, with the measured CO-combining capacity being slightly lower than the calculated value based upon the hemoglobin concentration. This is due to the presence of methemoglobin in the blood samples (approximately 0.5%), which would not combine with CO but is measured in the spectrophotometric assay for hemoglobin.

Table E.1. Results of the Comparison of the Measured Carbon-Monoxide-Combining Capacity of Blood Samples with the Calculated Capacity Based on Hemoglobin Concentration[a]

Sample ID	Date	GC Capacity	CNMet THb[b]	CNMet Capacity (g/dl)	CN Met Capacity[c] / GC Capacity (%)
TB	10-18-84	24.65	18.94	26.31	107
ARC-5	1-31-85	22.09	15.76	21.89	99
ARC-15	4-24-85	13.64	10.15	14.10	103
ARC-17	5-07-85	18.35	13.44	18.67	102
ARC-17	5-08-85	12.69	10.36	14.39	113
ARC-17 (200 μl)	5-10-85	14.23	10.45	14.52	102
ARC-17 (100 μl)	5-10-85	13.81	10.45	14.52	105
ARC-17 (10 μl)	5-10-85	15.15	10.45	14.52	96
ARC-18	6-10-85	19.05	13.64	18.95	99
ARC-25	7-23-85	10.49	8.90	12.36	118
ARC-25	7-24-85	9.23	7.67	10.65	115
ARC-25	7-24-85	14.60	11.15	15.49	106
ARC-25	7-24-85	13.67	11.03	15.32	112
HU-2	8-22-85	9.97	7.2	10.00	100
HU-1	8-22-85	10.27	7.6	10.56	103
RLA 214C	3-20-86	22.84	17.5	24.31	106
RLA 215L	3-20-86	24.78	19.3	26.81	108
SLC-11	4-30-86	22.83	17.7	24.59	108
Sample 1	5-22-86	23.10	17.3	24.03	104
CC1	7-07-86	18.10	13.3	18.47	102
CC2	7-07-86	17.40	13.1	18.20	105
CC3	10-09-86	14.83	10.5	14.58	98
CC4	10-09-86	14.67	10.4	14.45	99
CC5	11-24-86	18.06	12.9	17.92	99
CC6	11-24-86	20.09	14.5	20.14	100
CC7	1-21-87	18.37	13.3	18.47	101
CC8	1-21-87	17.60	12.9	17.92	102
CC9	3-03-87	19.50	13.9	19.31	99
CC10	3-03-87	19.06	13.2	18.33	96
CC11	4-27-87	17.77	13.0	18.06	102
CC12	4-27-87	17.76	13.0	18.06	102
CC13	6-11-87	17.37	12.7	17.64	102
CC14	6-11-87	17.53	12.7	17.64	101
CC15	8-18-87	12.31	8.6	11.95	97
CC16	8-18-87	12.29	8.2	11.39	93
CC17	9-24-87	16.45	12.0	16.67	101
CC18	9-24-87	16.30	11.6	16.11	99

[a] Each value represents the average of three measurements by both techniques.

[b] CNMet THb = total hemoglobin measured by the cyanmethemoglobin technique.

[c] Mean = 103%; SD = 5.3%; n = 37.

CONCLUSION

The technique used to calculate CO capacity for the study samples in the St. Louis Reference Laboratory (cyanmethemoglobin) was found to be accurate. The means of measuring CO content (GC) was also found to be valid. Therefore, the values reported for %COHb based on GC values of CO content are valid.

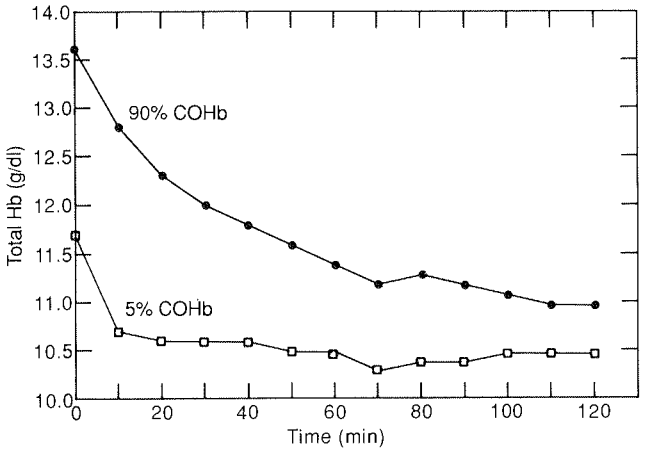


Figure E.1. Effect of time (up to two hours) on the production of cyanmethemoglobin for the measurement of hemoglobin in blood. This reaction was carried out both at a low %COHb (5%) and a high %COHb (90%) at a room temperature of 19° to 22°C.

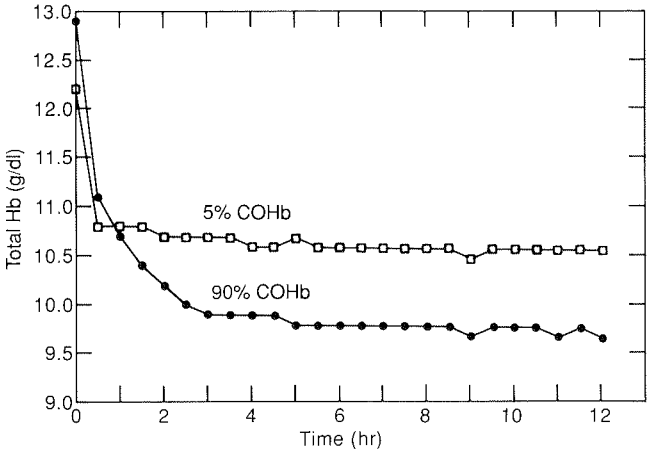


Figure E.2. Effect of time (up to 12 hours) on the production of cyanmethemoglobin for the measurement of hemoglobin in blood. This reaction was carried out both at a low %COHb (5%) and a high %COHb (90%) at a room temperature of 19° to 22°C.

APPENDIX F. Comparison of St. Louis University and Stanford University Gas-Chromatographic Techniques for Quantifying Carbon Monoxide in Blood

Because of concern about the accuracy of the techniques employed in the multicenter study, an attempt was made to validate the GC analysis performed by the St. Louis Reference Laboratory. The Stanford GC technique was chosen because it was another method for direct analysis of CO in blood. Duplicate samples from study subjects at all three centers were collected and shipped to Stanford University and to St. Louis University.

The technique used in St. Louis has been described in the Methods section of this document. The major differences between the two methods are: (1) the Stanford detector uses the ultraviolet detection of mercury vapor and is much more sensitive; (2) a much smaller volume of sample is used at Stanford; (3) for analysis of samples containing more than 2% to 3% COHb at Stanford, the blood must be diluted; and (4) the Stanford values are not corrected to STPD conditions.

The CO content of venous blood samples was measured by GC using the technique of Dahms and Horvath (1974). The blood gases were quantified by a headspace analysis technique after vortex extraction under a column head pressure of 40 psi. Blood gases were extracted in a 1.8-ml glass vial that was sealed with a rubber stopper held in place by an aluminum retainer. Prior to sealing the vial, the following reagents were added: a magnetic stir-bar, 100 μl of saponin-ferricyanide solution (2 g of saponin + 8 g of potassium ferricyanide [$K_3Fe(CN)_6$] in 42 ml water), 100 μl of lactic acid (0.5 mol/liter), and two drops of 2-octanol. The sealed vials were made fresh daily. The contents of the vial were purged with the chromatographic carrier gas, helium, for three minutes, while the vial contents were stirred in order to produce a vortex to increase the surface area for removal of dissolved gases in the extraction mixture. The flow rate of the purge gas was greater than 50 ml/min. The vials were purged immediately prior to the addition of the blood sample, in order to remove the nitrogen from the vial. This enabled better separation of the nitrogen and CO peaks as they eluted from the column. The blood sample was added to the purged vial, with anaerobic methods used as a precaution against loss of CO. Well-mixed or hemolyzed blood (200 μl) was added to the purged vial with a calibrated microsyringe. The vial, containing the reagents and the blood, was placed on a dual needle assembly and was extracted for three minutes while being stirred to a vortex. The headspace in the vial, of approximately 1.5 ml, was brought up to the column pressure of 40 psi during the extraction period.

After elution from the sample, the gases released from the blood sample, along with the carrier gas, traveled through 360 cm of hypodermic tubing to the gas sampling valve, and then onto the precolumn strippers and the columns. The precolumns and the columns (all 1/4-inch outer diameter) are serially connected in the following sequence: Water stripper: 30 cm, filled with 10-20 mesh Drierite; Column 1: 90 cm, filled with 80-100 mesh Porapak Q; CO_2 stripper: 30 cm, filled with either 5A or 13X molecular sieve; and Column 2: 360 cm, filled with 30-60 mesh 13X molecular sieve. The sample components passed by hot wire thermal conductivity detectors after leaving Column 1, and by a second set of detectors after Column 2. The chromatograph was operated under the following conditions: Oven temperature of 45°C or 85°C (appropriate conditions were chosen to provide for adequate separation of gases); detector current of 125 mA; column driving pressure

of 277 kPa (40 psi); and a helium carrier gas flow rate of 100 ml/min. Reinjection of the reaction vial containing a blood sample did not result in any CO peak. This suggests complete extraction and elution with no production of CO by the reagents.

The GC was calibrated by the addition of a CO gas standard to a prepared reaction vial, which was then processed identically to the vials that contained blood samples. Then 250 μl of 10% CO with the balance as helium (analyzed by Matheson Scientific, Chicago, IL) were added to the purged sample vial with a gas-flush Hamilton microsyringe. This technique has been shown to produce a linear relationship for peak heights throughout the entire expected range (Dahms and Horvath 1974), which enables use of a single calibration point each day. The volume of CO added to the vial was corrected to an STPD volume. The accuracy of this procedure was verified by the Van Slyke technique of Horvath and Roughton (1942). When the values for CO content derived by both methods were compared over a wide range of values, the correlation coefficient was 0.999, with an intercept of 0.05 ml/dl of blood (Dahms and Horvath 1974). Because of the difference in resolution capability of these two techniques (U.S. Environmental Protection Agency 1979), it is difficult to carry out a meaningful comparison of data from samples with only low levels of CO in blood.

The method used by Vreman and associates (1984) at Stanford University is described below. The blood samples were collected and aliquots were placed in microhematocrit tubes that contained dried heparin and saponin, as well as stainless-steel bars that are used for mixing the samples. The storage tubes were capped with tube closures. (These stored samples were in the form in which samples were shipped from the individual centers to the laboratory at Stanford.)

The analytical method basically involved extraction of CO for 30 minutes, followed by conventional headspace analysis. The liberation of CO from hemoglobin was performed in a 1.5-ml glass vial that was fitted with an open-center screw cap used to hold a high-temperature septum (8-mm diameter, blue silicone sheet; Alltech Associates, Los Altos, CA). Prior to sealing, 20 μl of a mixture of the following reagents were added to the empty vial: 100 ml/l Triton X-100, 100 g/l $K_3Fe(CN)_6$, and capryl alcohol. The vial with the reagents was then purged with CO-free carrier gas for two minutes, at a flow rate of 200 ml/minute. The blood sample, 2 μl, was then added to the vial with the use of a 5-μl Hamilton syringe. These vials were then held at room temperature for 10 to 30 minutes prior to analysis.

The amount of CO released into the headspace was determined with a GC equipped with a reduction gas detector (Trace Analytical, Palo Alto, CA). The gas sample from the headspace of the reaction vial was injected onto the chromatographic column by a pneumatic sample-injection valve controlled by a digital valve-sequence controller. The column used for separation of the headspace gas was a 90 × 0.53-cm (inner diameter) stainless-steel column packed with 60-80 mesh 5A molecular sieve. Column temperature was maintained at 110°C, with a flow rate of 50 ml/min of air carrier gas. Under these conditions, the minimal detectable volume of CO is 0.1 nl. The headspace gas passed through a moisture trap of methylene-blue-dusted magnesium perchlorate [$Mg(ClO_4)_2$].

The Stanford system was calibrated with a standard gas mixture containing 23.4 μl CO/liter, with the balance as nitrogen gas. Calibration was performed by injecting 0, 25, 50, 75, or 100 μl of the calibrated gas into a purged vial containing the reaction mixture. Concentrations of CO were calculated from peak height analysis of the data and are represented in ATPS (ambient temperature and pressure, saturated) units.

COMPARISON OF THE RESULTS FROM STUDY SAMPLES ANALYZED BY BOTH LABORATORIES

Data comparisons were made for both the CO content and the hemoglobin concentrations of the blood samples. The assessment of the two chromatographic methods can be directly made by comparing the CO content values for each sample. There were 108 samples from subjects in the multicenter study that were analyzed at both laboratories and that resulted in valid, unequivocal data points. Linear regression analysis of these values shows that the results from the two techniques are highly correlated, with a coefficient of 0.987. The Stanford values for CO content are linearly offset from the St. Louis values with a linear regression, $y = 0.066 + 1.098x$, where y = Stanford CO content in ml/dl of blood at ATPS conditions and x = St. Louis CO content in ml/dl of blood at STPD conditions. Figure F.1 illustrates the relationship between the values obtained at the two laboratories. The 9.8% higher CO value from the Stanford laboratory can be accounted for by incorporating a correction factor for the expected conditions in the Stanford laboratory. For example, to correct a sample at 23°C, 750 mm Hg, and dry to STPD, a correction factor of 0.919 would be required. Since daily records of barometric pressure and laboratory temperature do not exist for the Stanford laboratory, this explanation must remain as conjecture. However, if all the Stanford values are corrected by this factor, the linear regression becomes $y = 0.05 + 0.99x$. This remaining offset of 0.05 ml/dl (Stanford being higher) is not understood. This plot of "corrected" data is shown in Figure F.2. The y intercept represents approximately 0.2% COHb in these samples.

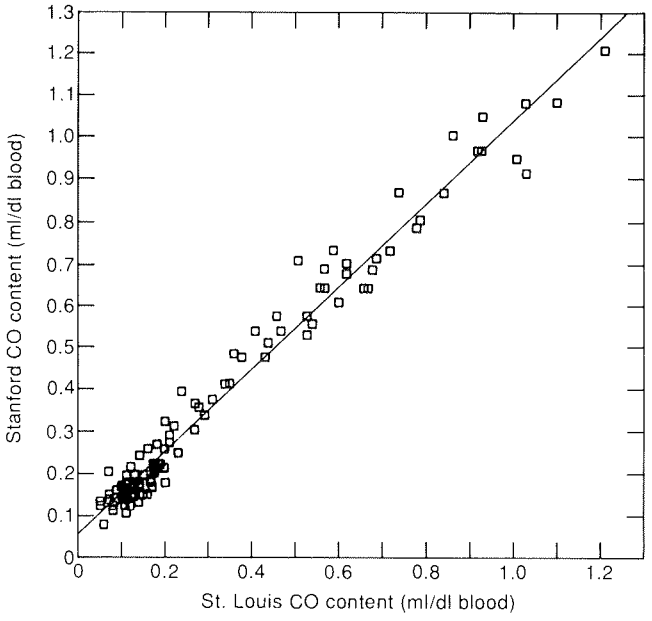


Figure F.1. Comparison of CO content in subject samples measured by GC at the St. Louis University and the Stanford University Reference Laboratories. The St. Louis values are corrected for STPD conditions, and the Stanford values are not corrected to STPD. The regression analysis for these data shows: n = 108; r = 0.987; and $y = 0.066 + 1.098x$.

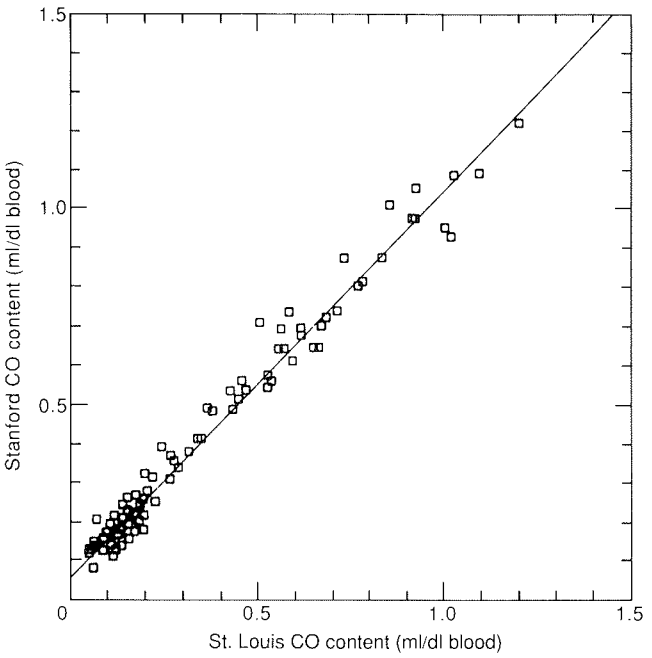


Figure F.2. Comparison of CO content in samples measured by GC at St. Louis University and Stanford University laboratories. The St. Louis values are corrected for STPD conditions, and the Stanford values were estimated at STPD conditions. The regression analysis for these data shows: n = 108; r = 0.99; and $y = 0.054 + 0.99x$.

APPENDIX G. Stability of Carbon Monoxide in Blood Samples

STABILITY OF CARBON MONOXIDE CONTENT IN BLOOD SPECIMENS

In order to carry out the standardization and sample-analysis aspects of this study at the St. Louis Reference Laboratory, the COHb content in blood samples had to remain stable, during sample shipment and storage, for at least one week. Previous data in the literature indicate that the CO content does not change in anaerobically stored, sterilely collected blood samples for at least a month (Dahms and Horvath 1974; Vreman et al. 1984). Additional information exists that COHb in blood is stable for six months (H.J. Vreman, personal communition).

Data have been collected in this study on the calibration standards that show that the CO content is unchanged with storage for at least 10 days. Routinely, samples were shown to be unchanged with storage for five to seven days. In addition, it was demonstrated that standard samples shipped from the Reference Laboratory to the Rancho Los Amigos center, and back, were unchanged in their CO content. Subjects' samples were routinely shipped to the St. Louis Reference Laboratory and it is unlikely that the CO content in these specimens changed as a result of handling.

It can be concluded that none of the procedures used in this study changed the COHb content of the blood samples once they were collected.

LOSS OF OPTICAL STABILITY OF BLOOD SAMPLES: PERCENT CARBOXYHEMOGLOBIN

A natural mechanism for checking the values collected on the subjects' samples at each center would have been to make measurements on each sample at the Reference Laboratory as well. For this analysis to be meaningful, the values obtained at each center laboratory would have to remain constant on repeated daily measurements made over a three- or four-day period, the expected time required for shipment and analysis on a routine basis.

When analyses of freshly drawn samples were made on a daily basis, it became clear that the %COHb by CO-oximetry did not remain sufficiently stable for this form of quality control. Table G.1 shows that samples stored at 4°C did not remain stable for 24 hours. Therefore, the blood samples could not be checked at the center laboratories and the Reference Laboratory. These data resulted in the need for the development of optically stable standards, as described in Appendix J.

Table G.1. Effects of Storage of Blood Samples at 4°C on the Reproducibility of IL 282 CO-Oximeter Measurements of Percent Carboxyhemoglobin

Sample	Day 0[a]	Day 1[a]	Day 2
AS 15	1.9	1.6	1.5
AS 30	2.8	2.7	2.4
AS 45	3.9	3.8	3.7
MV 15	2.2	1.8	
MV 30	3.5	3.0	
MV 45	4.3	3.9	
EO C	1.5	—[b]	1.2
EO 60	2.9	—	2.8
EO PE	2.7	—	2.4
MV C	1.3	0.8	
MV 60	3.4	2.9	
MV PE	2.9	2.3	

[a] IL 282 %COHB.

[b] — = not tested.

APPENDIX H. Evaluation of the IL 282 CO-Oximeter

There is always a need to obtain rapid information about subject exposure to CO during an experiment. The need is generally for both experimental control and subject safety. The optical method employed by the various CO-Oximeters available provides a means of obtaining such rapid results with good precision. The IL 282 CO-Oximeter was used in this study because of the extensive use of this instrument in this area of research. However, the accuracy of this instrument was a source of concern because of the manufacturer's stated limits of accuracy and the high base-line values of %COHb reported in the blood of nonsmokers with these instruments. Therefore, it was important to evaluate the accuracy of the IL 282 CO-Oximeter measurements of values of CO in blood relative to the values obtained by the more sensitive GC technique used in this study as the reference technique.

A comparison of values for %COHb on subject samples analyzed by CO-Oximeter and by the GC-cyanmethemoglobin technique (hereafter referred to as the GC method) was carried out. As will be discussed later, several factors are known to influence the optical measurement of %COHb in blood samples. The comparison of data points determined by the two methods, uncorrected for any influencing factors, gives an approximation of the error of the estimate for %COHb involved in the routine laboratory use of the individual IL 282 CO-Oximeters at each laboratory involved in this study.

All blood samples used in this paired analysis were measured by CO-oximetry within 15 minutes of their collection. The samples were analyzed five times, and the high and low values were discarded. The CO-Oximeter values reported are the average of the three remaining values. All of the samples consisted of 7 ml of blood collected, through a low-deadspace indwelling catheter in an antecubital vein, into a tube containing 100 units of heparin. Samples were analyzed at the St. Louis Reference Laboratory by GC within one week after collection. The final value for the GC method represents the mean of three analyses by GC and three separate analyses for hemoglobin content on the same well-mixed samples.

The comparisons described in this appendix were all made in the St. Louis Reference Laboratory with the same CO-Oximeter (the "St. Louis local instrument") for the entire study. (An additional CO-Oximeter was used in the Reference Laboratory for part of the study. Its use was discontinued because of unresolvable technical difficulties.) At different periods in this study, comparisons between CO-Oximeter and GC data were carried out and the regressions were used in the formulation of amendments to the experimental protocol. The data in this section include all of these analyses. The data base for each analysis will also be discussed. The primary difference among the sets of data is a continually increasing number of samples in the data base.

The initial comparison between CO-Oximeter and GC measurements was carried out on 66 data points from normal volunteers who were involved in the protocol-development phase of this study. (This comparison appears in the Procedures Manual for the study). Figure H.1 is a graph of these data. As more data were collected on normal subjects, a more complete comparison of the data collected by these two techniques could be made. In February of 1986, the data from 261 blood samples collected from control subjects were compared. These data are shown in Figure H.2, which includes the data in Figure H.1. There was very little difference in the nature of the data as the sample size increased.

The initial analysis of data (in January 1986), from 106 samples from angina subjects, indicated a larger discrepancy between the two techniques than was found with the normal subjects (Table H.1). This is shown in Figure H.3, where the intercept of the regression line is greater than 1% COHb for the angina subjects, whereas in Figure H.2 it is below 1% COHb for the normal subjects. However, an analysis of data from angina subjects, carried out on more samples (342) at the conclusion of the study, showed no difference between the control subjects' data and the angina subjects' data. The plot of the comparison of %COHb from both techniques for all subject samples from the St. Louis center is shown in Figure H.4.

It is clear from Table H.1 that the only aberrant comparison is that for the patient samples in February 1986. All of the

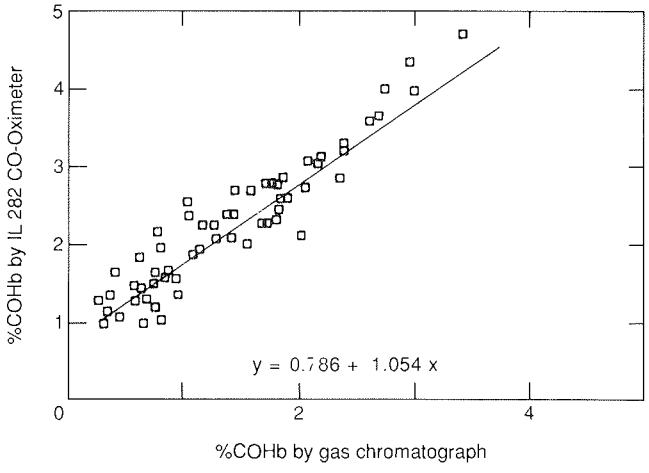


Figure H.1. Comparison of GC-based %COHb values with values determined with the IL 282 CO-Oximeter at St. Louis center. These 66 samples were obtained from control subjects. The CO-Oximeter values were obtained witbin 15 minutes of collection. Each data point is the average of three measurements by each technique. This analysis was made on the cumulative data base available as of March 1985.

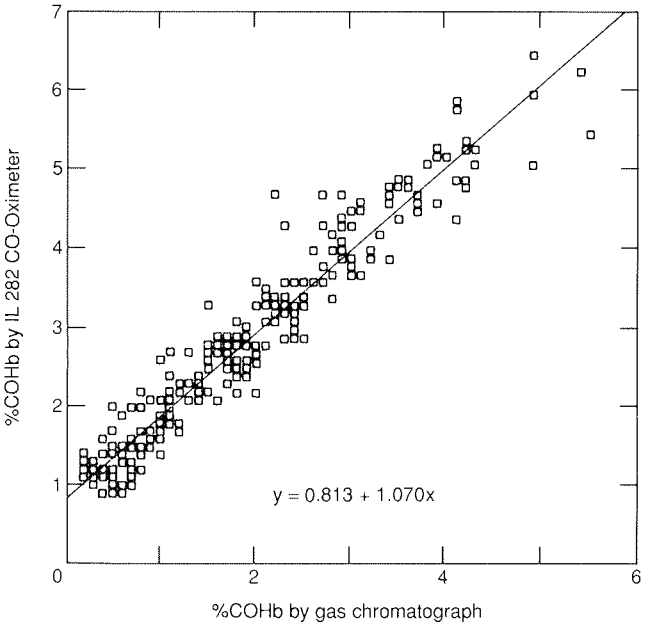


Figure H.2. Comparison of IL 282 CO-Oximeter values for %COHb with GC-based values for %COHb on the same samples. These 261 comparisons were made on samples collected from control subjects. The CO-Oximeter values were obtained within 15 minutes of collection. Each data point is the average of three measurements by each technique. This analysis was made on the cumulative data base available as of February 1986.

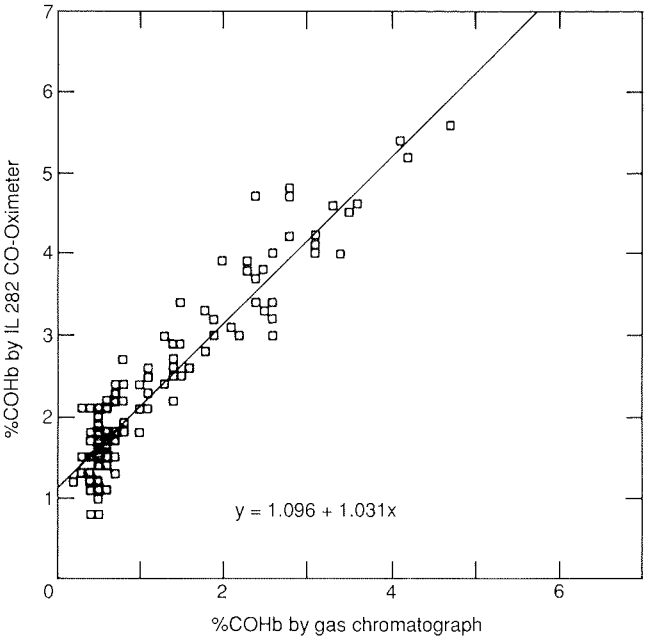


Figure H.3. Comparison of COHb values measured by both the GC and the IL 282 CO-Oximeter on the same samples. These samples were obtained from patients entered in the multicenter protocol at the St. Louis center. Each data point is the average of three measurements by each technique. These 106 comparisons were carried out before February 1986.

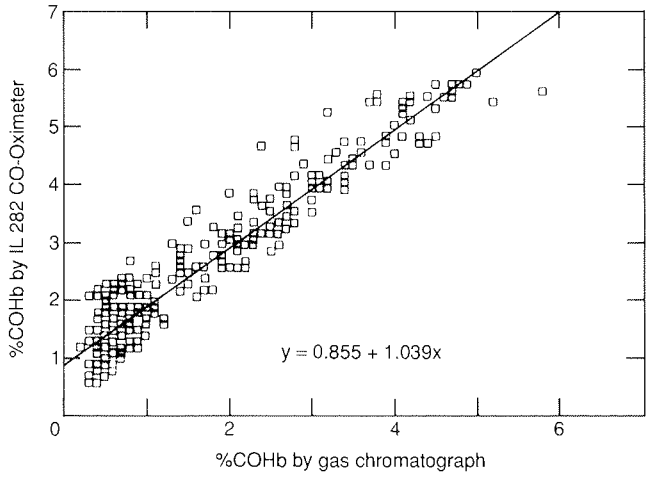


Figure H.4. Plot of the comparison of the GC-based %COHb values vs. values obtained on the same samples measured by IL 282 CO-Oximeter. These values are from 342 samples collected on study subjects at all three center laboratories. The CO-Oximeter values were obtained at the local laboratories within one hour of sample collection and the GC values were all determined at the St. Louis Reference Laboratory. Each data point is the average of three measurements by each technique. Tbese values represent all the stndy samples available at the conclusiou of the study.

Table H.1. Comparison of Regression Analysis of the Data Collected in St. Louis for Percent Carboxyhemoglobin by CO-Oximeter and by Gas Chromatography

Subjects	Analysis Date	n	Regression[a]
Control	3/85	66	$y = 0.786 + 1.054x$
Control	2/86	261	$y = 0.813 + 1.070x$
Patient	2/86	106	$y = 1.096 + 1.031x$
Patient	6/87	342	$y = 0.855 + 1.039x$

[a] Where y = %COHb by CO-Oximeter and x = %COHb by GC.

Table H.2. Corresponding Percent Carboxyhemoglobin Values Between CO-Oximetry and Gas Chromatography (Using the Appropriate Regressions in Table H.1)

	GC Values (%)		
Subjects	1	2.2	4.4
	CO-Oximeter Values (%)		
Normal (2/86)	1.9	3.2	5.5
Patient (6/87)	1.9	3.1	5.4

data used have been checked for inconsistencies in technique, but none was found. The reasons for this discrepancy are unknown, but are undoubtedly due to normal variability.

With the above regressions, it is possible to determine CO-Oximeter values that represent absolute GC %COHb values. The key values for %COHb that were used in this study were GC levels of 1%, 2.2%, and 4.4% COHb. Table H.2 provides these values based upon the regression equations shown in Table H.1.

If all these data had been available at the start of this study, the values in Table H.2 could have been used as guidelines for exposing these subjects to CO.

APPENDIX I. Evaluation of Factors Affecting CO-Oximeter Percent Carboxyhemoglobin Values

Additional tests were performed to identify the factors responsible for the discrepancy between techniques for measuring COHb. Information from these studies could be used to minimize the discrepancies between the methods, as well as to minimize the variability in the optical method. The following possible influences on the detection of %COHb by the optical method were investigated: methemoglobin concentration, pH, hemoglobin concentration, oxyhemoglobin concentration, cholesterol and triglyceride concentration in plasma, and cuvette temperature.

EFFECT OF METHEMOGLOBIN ON DETECTION OF PERCENT CARBOXYHEMOGLOBIN

A fresh sample of blood was treated with sodium nitrite ($NaNO_2$) in order to produce low fixed levels of methemoglobin, which were quantified by CO-oximetry. In a sample containing 10% COHb, increasing the methemoglobin levels up to 9.5% had very little influence on the readings obtained for %COHb. This is illustrated in Figure I.1. Because the samples from the subjects studied contained less than 1.2% methemoglobin, it was concluded that slight variations in levels of methemoglobin could not have contributed to the variability seen in the CO-Oximeter data.

EFFECT OF HEMOGLOBIN CONCENTRATION ON DETECTION OF PERCENT CARBOXYHEMOGLOBIN

During the course of these experiments, the subjects changed posture several times. This can produce slight, but significant, changes in distribution of body fluids (Thompson et al. 1928). Also, all of the subjects demonstrated some degree of hemoconcentration as a result of exercise. Therefore, it was important to determine whether or not changes in hemoglobin concentration influenced %COHb readings on the CO-Oximeter.

Fresh whole-blood samples containing 2.9%, 3.5%, and 4.1% COHb were diluted with physiological Krebs-Hensleit buffer containing 6% albumin. The original samples contained 16.2 g/dl. As shown in Figure I.2, the %COHb readings were unaffected by hemodilution. The initial dilution of the 4.1%-COHb sample resulted in a 0.3%-COHb increase, which is a very small change for such a large change in hemoglobin concentration. It is unlikely that the changes in hemoglobin levels in these subjects influenced the ability of the CO-Oximeter to determine %COHb.

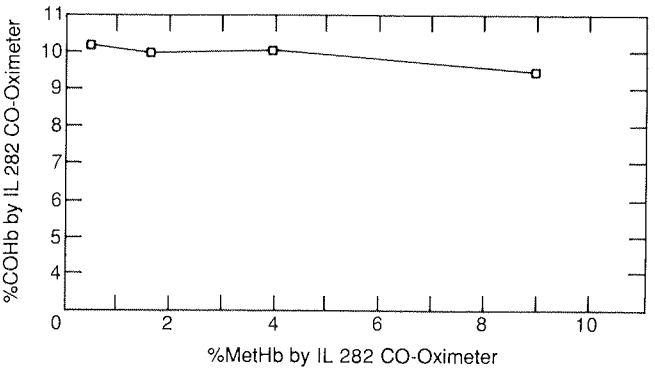


Figure I.1. Effect of increased methemoglobin on intact whole-blood samples on the ability of the IL 282 CO-Oximeter to measure %COHb. Methemoglobin values were increased by the addition of sodium nitrite ($NaNO_2$) to the whole-blood samples.

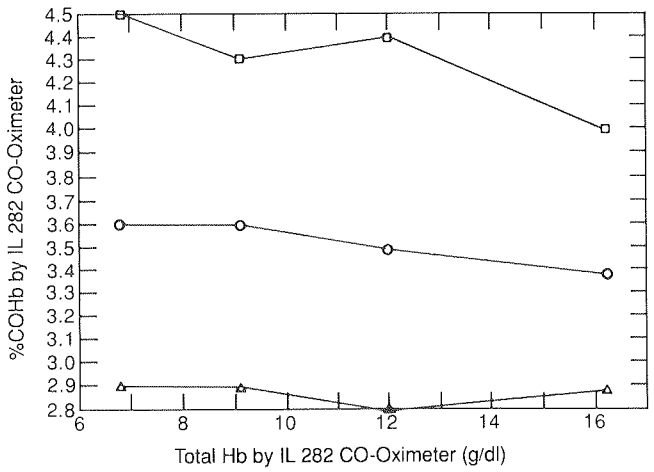


Figure I.2. The effect of dilution of three freshly collected blood specimeus on the ability of the IL 282 CO-Oximeter to measure %COHb. Whole blood was diluted with Krebs-Hensleit buffer containing 6% albumin.

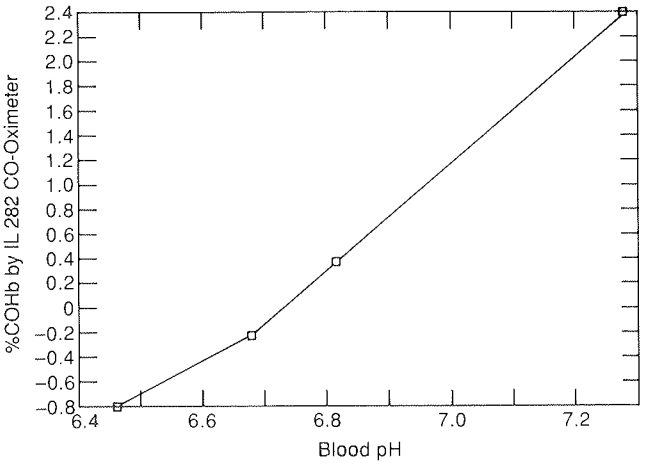


Figure I.3. Effect of pH on the ability of the IL 282 CO-Oximeter to measure %COHb. The pH was varied by the addition of phosphate buffers to the fresh whole-blood samples.

EFFECT OF pH ON THE DETECTION OF PERCENT CARBOXYHEMOGLOBIN

During the course of an exercise test, the hydrogen-ion concentration of the blood can be increased by the production of organic acids and by the influence of the bicarbonate buffer system. Also, samples of stored blood from the blood bank were very acidotic because they were stored in an acid citrate dextrose solution. Analysis of a few units of blood from the local American Red Cross, which were collected from nonsmokers, indicated normal control levels of CO by GC but negative values for %COHb by CO-oximetry. The pH of these stored units of blood can vary from 6.4 to 6.8.

To determine the influence of pH on %COHb readings obtained by the IL 282 CO-Oximeter, a freshly drawn blood sample was collected over acid citrate dextrose solution. The pH was determined with the use of a Radiometer (model BMS3 Mk2, Copenhagen, Denmark) pH meter. The pH of this whole-blood specimen was then increased by the addition of phosphate buffers. Control samples were diluted with normal saline.

Figure I.3 shows the dramatic effect of large changes in pH on the ability of the CO-Oximeter to detect %COHb. It is obvious that the structure of the hemoglobin molecule is influenced by these pH changes, resulting in varying absorption spectra for each species. Therefore, values obtained with the IL 282 CO-Oximeter should be made over a very narrow pH range. One can see from Figure I.3 that a change in 0.1 pH unit results in a change in apparent %COHb of 0.4%.

The significance of the influence of pH on the determination of %COHb in the subject samples is minimal. No differences were detected when the pH of the antecubital venous blood was measured before and after exercise. Although pH measurements were not made in all of the subjects, the lack of influence in those subjects suggests that pH did not play a major role in the variation of %COHb. Furthermore, subjects did not exercise during these tests to levels of oxygen consumption that would have produced transient changes in blood pH.

EFFECT OF OXYHEMOGLOBIN CONCENTRATION ON THE DETECTION OF PERCENT CARBOXYHEMOGLOBIN

The collection of antecubital venous blood for the detection of %COHb would be expected to vary in the oxygenation of the samples, both within and among subjects. The blood supply to the forearm is determined by the autonomic nervous system, the level of exertion of the muscles in the forearm, and the thermoregulatory demands of the body. When the arm is at rest, a high flow state will result in very little extraction of the arterial oxygen and a high $\%O_2Hb$ in the venous blood. A quiet, cool forearm, however, would have a low level of $\%O_2Hb$. Because these varying conditions would exist in the course of this study, the influence of oxygenation on detection of %COHb had to be determined.

Carbon monoxide is removed less readily than is oxygen from hemoglobin because of the high affinity of CO for hemoglobin, which was the basis for these experiments. Blood samples containing 0.7% and 4.1% COHb by GC were equilibrated with room air, in a ratio of air to blood of 1:1 for 10 minutes at room temperature. This changed the $\%O_2Hb$ from 25% to 95% in these samples without changing the %COHb. Mixtures were made with low and high oxygen content, and these samples were analyzed by both CO-oximetry and GC. As shown in Figure I.4, changing the oxygen content of the samples had a significant effect on the %COHb value obtained

from the CO-Oximeter, but the GC-based level of CO remained unchanged. These data suggest that a considerable portion of the variability of the %COHb data based on the CO-Oximeter is due to varying levels of $\%O_2Hb$.

The interaction between $\%O_2Hb$ and %COHb on the CO-Oximeter was also demonstrated by exposing to room air all the samples from a control subject who performed the experimental protocol. Samples were analyzed immediately after collection. Then room air was drawn into the syringe containing 4 to 5 ml of blood, so that the ratio of blood to air was 1:1. The samples were mixed for 10 minutes, and the gas phase was then ejected. The results are shown in Figure I.5. All of the levels of %COHb increased with increasing oxygenation. The variability in the antecubital venous $\%O_2Hb$ is also indicated, in Figure I.5, by the start-points for each sample prior to air exposure. The slopes of the lines all appear to be consistent.

The influence of oxygen on %COHb as determined by CO-oximetry was evaluated in many of the subjects studied at the St. Louis center. Nonessential samples were treated, as described above, for increasing $\%O_2Hb$. A plot of the change in %COHb versus the change in $\%O_2Hb$ is shown in Figure I.6. This is a significant relationship; however, the variability is great. The influence of oxygen among subjects can vary by three-fold. Therefore, it is essential to know the influence of oxygen in each individual.

EFFECT OF CHOLESTEROL AND TRIGLYCERIDES ON DETECTION OF PERCENT CARBOXYHEMOGLOBIN

Specimens of plasma were analyzed for total cholesterol and triglycerides for each subject. The absolute level of either, or both, of these lipids was compared with the discrepancy between the GC and CO-Oximeter methods of determining %COHb. No influence of these lipids was found. These observations are not in agreement with the statement in the laboratory manual for the IL 282 CO-Oximeter. However, the range of values for these lipids in the subjects in this study may have been less than those referred to in the manual.

EFFECT OF CUVETTE TEMPERATURE ON DETECTION OF PERCENT CARBOXYHEMOGLOBIN

The detection cell containing the cuvette in the IL 282 CO-Oximeter is maintained at 37°C. The adjustment of this temperature is possible with the use of an available test cuvette. Generally, this temperature is accurately set at the factory and does not need further adjustment. The test cuvette used for this study was modified to accept a copper-constantan thermocouple in the form of a (29-gauge) solid needle-like probe (Bailey Instruments). This enabled the direct conversion of the resistance (ohms) obtained from the test cuvette into cuvette temperature. The temperature of the cuvette was changed by adjusting the appropriate circuits in the instrument according to the field service manual. Three blood samples were analyzed at three different temperatures, allowing for an estimate of the influence of cuvette temperature on detection of %COHb. The results indicated that a 1°C change in cuvette temperature resulted in a change of + 0.6% COHb, regardless of the quantity of CO present. This temperature effect would, then, have its greatest influence on the blood samples containing less than 1.0% COHb. A check of six laboratories that were using the IL 282 CO-Oximeter indicated that all the instruments, except the two in St. Louis, were set at the factory to be within 0.5°C of each other. The St. Louis instruments were about 10°C lower than the other instruments. The St. Louis instruments were adjusted at that time to have the same cuvette temperature (36.2°C) as all the other instruments. Although the cuvette temperature is a possible source of error, no detectable difference in sample comparisons was noted after adjustment of the St. Louis instrument. This change in cuvette temperature may have accounted for the different regression equations presented above. It is not likely that the differences seen between instruments in the different laboratories are due to differences in cuvette temperature.

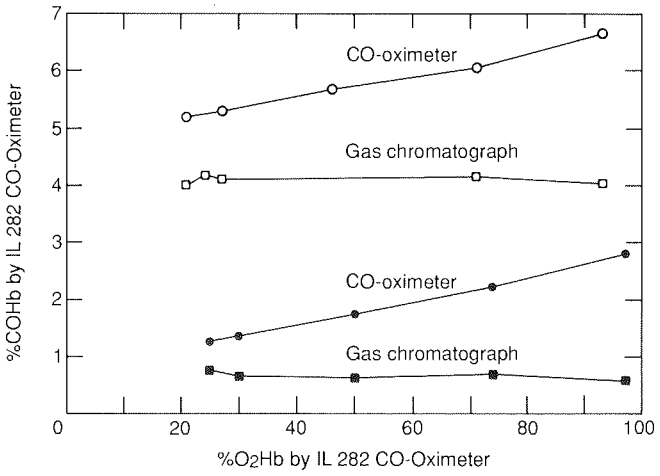


Figure I.4. Interaction of $\%O_2Hb$ determinations by the IL 282 CO-Oximeter. The oxygen saturation in aliquots of the same blood samples were varied, while COHb concentration was held constant (GC values).

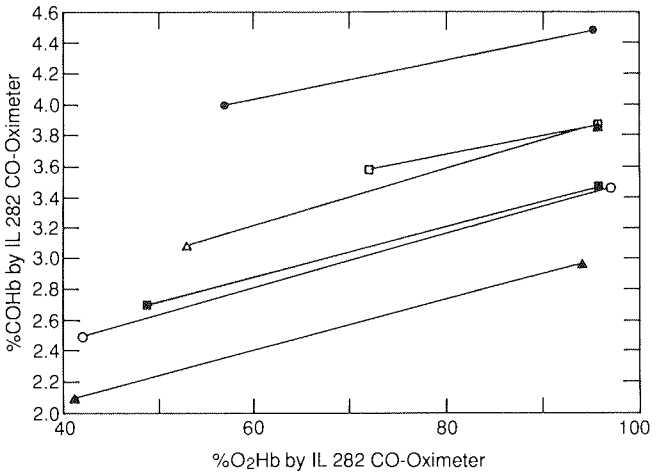


Figure I.5. Effect of oxygenating several samples from the same subject during a CO-exposure protocol (increasing initial COHb values). Note the consistency in the slopes of the lines. Also note the variability in the initial $\%O_2Hb$ values.

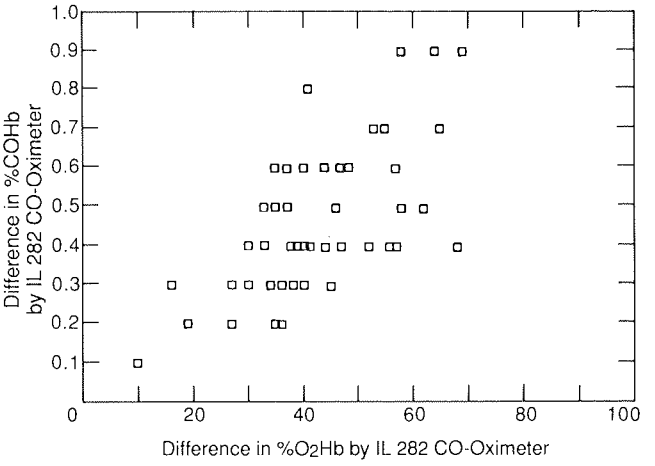


Figure I.6. Effect of increasing oxygen saturation on the increase in %COHb detected by the IL 282 CO-Oximeter. The oxygen saturation was increased from the level when collected by equilibration with increased oxygen tension, without loss of CO content, as measured by GC. These 47 subject samples were all analyzed at the St. Louis center. The regression analysis for these data shows: n = 48; r = 0.66; and $y = 0.047 + 0.0096x$.

APPENDIX J. Development of Optically Stable Low-Level Carboxyhemoglobin Standards

PREPARATION OF STANDARD BLOOD SAMPLES

Freshly drawn whole human blood was obtained from nonsmokers through the American Red Cross and from volunteers in the laboratory. The blood samples were heparinized (10 units/ml of blood) and analyzed by CO-oximetry to assure that the subject was a nonsmoker. If necessary, the pH of the sample was corrected to 7.3 to 7.4 by the addition of 1 M sodium phosphate (Na_2HPO_4). For each set of standards, 180 to 200 ml of blood were prepared. The blood was split into two aliquots, and 5 to 7 ml of one were equilibrated with 100% CO for 15 minutes. This blood, which was saturated with CO, was then added to the half of the initial stock designated as the high standard (resulting in the entire high standard containing approximately 7% COHb). The high and low stock standards were then centrifuged at 1200 × g for 30 minutes in order to pellet the blood cells. Then the plasma, which also contained the volume of phosphate buffer that had been added for pH adjustment, was removed. The volume representing the plasma, but not the buffer, was then replaced with an equal volume of a sterile Krebs-Hensleit buffer at pH 7.4, which contained 6% bovine serum albumin. This buffer represents a synthetic plasma that is isoosmotic and isooncotic with normal plasma.

The final mixture resulted in whole blood cells that were suspended in an artificial plasma, with a viscosity similar to that of normal whole blood. These properties were essential for these standards to be used to assess the functional capabilities of the IL 282 CO-Oximeter. This instrument uses an oscillator to assist in the lysis of cells. The extent of cell lysis is determined by measuring the number of whole red cells present. Another essential feature of this instrument is a series of roller pumps for aspirating the sample into the measuring cuvette. Proper functioning requires that a standard sample have a viscosity similar to blood.

Mixtures of the high and low standards were made in one-third/two-thirds ratios, resulting in four samples with from 0.5% to 7% or 8% COHb. After these well-mixed stock samples were stored in the dark for at least 24 hours at 4°C, aliquots were taken from them in a random order and sent to the center laboratories. All of the samples were exposed to room air during the preparation phase, and had $\%O_2Hb$ levels above 90%.

The entire procedure was carried out with sterile disposable materials. The synthetic plasma was filtered with 0.22 micron filters (Nalgene, Rochester, NY) in a laminar flow hood. These precautions were taken in order to minimize the bacterial contamination of these samples, which was the biggest factor affecting optical stability. When a sample was contaminated, the oxygen saturation fell and the %COHb changed, presumably reflecting both a deterioration of the optical quality of the sample and the interactive effect of the $\%O_2Hb$ on %COHb (Appendix I). The changes in %COHb detected by CO-oximetry were not accompanied by changes in CO content, as measured by GC.

Prior to shipment, standards were analyzed for pH (Radiometer BMS3 M2) and for %COHb by CO-oximetry and GC. This testing also permitted the determination of the absence of clots in the samples. The heparinization, and the removal of the bulk of the clotting factors by substitution of the synthetic plasma, reduced the clotting problems.

STABILITY OF THE ST. LOUIS STANDARDS

All of the standards were read on the CO-Oximeters at all three laboratories within three days of shipment. It was essential that these samples remain stable for three days. A comparison of the results obtained from early sets of standards over the three days of analysis in the Reference Laboratory is presented in Table J.1. These data were analyzed by averaging all of the data collected for all three days for each sample and then computing the daily values as a percentage of the mean value. If there was a clear trend in the values obtained for each sample, the percentage values would have either systematically increased or decreased. The normalization of the data (presenting them as percentages) was necessary because of the wide range of values included in the analysis. It is clear from the data in Table J.1 that these standards were stable for three days.

An additional test of the stability of these standards was carried out more recently. A set of four standards was analyzed every day for five days on the CO-Oximeter at the St. Louis center (designated as the local instrument). These samples contained 1.5%, 3.7%, 6.0%, and 8.3% COHb and, as shown in Figure J.1, did not vary by more than 0.1% to 0.2% over the entire week. These data indicate that the standards and the instrument were very stable.

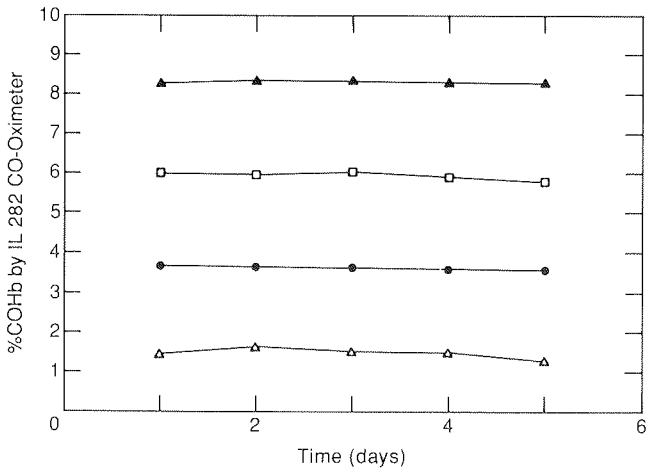


Figure J.1. Stability of the optical quality of standard samples for %COHb analysis by the IL 282 CO-Oximeter. Four standards were prepared, as described in Appendix J, and the %COHb was measured daily. The samples were stored in capped disposable syringes in the dark at 4°C. ▲ = standard 180; □ = standard 177; ● = standard 179; and Δ = standard 170.

EFFECT OF SAMPLE SHIPMENT

The concern about the effect of shipping these standards was addressed by sending out a standard set to Rancho Los Amigos and having the laboratory immediately return them to the St. Louis Reference Laboratory. This resulted in a double shipment of the samples. The results of the standards were compared between the two laboratories at the point when the oxygen content of the samples was the same. The CO-Oximeter values for %COHb were 4.5% at Rancho Los Amigos and 4.6% at the St. Louis Reference Laboratory. The total hemoglobin, determined by cyanmethemoglobin, and the CO content, determined by GC, were unchanged as a result of the double shipment.

Table J.1. Effect of Storage on Carbon Monoxide Content and Percent Carboxyhemoglobin Reference Standards as Measured by Gas Chromatography[a]

Standard No.	CO Content as % of Mean			%COHb as % of Mean		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Samples with Greater Than 2% COHb						
21	100	102	98	98	110	92
22	102	100	98	104	104	91
25	95	100	105	97	97	106
27	99	100	100	100	100	100
28	100	100	100	99	103	99
29	91	103	107	87	114	99
31	97	102	102	95	106	99
41	101	100	99	104	96	101
42	100	100	99	101	98	101
44	99	100	100	99	99	102
45	99	101	101	102	98	100
46	102	100	98	104	99	97
47	98	104	98	100	100	100
54	96	102	102	102	100	98
55	97	104	99	105	102	93
56	98	102	100	106	99	96
n	16	16	16	16	16	16
Mean	98.4	101.3	100.4	100.2	101.6	98.4
SD	2.7	1.4	2.5	4.5	4.7	3.8
Samples with Less Than 2% COHb						
23	104	104	91	107	107	86
26	96	108	96	94	113	94
30	91	104	104	86	107	107
43	95	102	102	100	100	100
48	89	111	100	100	100	100
53	94	113	94	109	109	82
n	6	6	6	6	6	6
Mean	94.8	107	97.8	99.3	106	94.8
SD	4.7	4.0	4.6	7.7	4.7	8.6

[a] Values were measured for all three days, and each value was represented as a percentage of the mean. Each data point represents the average of three determinations.

APPENDIX K. Comparison of IL 282 Results at the Center Laboratories and the Gas-Chromatographic Values for the Standard Samples

Regression analyses were carried out on CO-Oximeter values for the standard samples that were periodically sent to each center laboratory, comparing them to the GC values obtained on each sample in the Reference Laboratory prior to shipment. The key aspects of the analyses were the CO-Oximeter value of the linear intercept for each instrument (the point at which the GC value would be zero) and the slope of the regression. For the Johns Hopkins center: intercept = 0.28% COHb, slope = 1.07, and n = 211. For the Rancho Los Amigos center: intercept = 0.20% COHb, slope = 1.07, and n = 205. For the St. Louis center: intercept = 0.44% COHb, slope = 1.07, and n = 308.

From this information, it can be seen that the slopes of the instruments are identical, but the intercept values are not. This implies that the instruments are operating virtually identically. However, the absolute value will be offset, based on the intercept values. The intercept values can be adjusted electronically, for example, by adjusting the cuvette temperature, so the intercept variability could be due to minor differences in factory adjustments.

Similar regression analyses were done for the samples collected during the study that were analyzed immediately by CO-oximetry and shipped to the Reference Laboratory for analysis by GC. In both analyses, the GC-derived value was considered to be the independent value. The following results are for the whole-blood samples analyzed immediately by CO-Oximeter. For the Johns Hopkins center: intercept = 0.55% COHb, slope = 1.05, and n = 192. For the Rancho Los Amigos center: intercept = 0.47% COHb, slope = 1.05, and n = 162. For the St. Louis center: intercept = 0.79% COHb, slope = 1.05, and n = 203.

For these data, as well as for the reference samples, the slopes of the regressions for all three instruments were identical. This is not the case for the intercepts. It should also be pointed out that the absolute values of both parameters are not the same for the standards and the freshly analyzed subject samples. This further emphasizes that the reference samples were intended to be used only to standardize the instruments. These standards did not precisely reflect the ability of these instruments to analyze freshly drawn samples of whole blood.

APPENDIX L. Results of the Round-Robin Analysis of Cylinders of Unknown Carbon Monoxide Content

In order to evaluate the consistency among the three centers in the measurement of CO concentration in air, the following procedure was developed. On five occasions throughout the study, two coded cylinders of analyzed CO concentration were blindly analyzed in all three centers. This procedure provided assurance that a given CO level measured at one center was similarly measured at the other two centers. The originating laboratory, Rancho Los Amigos, analyzed these cylinders at the beginning of each series and upon the return of the cylinders to that laboratory. These cylinders were also reanalyzed by the manufacturer, Scott Specialty Gases. At all three centers the personnel carried out the analyses according to the uniform standard operating procedure and were blinded as to the concentrations in the cylinders. The cylinders were labeled A and B, and it was known in advance that one cylinder would be high and one low in CO concentration.

The interlaboratory comparison was carried out according to the protocol given below. The Rancho Los Amigos laboratory purchased two cylinders of CO calibration gas (EPA Protocol analytical quality, ± 1%). These cylinders were delivered from the commercial supplier, Scott Specialty Gases, directly to Rancho Los Amigos. One of the cylinders contained a CO concentration in the range of 0 to 100 ppm, and the other contained a CO concentration in the range of 0 to 250 ppm. Because of the requirements of the CO analyzer in use at Rancho Los Amigos, these cylinders also contained 350 ppm CO_2, with the balance pure air. The technical information regarding the analysis of CO concentration was removed from the cylinder upon receipt of the cylinders at Rancho Los Amigos. The cylinders thereafter were identified only by the letters A and B and by their Department of Transportation cylinder identification numbers. These cylinders were then analyzed at Rancho Los Amigos and shipped, along with the appropriate regulators, to the next laboratory by Federal Express delivery.

At each center laboratory, the CO monitors were prepared and calibrated according to the standard operating procedure for monitoring the CO concentration in the chamber. That is, the working standard gases were used to calibrate the instrument, and the instrument was determined to be in working order according to the guidelines established for the multicenter study. These unknown gases were then treated as though they were additional standard gases. A CO concentration was determined for each cylinder three times, and the values reported in Table L.1 represent the averages of these three values. The cylinders were then shipped to the next designated laboratory. Each round-robin was completed within 30 days. These data indicate that the instruments at the three centers produced comparable results.

Table L.1. Results of Round-Robin Analysis of Carbon Monoxide at the Three Center Laboratories

Date	Cylinder	Scott[a] Before	Rancho Los Amigos	St. Louis	Johns Hopkins	Rancho Los Amigos	Scott After
5/85	A	69	70	70	72	76	70
5/85	B	176	172	175	172	180	177
1/86	A	110	111	111	123	110	112
1/86	B	299	297	295	320	295	299
7/86	A	122	123	119	125	122	122
7/86	B	268	271	271	280	271	268
11/86	A	83	82	81	83	83	81
11/86	B	227	233	225	226	234	226
4/87	A	70	70	71	71	70	69
4/87	B	251	255	246	261	256	251

[a] Scott = Scott Specialty Gases (originator of the cylinder).

APPENDIX M. Attainment of Target Levels of Carboxyhemoglobin

As described in the main body of this paper, several protocol changes were made in the early phase of this investigation that had an impact on the levels of %COHb at the end of CO exposure. The desired levels of %COHb, as measured by GC, were set by the protocol (target levels of COHb.) The description given here will be limited to data collected on subjects enrolled in the study after August 29, 1985, when the target levels of %COHb were 3.2% and 5.7% by CO-Oximeter. The exposure protocol was designed to elevate all the subjects in the study to two levels of COHb: 2.2% and 4.4% by GC. These levels were chosen based on (1) the results obtained on normal subjects, who showed a 10% decline in COHb with 16 minutes of exercise on the study protocol; and (2) the desire to have 2.0% and 4.0% COHb at the end of the exercise protocol. The target levels were based on the discrepancy observed between the values recorded by the St. Louis IL 282 CO-Oximeter on all the samples collected prior to August 1985 and those found by the GC technique (reference method) for assessment of %COHb. There were several unproven components of the exposure protocol that made this approach subject to question: the linearity of the uptake, the variability in the uptake of each person, and the potential problems of the CO-Oximeter for use in this range of COHb values.

The results of the use of the exposure protocol are shown in Table M.1 for the 56 subjects studied under this final protocol revision. With a target of 3.2% COHb on the low day, an average of 3.26% COHb was obtained. All the centers were within 0.1% COHb, which was better than had been predicted at the outset.

With a target of 5.7% on the high-target day, the group mean was 5.68% COHb. The Johns Hopkins and Rancho Los Amigos centers were about 0.1%-COHb high, and the St. Louis center was 0.2% low. Based upon the differences seen in the CO-Oximeter units at the centers, this discrepancy would be predictable. As seen in Table M.1, the base-line (sample 2) %COHb levels for the St. Louis center were higher, which means that the chamber level of CO would be computed to be lower than at the other centers. Therefore, with a lower range of atmospheric CO, one might expect the subjects to take up less CO in the exposure period than at the other two centers, where the exposure levels were higher. These CO-Oximeter results would indicate that the variability in uptake rate for each subject on different days was corrected for by varying the time of exposure, and that the linearity of the uptake rate was a correct assumption.

The mean, standard deviation, and range of values at each center are shown in Table M.2. These data show that all the centers had essentially the same dispersion of values for both the 2%- and 4%-COHb-target days of exposure. In this group of subjects, none of the values for the two exposures overlap; except for St.Louis, there is a difference of greater than 1.0% COHb between the maximum of the 2%-COHb-target day and the minimum of the 4%-COHb-target day. The protocol chosen was, therefore, able to do what it was designed to do: give two separate populations of low levels of %COHb.

The actual levels of %COHb, as determined by GC, are shown in Table M.1. The variability between the GC and CO-Oximeter readings at the three centers is large because of the discrepancy between the CO-Oximeter units at the different laboratories. Clearly, the St. Louis center instrument had a much greater offset when compared to the GC results obtained in the Reference Laboratory. The smaller offset at Johns Hopkins and Rancho Los Amigos resulted in levels of %COHb by GC that were higher than expected for the group of 56 subjects: 2.43% vs. 2.2% and 4.77% vs. 4.4%. The experimental subjects lost more CO from their blood as a result of the exercise than expected (based on the pilot studies) and more than did the control subjects. This resulted in group mean values of 2.06% and 4.00% COHb at the end of exercise, which were the desired endpoints. However, it can be seen that the St. Louis values were less than those at other centers, resulting in a wider range of values than originally planned. The same range of values for the GC-based levels of %COHb existed as for the CO-oximetry values, discussed in detail above: approximately 1%-COHb difference between the maximum of the low level and the minimum of the high level %COHb.

Table M.1. Carboxyhemoglobin Values for the Blood Samples from Subjects Prior to and at the End of Exposure to Carbon Monoxide and After Exercise[a]

	Sample	Group	Johns Hopkins	Rancho Los Amigos	St. Louis
Co-Oximeter Values for %COHb					
3.2% Target[b]	2	1.21	1.18	1.09	1.35
	5	3.26	3.33	3.23	3.20
	6	2.67	2.79	2.61	2.57
5.7% Target[b]	2	1.20	1.13	1.15	1.31
	5	5.68	5.78	5.75	5.52
	6	4.76	4.80	4.84	4.65
Gas-Chromatograph-Based Values for %COHb					
2.2% Target[b]	2	0.62	0.60	0.69	0.59
	5	2.43	2.56	2.59	2.18
	6	2.06	2.25	2.15	1.78
4.4% Target[b]	2	0.67	0.66	0.76	0.61
	5	4.77	4.91	4.99	4.45
	6	4.00	4.04	4.25	3.76

[a] Sample 2 was taken before exposure, sample 5 at the end of exercise test 1, and sample 6 after excercise test 2. Determinations were made by both IL 282 CO-Oximeter and by GC. Average values are shown for the entire group (n = 62 or 63) and for each center laboratory.

[b] The target end-of-exposure (sample 5) COHb levels were 3.2% and 5.7% when measured by CO-Oximeter, corresponding to 2.2% and 4.4%, respectively, when measured by GC. These COHb-target levels are 10% greater than the desired levels at the end of the second exercise test (sample 6), which were 2.0% and 4.0% COHb, to compensate for the loss of CO during exercise.

Table M.2. Average Carboxyhemoglobin Values as Determined by IL 282 CO-Oximeter on Subject Blood Samples at the End of Carbon Monoxide Exposure[a]

			%COHb	
Center	Mean	SEM	Minimum	Maximum
CO Exposure To Attain 3.2% COHb				
Johns Hopkins	3.33	0.29	3.00	4.10
Rancho Los Amigos	3.23	0.22	2.80	3.60
St. Louis	3.20	0.24	2.80	3.60
CO Exposure To Attain 5.7% COHb				
Johns Hopkins	5.78	0.39	5.20	6.50
Rancho Los Amigos	5.75	0.33	5.20	6.30
St. Louis	5.52	0.34	4.40	6.00

[a] These values were obtained under randomized blinded conditions after August 29, 1985.

APPENDIX N. Carbon-Monoxide-Uptake Rates

The rate at which an individual subject took up CO was an important aspect of the experimental protocol used in this study. The exposure protocol was designed to limit the increases in COHb to two levels at the end of exposure, 2.2% and 4.4% (by GC), with as little deviation from these values as possible. Based upon limited preliminary studies on normal subjects, the CO-uptake rate was found to be more variable than expected. These pilot data led to the incorporation of the measurement of uptake rates on each subject, and then the use of these individual rates to control the exposure conditions in order to attain the desired COHb endpoint. This appendix discusses the subjects in the final data set at each of the centers.

Individual CO-uptake rates were determined for each of the subjects in the study. At the end of the qualifying visit (visit 1), each subject was exposed in the chamber at the respective center to 150 ppm CO for 60 minutes. During this portion of the visit, venous blood samples were collected both prior to exposure and at 15-minute intervals during the exposure. The blood collection was carried out while the subject remained in the chamber; the person collecting the samples briefly entered the chamber for this purpose. The samples were collected via a low-volume indwelling venous catheter. The

subject remained comfortably seated in the chamber for the whole period of exposure. The blood samples were analyzed immediately by CO-oximetry. The uptake rate was assumed to be linear over this time period. Linear-regression analysis of these data points was carried out and the uptake rate for CO was determined by the plotted slope of the %COHb vs. time.

The chamber CO levels were monitored continuously during the exposure and were recorded. The actual chamber level of CO was averaged over a 15-minute time interval, and the time-weighted levels of CO, in ppm, were averaged for the one-hour exposure.

A CO-uptake rate constant was calculated from the above set of data. The change in %COHb per minute was multiplied by 60 minutes. The resulting value, in %COHb per hour, was then divided by the one-hour average chamber CO concentration. The resultant uptake constant has the units of change in %COHb per hour per ppm CO. This constant was chosen so that it could be used easily in the experimental protocol to determine a chamber CO concentration. The CO level in the chamber was then calculated to bring about a desired change in %COHb in a one-hour period.

This analysis of the rate of CO uptake and CO uptake constants is based entirely upon CO-Oximeter data. It would be assumed that GC analysis of these samples would produce very similar findings. This is based upon the fact that the slope of the CO-Oximeter data vs. the GC data from the same samples is very close to a value of 1 (Appendix H). The before- and end-of-exposure blood samples for visit 1 were analyzed by GC only at the St. Louis center. Therefore, a comparison of GC uptake rates is not possible among the three centers.

Further analysis of uptake data is possible through the computation of uptake rates for CO during the randomized exposure test days. By including the analysis of the randomized days along with the visit-1 exposures, the influence of CO concentration and the possibility of protocol familiarization (loss of apprehension) on the uptake rate of CO could be determined. The latter issue is of importance because the rate of alveolar ventilation is one of the most important physiological variables in the determination of CO uptake. Alveolar ventilation would definitely be increased in a subject who was anxious about being in the chamber and being exposed to CO.

RESULTS

The data presented in Table N.1 indicate that, for visit 1, the chamber exposures were very close to the desired level of 150 ppm. These reported values are considered to be accurate because of the round-robin analysis of unknown gas cylinders (Appendix L). These blinded tests of the instruments used for atmospheric monitoring indicated that all three local instruments gave similar readings. However, it is possible that the Johns Hopkins chamber levels were overestimated by approximately 4%: the average overestimate of the blinded standards was 6 ppm (Appendix L). This means that the Johns Hopkins atmospheric levels during the visit-1 chamber exposures may have been 144 ppm, and not the 150 ppm reported.

The average blood levels of %COHb at the different times of exposure during visit 1 were identical at all three centers, as shown in Table N.2. Therefore, the computed uptake constants were all the same: There were no differences between the mean values at each center. This is substantiated by the comparison of the reported uptake-rate constants, which shows no difference among centers. There is some concern about the data presented in Table N.2 for the preexposure levels of %COHb. In the randomized trials, this sample is roughly comparable to sample 2 taken at the end of exercise test 1. The randomized-visit sample-2 data reflect the differences in the function of the CO-Oximeters at the different laboratories: the St. Louis values are consistently higher than those values at the other laboratories. Attempts to resolve this lack of difference, shown in Table N.1, have failed.

It should be pointed out that the range of uptake constants for CO in this group of subjects is large. This wide range can be inferred from the range of CO-exposure levels shown in Table N.1. With a range of required CO levels of 42 to 202 ppm to obtain the 3.2%-COHb target, it can be seen that the uptake constant varied by 116% (range/mean × 100.) The potential factors involved in this variability have been delineated by Coburn and coworkers (1965), but there are inadequate data on these parameters in these subjects to speculate about the reasons for this wide range of values.

Table N.1. Chamber Carbon Monoxide Concentration During Subject Exposures at All Three Centers[a]

	Group		Individual Centers					
			Johns Hopkins		Rancho Los Amigos		St. Louis	
	CO Concentration (ppm)	n	CO Concentration (ppm)	n	CO Concentration (ppm)	n	CO Concentration (ppm)	n
Visit 1	149.8 ± 2.6	63	150.2 ± 3.3	22	149.6 ± 1.1	18	149.6 ± 2.6	23
Air day	0.7 ± 0.6	62	0.1 ± 0.4	22	1.3 ± 0.5	18	0.8 ± 0.5	22
2%-COHb-target day	117.4 ± 34.6	62	113.9 ± 31.6	22	115.7 ± 35.3	18	102.3 ± 30.6	22
4%-COHb-target day	252.9 ± 48.6	63	267.1 ± 45.3	22	255.8 ± 53.9	18	237.0 ± 44.4	23

[a] The values represent means ± SD of the time-weighted averages for each subject.

Table N.2. Visit-1 Uptake Rate: Percent Carboxyhemoglobin Values

				Individual Centers					
		Group		Johns Hopkins		Rancho Los Amigos		St. Louis	
Sample	Minutes	%COHb	n	%COHb	n	%COHb	n	%COHb	n
2	0	1.4 ± 0.4	62	1.4 ± 0.5	22	1.4 ± 0.4	18	1.4 ± 0.4	22
3	15	2.2 ± 0.4	62	2.2 ± 0.4	22	2.2 ± 0.5	18	2.3 ± 0.4	22
4	30	2.8 ± 0.4	62	2.8 ± 0.4	22	2.8 ± 0.5	18	2.9 ± 0.4	22
5	45	3.4 ± 0.5	62	3.4 ± 0.5	22	3.4 ± 0.5	18	3.5 ± 0.4	22
6	60	4.0 ± 0.5	62	4.0 ± 0.5	22	4.0 ± 0.6	18	4.1 ± 0.5	22

APPENDIX O. Effectiveness of Exposure Protocol

Minimizing the range of end-of-exposure COHb levels in this study was desirable because the primary analysis compared cardiovascular changes at 0.7% COHb to changes at 2.0% and 4.0% COHb. The more these exposures could be cleanly separated, the better. The extent to which the protocol maneuvers, of varying exposure concentration and time, were effective in accomplishing this reduction in variability can only be estimated. We have taken two approaches to estimating the scatter that would have been obtained if these subjects had been exposed to fixed levels of CO for a fixed time.

The first approach was to use the data from visit 1, when all subjects were exposed to 150 ppm for one hour. The range of COHb values obtained after 60 minutes of exposure demonstrates considerable variability among individuals in CO uptake. Analysis of data from visit 1 from the St. Louis center shows that, for 24 subjects studied, the end-of-exposure COHb values ranged from 3.8% to 5.8%, with a mean of 4.1% (SD = 0.49). This range of 2.0% among subjects at the end of a 60-minute exposure equals the difference between the two desired levels after exposure to CO used in this study. Therefore, it suggests that the use of a fixed-exposure/fixed-time protocol would have been unacceptable.

A second approach was the use of the individual uptake rates actually measured on visit 1, combined with the actual starting COHb levels, to project a theoretical end-of-exposure COHb. The predicted levels of COHb for the St. Louis subjects were calculated for fixed atmospheric levels of CO for 60 minutes of exposure. The calculated end-of-exposure levels were obtained by using the average chamber levels of 102 ppm and 237 ppm CO that were used in the study. The uptake-rate constants determined during the first visit were used to predict the 60-minute values. The values of %COHb determined prior to exposure on each day were used as the start values. This process predicts the effect of exposing these subjects to a constant level of CO for 60 minutes given the variability in their base-line levels of %COHb. The calculated results are compared with the actual results in Table O.1. The range, standard deviation, and standard error are all reduced about two-fold at each target level in the actual, compared to the calculated, results.

Table O.1. Comparison of Actual Carboxyhemoglobin Results with Projected Results if a Fixed-Concentration/Fixed-Time Protocol Had Been Used

	2%-COHb-Target Day		4%-COHb-Target Day	
	Actual[a] %COHb	Calculated[b] %COHb	Actual[a] %COHb	Calculated[b] %COHb
Mean	3.1	3.2	5.4	5.6
Median	3.1	3.1	5.6	5.8
Range	2.7 – 3.6	2.1 – 4.4	4.4 – 6.0	4.4 – 7.7
SD	0.26	0.59	0.41	0.79
SEM	0.05	0.12	0.08	0.16

[a] Actual experimental data (individual CO levels/variable time).

[b] Calculated data using uptake rates determined at visit 1 and assuming a single CO-exposure level and constant time.

APPENDIX P. Elimination of Carbon Monoxide During Exercise

The previous studies that have investigated the effect of CO on the exercise performance of subjects with coronary artery disease all used protocols that had the time of onset of angina as the end of the exercise test. Therefore, the COHb level measured at the end of the experiment reflected the level at which the pain occurred. The exercise protocol in the HEI multicenter experiments required the subjects to continue to exercise after angina developed and after the ischemic ST-segment change in the ECG had occurred. Therefore, the %COHb measurement made at the end of exercise does not precisely reflect the level at which the cardiovascular endpoints occurred. An understanding of the nature of the change in %COHb that occurred during the exercise protocol is needed for determination of the levels of %COHb that occurred during the exercise protocol at which any cardiovascular changes may have occurred.

At the outset of this study, the basic assumption about the loss of CO as a result of exercising in room air was that the rate of loss would be predictable. It was presumed that this loss of CO would have occurred primarily through the respiratory system. With the above considerations in mind, the end-of-exposure (target) levels of COHb were based upon the desire to have end-of-exercise levels of COHb of 2.0% and 4.0% for the two levels of CO exposure. The attainment of these levels was thought to require approximately a 10% overshoot, that is, 2.2% and 4.4% COHb. This overshoot was based upon the approximate decrease of 10% in COHb in normal subjects who followed the protocol during pilot studies.

This appendix describes briefly a series of experiments carried out in normal subjects in a more detailed protocol, designed to follow the changes in %COHb during the modified Naughton protocol used in the multicenter study. Similar experiments were not carried out in the subjects with angina, but it is assumed that the data presented below would, in general, pertain to the subjects observed in the HEI multicenter study protocol.

METHODS

The studies described below were designed to follow the changes in %COHb as a result of performing a standard treadmill exercise test according to the modified Naughton protocol. Four male, nonsmoking, healthy subjects between the ages of 24 and 33 years, who had given informed consent, were given a standard 12-lead ECG. An antecubital venous catheter was inserted to enable repeated blood collection. The subjects were given 50 ml of 99.5% CO into the inhaled air via a rebreathing system; this procedure elevated their %COHb by CO-oximetry to 5.6% after 10 minutes.

Once the subjects had been given this dose of CO, which represented the amount used in the multicenter protocol, they entered the exposure chamber. After the subjects were removed from the rebreathing system, they breathed room air containing less than 1 ppm CO for the duration of the study. These subjects were connected to a standard open-circuit breathing apparatus, used conventionally for monitoring minute ventilation and oxygen consumption. In this case, the minute CO excretion was monitored. Based upon the expected performance of the experimental subjects with exercise-induced angina, these healthy subjects were not exercised longer than 18 minutes on the protocol (3 mph at 15% grade), which represented approximately 50% to 60% of the maximal effort of these normal subjects.

Carbon Monoxide Excretion from the Respiratory System

After the CO loading via the rebreathing system, the subjects then entered the environmental chamber and were attached to the respiratory monitoring system. This consisted of a low-volume directional breathing valve connected, by low-resistance tubing, to a 5-liter baffled mixing chamber. The expired minute ventilation was monitored with a Pneumoscan model 301 (Los Angeles, CA), which was calibrated against a Tissot spirometer (W.E. Collins, Braintree, MA). The temperature of the expired gas was measured with a Bailey Instruments (model BAT 8) monitor that was connected to a thermocouple in the exhaled air as it passed by the ventilation-sensing head. The mixed expired CO concentration was monitored from the mixing chamber with the use of a Bendix infrared analyzer, set at a very high flow rate to minimize the delay in response time of the analyzer. The delay in response time was taken into account in the analysis of the continuous record of the mixed expired CO concentration.

Carbon Monoxide Loss from the Blood

The subject's venous blood was sampled by a low-deadspace (100-μl) catheter, in the antecubital vein, which was flushed with 10 units/ml heparin in sterile saline after each sample. Samples of blood (10 ml) were collected into heparinized syringes (100 units of heparin). These samples were analyzed immediately by CO-oximetry for %COHb. All values represent averages of triplicates for each sample. Blood samples were taken prior to exercise and at 4, 8, 12, and 16 minutes of exercise. Often the oxygen saturation in the venous blood varied during the exercise period. Therefore, the analysis of the CO-Oximeter data was carried out with the use of a correction factor for the $\%O_2Hb$ effect on the %COHb, as described in Appendix I.

RESULTS

The loss of CO during the exercise test in four normal subjects was monitored by CO-oximetry. The %COHb values, corrected to 80% O_2Hb, are shown in Figure P.1. They suggest that the loss of CO from the blood may be linear for this

type of exercise. If more frequent samples had been collected, the linearity of the results could have been tested with more confidence. These results were treated as though the relationship of COHb and time of exercise were linear. Linear-regression analysis indicated that %COHb was correlated with duration of exercise with a correlation coefficient of –0.98, where $\%COHb = 5.66 - 0.038x$, and x = minutes of exercise.

The minute-by-minute CO excretion was summed for the duration of the exercise test and is shown in Figure P.2. It is apparent that the linear loss of CO from the blood is accompanied by a linear loss of CO from the respiratory system.

CONCLUSION

Since the change in %COHb is linear for subjects undergoing the exercise protocol used, one could extrapolate from the change in blood %COHb levels at specified times of exercise to obtain intermediate values of %COHb.

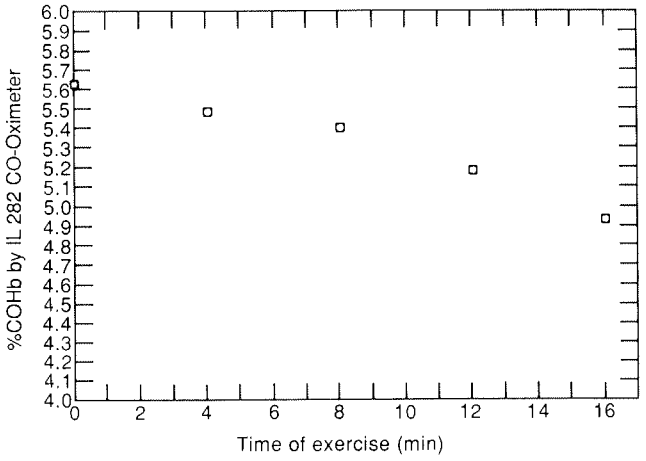


Figure P.1. Effect of modified Naughton treadmill exercise test on the change in %COHb in subjects. The %COHb was determined by CO-oximetry. Each data point is the average value of four subjects.

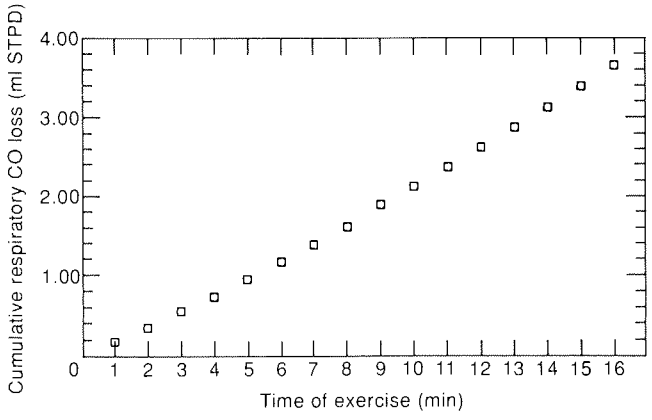


Figure P.2. Effect of modified Naughton exercise treadmill test on tbe cumulative respiratory loss of CO in normal subjects. Each data point reflects the average value for four subjects.

APPENDIX Q. Exposure Chamber Descriptions

EXPOSURE CHAMBER SPECIFICATIONS AT JOHNS HOPKINS UNIVERSITY

Physical Description

Dimensions. Ten by 20 by 10 feet (2,000 cubic feet).

Features.

- Restroom that doubles as an entry air lock.
- Additional door with one-way mirror for observation.
- Large outside sealed window.
- Partially carpeted floor.
- Intercom system between chamber control and exposure areas allows monitoring of subject.
- CO-monitoring equipment and CO cylinders screened from view during exposure.

Environmental Conditions

Operated at 65° to 75°F and 45% to 70% relative humidity.

Air Handling and Sampling

Air handling at 1,850 cubic feet per minute; air sampling at 1 to 2 feet from subject's head while seated.

Location with Respect to Other Study Functions

Analysis of blood samples by CO-oximetry was carried out within a screened area containing CO-monitoring equipment. Exercise testing was conducted in the chamber under ambient air conditions.

EXPOSURE CHAMBER SPECIFICATIONS AT RANCHO LOS AMIGOS MEDICAL CENTER

Physical Description

Dimensions. Sixteen by 13.3 by 9.5 feet (2,022 cubic feet).

Features.

- Controlled access through slightly pressurized airlock (dimensions 10.5 by 4.5 by 9.5 feet).
- Large window allows for observation of subject during exposure.
- CO-monitoring equipment screened from view during exposure.

Environmental Conditions

Operated at 71.7° ± 1°F and 51.2% ± 4.3% relative humidity.

Air Handling and Sampling

Air handling at 500 cubic feet per minute; and air sampling at 3 feet right of chamber center at a height of 7 feet. Quarterly homogeneity measurements were made at seven loca-

tions in the chamber, including the probable seating location of the subject, to document the appropriate use of remote sampling.

Location with Respect to Other Study Functions

Analysis of blood samples by CO-oximetry and exercise testing was carried out in separate laboratories.

EXPOSURE CHAMBER SPECIFICATIONS AT ST. LOUIS UNIVERSITY

Physical Description

Dimensions. Eight by 8 by 10 feet (640 cubic feet).

Features.

- Most walls (80%) made of clear lucite to allow observation of subject and reduce feeling of claustrophobia.
- CO-monitoring equipment and CO cylinders screened from view during exposure.

Environmental Conditions

Controlled for temperature at 60° to 85°F and for relative humidity at 60% to 80%.

Air Handling and Sampling

Air handling turnover once every five minutes; and air sampling at 1 to 2 feet from subject's head while seated. After chamber reaches desired CO level at the subject sampling site, a floor-to-ceiling and front-to-back gradient of less than 5 ppm exists.

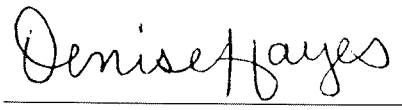
Location with Respect to Other Study Functions

Analysis of blood samples by CO-oximetry was carried out in a separate laboratory. Exercise testing was conducted in the chamber under ambient air conditions.

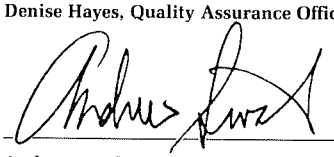
APPENDIX R. Quality Assurance Report

The conduct of this study has been subjected to periodic inspections by the Quality Assurance Team from Arthur D. Little, Inc. The dates of inspection visit, study center, nature of visit, and QA personnel participating in the inspection are listed in Table R.1. The results of the inspections were reported to the Executive Director of HEI, reviewed by the HEI project manager, and transmitted to the Principal Investigator at the study center by the HEI project manager.

Observations made during these visits indicate that the study is well documented and that the report describes the methods and standard operating procedures used. The final data audit and review of the final report indicated that the report accurately reflects the raw data, and that deviations from the protocol and standard operating procedures have been considered and addressed, as appropriate, in the analysis of the data and interpretation of the results of the study.



Denise Hayes, Quality Assurance Officer



Andrew Sivak, Vice President, Arthur D. Little, Inc.

November 6, 1987

Table R.1. Visits by Quality Assurance Team

Date	Participating Personnel	Study Center[a]	Nature of Visit
12/84	D. Hayes A. Sivak	HSPH	Discussion of data management procedures
1/8/84	D. Hayes	STL	Discussion of draft standard operating procedures
1/15/85	D. Hayes A. Sivak	RLA	Prestudy visit
2/27/85	D. Hayes A. Sivak	STL	Prestudy visit
3/5/85	D. Hayes S. Battista	JH	Prestudy visit
8/22/85	D. Hayes	Stanford University	Discussion and audit of Reference Laboratory functions
8/23/85	D. Hayes	RLA	Audit visit
9/12/85	D. Hayes	STL	Audit visit
11/8/85	D. Hayes	JH	Audit visit
2/20-21/86	D. Hayes	RLA	Audit visit
3/14/86	D. Hayes	HSPH	Review and discussion of data
3/27/86	D. Hayes	HSPH	Review of data and discussion of data management problems
4/9/86	D. Hayes	STL	Audit visit
5/23/86	D. Hayes	HSPH	Audit visit
5/30/86	D. Hayes	RLA	Audit visit
6/20/86	D. Hayes	STL	Audit visit
7/22/86	D. Hayes	JH	Audit visit
9/2/86	D. Hayes	STL	Audit visit
9/18/86	D. Hayes	JH	Audit visit
10/30/86	D. Hayes	JH	Audit visit
11/24/86	D. Hayes	JH	Audit visit
1/29-30/87	D. Hayes P. Capomaccio	STL	Audit visit
2/19-20/87	D. Hayes K. Findlen	RLA	Audit visit
4/14/87	D. Hayes	JH	Audit visit
5/21/87	D. Hayes	STL	Audit visit
7/17/87	D. Hayes	HSPH	Audit visit and discussion of final data audit
8/10-11/87	D. Hayes	STL	Final data audit
8/25/87	D. Hayes P. Capomaccio	JH	Final data audit
9/87	P. Capomaccio	HSPH	Final data audit of RLA data

[a] HSPH = Harvard School of Public Health, Health Sciences Computing Facility; STL = St. Louis University; RLA = Rancho Los Amigos Medical Center; and JH = Johns Hopkins University.

ABOUT THE AUTHORS

Ms. Elizabeth Allred received an M.S. in biostatistics from the Harvard School of Public Health in 1978. She is currently working as a statistician for the Department of Neuroepidemiology at Childrens Hospital in Boston. Her research interests involve data-base management and statistical analysis for longitudinal studies.

Dr. Eugene Bleecker received an M.D. from the State University of New York Downstate Medical Center in 1968. He was a research associate at the Gerontology Research Center at the National Institute of Aging and completed his training in pulmonary disease at the Cardiovascular Research Institute, University of California in San Francisco. He is currently an associate professor of medicine at the Johns Hopkins University School of Medicine. His research interests include respiratory and exercise physiology with specific interest in bronchial inflammation and the pathogenesis of asthma and chronic airflow obstruction.

Dr. Bernard Chaitman received an M.D. at McGill University in Montreal in 1969. He is currently professor of medicine at St. Louis University School of Medicine and director of cardiovascular research (Cardiology Division). His research interests involve exercise testing and nuclear cardiology procedures in the evaluation of patients with coronary artery disease and assessment of efficacy of therapeutic treatments in the management of patients with coronary disease.

Dr. Thomas Dahms received a Ph.D. in biological sciences at the University of California, Santa Barbara in 1970. He is serving as an associate professor in the Pulmonary Division at the Department of Internal Medicine and the Anesthesiology Division of Surgery at St. Louis University Medical Center. His research interests include pathophysiological effects of acute lung injury on vasoreactivity of the pulmonary circulation, and in health effects of carbon monoxide on the cardiovascular system.

Dr. Sidney Gottlieb received an M.D. from Emory University of Medicine in 1979, and all subsequent training in medicine and cardiology from Johns Hopkins University School of Medicine. He is an assistant professor of medicine in cardiology at the Johns Hopkins University School of Medicine. He is principally engaged in clinical research of the pathphysiology and treatment of ischemic heart disease.

Dr. Jack Hackney received an M.D. from St. Louis University School of Medicine in 1948. His postgraduate work was in internal medicine and pulmonary physiology. He is professor of medicine at the University of Southern California School of Medicine, and chief of the Environmental Health Service at Rancho Los Amigos Medical Center in Downey, CA. Since 1969, he and his associates have developed studies of human volunteers exposed to controlled environments in research on health effects of air pollutants.

Ms. Denise Hayes received an M.S. in pharmacology at Northeastern University in 1981. She is currently a quality assurance officer at Arthur D. Little, Inc. in Cambridge, MA. Her research interests include disposition and metabolism of xenobiotics, quality assurance, and regulatory affairs.

Dr. Marcello Pagano received a Ph.D. in mathematical statistics in 1970 from Johns Hopkins University. He is currently professor of statistical computing in the Department of Biostatistics at the Harvard University School of Public Health. His research interests include statistical methods in clinical trials and epidemic modeling, and statistical computing.

Dr. Ronald Selvester received an M.D. from Loma Linda University in 1950 completing postgraduate training in cardiology in 1958. He is currently professor of medicine at the University of Southern California and director of the biomathematics and ECG research group at Rancho Los Amigos Medical Center in Downey, CA. Major current research interests are in the detection and quantitation of regional myocardial ischemia and infarct size as it relates to prognosis, treatment, and rehabilitation of patients with ischemic heart disease.

Dr. Sandra Walden received an M.D. from Emory University School of Medicine in 1979. She finished her fellowship in pulmonary and critical care medicine in 1985 at Johns Hopkins University School of Medicine. She is currently an assistant professor of medicine in the Department of Pulmonary and Critical Care Medicine at the Johns Hopkins University School of Medicine. Her research focuses on the pathophysiology of asthma, chronic airflow obstruction, and related airways inflammatory diseases.

Dr. Jane Warren received a Ph.D. in biology from Princeton University in 1974. Since then her interests in genetics and developmental biology have broadened to include concerns about health effects of environmental agents. Since 1983 Dr. Warren has been at the Health Effects Institute, where she is now director of research and acting coexecutive director.

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ERRATA

Acute Effects of Carbon Monoxide Exposure on Individuals with Coronary Artery Disease

The HEI Multicenter CO Study Team

Research Report Number 25
November 1989

Investigators' Report:

p. 16 Right column, paragraph 3, line 3: change Raider to Naughton and Haider.

p. 42 Right column, Raider reference: change Raider R to Naughton J, Haider R.

Health Review Committee's Report:

p. 81 Left column, paragraph 3, last line: change 1973 to 1970.

p. 82 Right column, last line before heading: change 1985 to 1986.

p. 89 Right column, paragraph 3, line 9: change Raider to Naughton and Haider.

p. 92 Right column, paragraph 3, line 6: change Kleinman et al. 1973 to Kleinman and Whittenberger 1985.

p. 95 Right column, last line: change 9:415-417 to 49:415-417.

p. 96 Right column, Kannel and Abbott reference: change 1988 to 1984.

p. 97 Right column, Raider reference: change Raider R to Naughton J, Haider R.

BACKGROUND

REGULATORY BACKGROUND TO THE STUDY

The Clean Air Act mandates that the U.S. Environmental Protection Agency (EPA) establish primary standards for air pollutants based on health effects and at levels "requisite to protect the public health . . . allowing an adequate margin of safety." The legislative history of the act makes it clear that in setting the ambient air quality standards, the EPA is required to consider the health of particularly sensitive subgroups of the population. The Senate report on the legislation states: "An ambient air quality standard . . . should be the maximum permissible air level of an air pollution agent or class of such agents (related to a period of time) which will protect the health of any group of the population" (U.S. Senate 1970).

The identification of such groups is not clearly defined, but the Senate report does specify that "included among those persons whose health should be protected by the ambient standard are particularly sensitive citizens (such as bronchial asthmatics and emphysematics) who in the normal course of daily activity are exposed to the ambient environment." The report further states that "in establishing an ambient standard necessary to protect the health of these persons, reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group."

The current primary National Ambient Air Quality Standard for carbon monoxide (CO) is 9 parts per million (ppm), averaged over eight hours, and 35 ppm, averaged over one hour, both not to be exceeded more than once a year. Established in 1971, this standard was based on a study by Beard and Wertheim (1967) that reported an effect on the central nervous system (impairment in discrimination of time intervals) resulting from low-level CO exposures (50 and 100 ppm). However, several subsequent studies failed to replicate the Beard and Wertheim findings (O'Donnell et al. 1971; Stewart et al. 1973; Otto et al. 1979).

During the 1970s, three studies reported that relatively low levels of CO exposure (50 and 100 ppm for one to four hours) aggravated the symptoms associated with cardiovascular disease. The studies by Aronow and Isbell (1973) and by Anderson and coworkers (1973) reported more rapid onset of pain in angina patients, and another study by Aronow and coworkers (1974) reported a similar result in patients with intermittent claudication. The EPA also recognized the importance of "the more rapid accumulation of blood carboxyhemoglobin in moderately exercising sensitive persons compared to resting individuals. The impact of exercise, which is greater for short-duration exposures, was not considered in the original standard" (U.S. Environmental Protection Agency 1980). Therefore, the EPA proposed to lower the one-hour standard from 35 ppm to 25 ppm; this lower standard was never implemented.

Subsequently, an investigation of drug studies that were conducted by Aronow for the Food and Drug Administration revealed several serious problems with data quality. These findings raised questions about the validity of the Aronow CO studies (Aronow 1983; Horvath et al. 1983). Also, EPA scientists conducted an internal review of the Anderson study. They concluded that "several aspects of the study were above average quality; [but that] the study is surely in need of replication and extension" (O'Neil 1983). Thus, the scientific basis for the existing CO standard is uncertain because of questions raised about the reliability of the underlying data.

The EPA Request to HEI

In the summer of 1983, the Acting Assistant Administrator for Research and Development at the EPA, Mr. Courtney Riordan, wrote to the Health Effects Institute and asked the Institute to explore "possible approaches for performing an independent study of carbon monoxide effects on angina patients" that would use an objective measure of the health effects of CO. He added that "because the Health Effects Institute was established to perform quality health effects research on motor vehicle pollutant concerns (e.g., CO), [the EPA] would like HEI to consider sponsoring this independent study." In addition, the HEI learned that its industrial sponsors were also interested in such a study.

HEI's Response to the EPA Request

The Health Effects Institute agreed to undertake a study that would examine the effect of CO exposure on subjects with angina pectoris. In order to enroll a sufficient number of subjects, and to rule out any institutional or geographical biases, the HEI decided that a multicenter approach would be most appropriate. Because of the urgency of the EPA request, and the need for coordinating the multicenter study, the HEI Research Committee and the staff took an active role in the design and oversight of the study. The Research Committee appointed an advisory committee, composed of three members of the Research Committee and three experts who were not affiliated with HEI, to work with HEI staff in planning the study, evaluating the proposals, and overseeing the research. Members of the advisory committee were Drs. John Tukey (chairman), Stephen Achuff, Stephen Ayres, Joseph Brain, Roger McClellan, and Steven Horvath.

A general plan for the study, along with a description of the HEI application procedure, was sent to 271 cardiology

departments at hospitals and medical schools throughout the United States. Three of the proposals received in response to this letter (from investigators at Johns Hopkins University, St. Louis University, and Rancho Los Amigos Medical Center) were found to be especially meritorious, and were funded by the HEI.

The HEI Multicenter CO Study began in 1984 with preliminary studies and the development of a protocol and a manual of standard operating procedures. Testing of subjects at the three centers began in the spring of 1985. The final report from the study was accepted by the HEI Review Committee in March 1988. Total expenditure on the study was approximately $2.5 million.

THE HEI REVIEW PROCESS

Upon completion of any study funded by the HEI, the investigators submit a final report on the work, which undergoes a detailed peer review by the Institute's Review Committee. The Review Committee's objective in reviewing the Investigators' Report is to help ensure that the report is as complete, precise, accurate, and understandable as possible. Because of the complexity of the Multicenter CO Study, the Review Committee appointed an outside Technical Review Panel (Table 1), composed of cardiologists, pulmonary physiologists, and biostatisticians, to help the committee evaluate the study.

The Technical Review Panel met twice in Cambridge, MA. At the first meeting, the members discussed the justification, organization, and protocol of the study. At their second meeting, they met with the investigators, and reviewed and discussed the first draft of the Investigators' Report. The investigators were asked by the panel to revise their report, and to analyze the data further. The revised Investigators' Report, as well as the investigators' response to the panel's comments, were then reviewed by the Review Committee.

The Review Committee then prepared this report, in accordance with standard HEI practice. The Review Committee Report is intended to place the investigators' work in a broad scientific context, to discuss the strengths and the weaknesses of the study, and to address the public health significance of the study findings. A draft of this report was circulated to the investigators, as well as to four members of the HEI Research Committee, for their information and comment. After appropriate revisions and approval by the full Review Committee, the Review Committee's Report, as well as the Investigators' Report, was sent to the HEI Board of Directors with a strong endorsement of the study, and a recommendation to publish the report as soon as possible.

SCIENTIFIC BACKGROUND TO THE STUDY

Ambient CO is derived mainly from incomplete combustion of carbonaceous materials. Motor vehicles are the major source, although combustion of coal, fuel oil, natural gas, wood, and solid waste, as well as industrial processes and miscellaneous types of fires also contribute to atmospheric CO. Carbon monoxide in indoor air comes from a variety of sources, such as stoves, furnaces, space heaters, and cigarette smoke. The typical range of outdoor levels of CO in urban areas is between 3 and 15 ppm (eight-hour average), with peak concentrations as high as 40 ppm (U.S. Environmental Protection Agency 1985).

Table 1. The Technical Review Panel for the Review of the HEI Multicenter CO Study

Robert I. Levy[a] (Chairman)
President, Sandoz Research Institute, East Hanover, NJ

Jeffrey Borer
Cornell Medical School, New York, NY

Ronald Coburn
University of Pennsylvania, Philadelphia, PA

Larry Cohen
Yale University School of Medicine, New Haven, CT

David DeMets
University of Wisconsin, Madison, WI

Stephen Fienberg
Carnegie Mellon University, Pittsburgh, PA

David Freedman
University of California, Berkeley, CA

Curt Furburg
Bowman-Gray School of Medicine, Winston-Salem, NC

Roland Ingram
Brigham and Women's Hospital, Boston, MA

Paul Meier[b]
University of Chicago, IL

Bertram Pitt
University Hospital, Ann Arbor, MI

[a] Also Chairman of the HEI Review Committee during the review of this study.
[b] Also a member of the HEI Review Committee.

Cardiovascular Effects of Carbon Monoxide

Oxygen in inhaled air diffuses rapidly through the lungs and binds to hemoglobin inside red blood cells to form oxyhemoglobin. This oxyhemoglobin complex is transported by the blood, and the oxygen is eventually released in the tissues. When CO is present in the air, it competes with oxygen for the four oxygen-binding sites of hemoglobin, and thus reduces the oxygen-carrying capacity of hemoglobin. Delivery of oxygen to the tissues is further impaired because CO alters the oxygen-binding property of hemoglobin so that oxygen is bound more tightly and hence is less available. The decrease in oxygen availability interferes with cellular respiration and results in tissue hypoxia, which may cause transient or permanent damage to tissues, especially in organs with high

oxygen demand such as the heart, brain, and fetal tissues. It should be noted that other heme-containing proteins (such as myoglobin and cytochrome oxidase) may also play a role in CO toxicity (Chance et al. 1970; Coburn et al. 1973); however, the evidence in support of this suggestion, particularly at low levels of blood carboxyhemoglobin (COHb), is limited and inconclusive.

The HEI CO study focused on the acute effects of CO exposure on the cardiovascular system. Tissue hypoxia in the myocardium (heart muscle) initiates a variety of compensatory responses. These compensatory responses include functional changes, such as increased cardiac output and vasodilation, which increase the rate of oxygen delivery to the tissues by increasing the volume of accessible blood. Such functional changes follow immediately upon CO exposure and continue for a period of time after exposure ends. Despite the existence of short-term compensatory responses, CO exposure may have harmful effects. If an individual's cardiovascular system is impaired, breathing low levels of CO may produce toxicity because the system is unable to adjust to the decreased availability of oxygen in the presence of CO. After prolonged exposure to CO, morphological and hematological changes occur, such as cardiac enlargement and increased production of red blood cells. However, the HEI study was not designed to address the long-term effects of CO exposure.

Populations Susceptible to the Cardiovascular Effects of Carbon Monoxide

Under the Clean Air Act, National Ambient Air Quality Standards are established to protect the health of sensitive groups in the population. Because of low blood-oxygen concentrations and the inability to increase cardiac output, fetuses comprise one of the groups especially susceptible to CO. Empirical evidence of adverse effects in the fetus is derived mainly from animal data and includes increased heart weight, a higher incidence of prenatal death, and decreased birth weight and length (Penney et al. 1974, 1984; Prigge and Hocharainer 1977; Fechter et al. 1980).

Individuals with a diseased heart or vascular system comprise another group that is especially susceptible to the adverse effects of CO. This group includes persons with myocardial ischemia, in whom the blood supply to the heart muscle is compromised. Ischemic heart disease is almost always due to thickened, and partially or completely obstructed, coronary arteries; in addition, such arteries usually cannot dilate to increase blood flow to the myocardium. Myocardial ischemia can be further exacerbated by increased metabolic demand, as with exercise, or by impairment in the oxygen-carrying capacity of the blood, as with CO-induced or anemic hypoxia.

Myocardial ischemia has four major clinical manifestations: angina (chest pain due to decreased oxygen supply relative to demand); myocardial infarction (heart attack); chronic ischemic cardiomyopathy (loss of myocardial function and increased ventricular instability due to cell death and fibrosis); and sudden death (Robbins and Coltran 1979). These different manifestations overlap somewhat. Both angina and myocardial infarction result from local heart-tissue hypoxia; angina, however, is usually reversible. Individuals who suffer from angina most often have some degree of coronary artery atherosclerosis, although other disorders that interfere with oxygen delivery to the myocardium may also cause angina. The symptoms of myocardial infarction range from sudden, severe chest pain, to less specific "heartburn," to an entirely asymptomatic condition that is recognized only later by electrocardiography or angiocardiography. Individuals with angina and recovered infarct patients are a distinct subset of individuals with coronary artery disease who can lead active, though sometimes somewhat restricted, lives, but who may be at risk from CO exposure during normal activities as well as on exertion.

Ischemic cardiomyopathy involves a slow, progressive decrease in coronary artery blood flow occurring in individuals with advanced coronary atherosclerosis who may or may not have had previous angina or overt myocardial infarction. Sudden death occurs sometimes as the first sign of ischemic heart disease and is thought often to be related to ventricular instability and dysrhythmia secondary to ischemia.

Quantifying Exposure and Dose of Carbon Monoxide

The blood COHb concentration is a marker of the integrated internal dose of CO. The blood COHb level resulting from CO inhalation is influenced by factors such as inhaled CO concentration, duration of exposure, blood hemoglobin level, minute ventilation, diffusing capacity of the lung, mean oxygen tension in the pulmonary capillaries, and endogenous CO production (Coburn et al. 1965). Because these physiological parameters vary among members of the population, exposure to the same concentration of CO may result in different COHb levels in different individuals (U.S. Environmental Protection Agency 1984) (also see the Blood Carboxyhemoglobin Versus Ambient Carbon Monoxide section of this report).

Based on a number of assumptions, the EPA has estimated that the ambient air quality standard of 35 ppm (one-hour average) would result in approximately 2% COHb in a healthy male who exercises moderately for one hour (U.S. Environmental Protection Agency 1984). Background levels of COHb in nonsmokers, with no environmental CO exposure, average between 0.5% and 1.0% (Urbanetti 1981) as a result of the endogenous production of CO (Coburn et al. 1963). Cigarette smoke contains a high concentration of CO; therefore, smokers

typically have higher COHb levels than nonsmokers, with a reported mean value of 4.5% COHb (Wald and Howard 1975; Radford 1983).

Issues in Designing Clinical Studies

Studies to determine whether or not specific groups of individuals are susceptible to the adverse effects of CO must be designed and conducted with care. Such studies involve exposing human subjects to relatively low levels of CO, defining the subject population, testing adequate numbers of subjects, and reliably and accurately measuring both the CO dose and the biological response.

The selection of a study sample has important implications for generalizing the results of a study. Carefully defined inclusion and exclusion criteria, which are essential to good study design, imply that the results of the study should be extended only to similar individuals in the general population. However, based upon the knowledge of disease mechanisms, it may sometimes be desirable for public health purposes to consider the implications of the results for groups that are different from the study sample.

Adequate sample size ensures statistical reliability and allows a more precise estimate of the "true"magnitude of the effect. Sufficient numbers of subjects also provide confidence that the adverse health effects documented are a characteristic of the population from which the subjects are derived, and not merely random fluctuations resulting from interindividual variability. Furthermore, to be meaningful as a health effect, the magnitude of the effect must be greater than, or comparable to, intraindividual variations in the response.

Accurate monitoring of both the exposure level and the resultant COHb levels are necessary for the safety of subjects, data reproducibility, and study interpretation. Among the methods used for the determination of blood COHb levels are spectrophotometry and gas chromatography. Spectrophotometric measurement by the CO-Oximeter is the most widely used technique because of its convenience and rapidity, but its accuracy is limited, particularly for samples with less than 5% COHb (Guillot et al. 1981; Kane 1985). Gas chromatography is highly accurate (Dahms and Horvath 1974), but requires complex equipment, is resource-intensive to operate, and does not provide readings rapidly.

To determine the health response in a particular study sample, the earliest measurable symptomatic change indicative of an adverse health outcome is recorded. Such a change must be capable of being measured objectively, or be a documented sign or symptom of the defined population's health status, or both. In addition, the change must be reversible, and the procedure leading to the measurement must be safe, ethical, and reproducible.

Patients with coronary artery disease commonly experience angina during activities that require moderate physical exertion. Often, investigators will monitor ischemia due to physical exertion by having subjects exercise in a standardized way while they record the changes in the subjects' electrocardiogram. A specific segment of the recorded wave, the initial takeoff of the ST segment, will often sweep lower than normal with left ventricular ischemia. Because the left ventricle supplies blood to the whole body (except the lungs), any impairment in its function can have profound physiological effects. Thus, the ST-segment change serves as an important and objective source of information on the physiology of the heart.

Investigators can obtain a relevant measure of a subject's exercise tolerance by recording the time until a defined ST-segment change or angina occurs in response to exercise. By comparing the exercise time when a subject is exposed to CO to the exercise time when the subject is exposed to room air, a conclusion can be drawn as to whether or not exposure to CO compromises a subject's ability to exercise.

OBJECTIVES OF THE STUDY

The HEI Multicenter CO Study was designed to answer the question: In men with coronary artery disease who have reproducible exertional angina, does CO exposure that produces about 2% or 4% COHb in the blood change the time to onset of myocardial ischemia during exercise, as indicated by angina or electrocardiographic ST-segment change?

DESCRIPTION OF THE STUDY

ORGANIZATION

The three clinical test centers were at the Johns Hopkins University School of Medicine, Baltimore; Rancho Los Amigos Medical Center, Downey, CA; and St. Louis University School of Medicine, St. Louis. Each center was staffed by a pulmonary physiologist, a cardiologist, and other personnel. A reference laboratory at St. Louis University tested the most important blood samples by gas chromatography and provided support for ensuring that the blood gas measurements were comparable among the centers. All data were collected in a standard way and were then sent to the data management and analysis facility at Harvard University School of Public Health. The conduct of the study at each center was monitored periodically by a quality assurance team from Arthur D. Little, Inc., Cambridge, MA. The project manager at HEI facilitated communication among the investigators, helped to alleviate any problems that arose during the study

and managed the preparation of the final report. Finally, during the course of the study, the investigators received advice from the advisory committee (see Figure 2 of the Investigators' Report).

DESIGN

The study was designed as a three-center, double-blind, placebo-controlled study of the effect of CO exposures sufficient to elevate blood COHb levels to 2% or 4% in subjects with coronary artery disease. Exposures to air or CO were randomized within each center.

The investigators planned to recruit 75 male subjects who had well-diagnosed, stable, and manageable coronary artery disease, and reproducible symptoms of exercise-induced ischemia. For enrollment in the study, the men had to meet stringent entry criteria. They had to be nonsmokers, between 35 and 75 years of age, and have stable, reproducible, exertional angina pectoris and positive exercise treadmill tests with reproducible ST-segment changes. In addition, the men had to meet at least one of three objective indicators of coronary artery disease: (1) angiographic evidence of 70% or more narrowing of at least one major coronary artery; (2) previously documented myocardial infarction; or (3) a positive exercise thallium test. The enrolled subjects were asked to remain on their normal dose and timing of medication.

Specific exclusion criteria were employed to avoid enrolling subjects whose personal habits or medical conditions might have confounded the results of the study or put the subjects at increased risk. Thus, smokers, patients with significant pulmonary disease, valvular heart disease, recent heart attack, stroke, or coronary bypass surgery, uncontrolled hypertension, severe anemia, or certain specific resting ECG abnormalities, and patients on digitalis therapy were excluded from the study sample. Women were also excluded from the study; this decision was based on reports that women frequently experience exertional chest pain without any of the objective indicators of coronary artery disease or myocardial ischemia (Pasternak et al. 1980; Ilsley et al. 1982; Val et al. 1982).

On the first visit to the laboratory, each subject underwent a physical examination and was screened with base-line blood tests, including COHb levels. In order to ensure that the exercise-induced ST-segment changes and angina were reproducible, the subject twice performed a standardized treadmill test, with a sham exposure period between the two tests. Subsequently, he was exposed to 150 ppm CO while at rest, and his rate of CO uptake was determined.

The subjects who met all entry criteria were asked to return for three additional visits. The test visits were identical, except that subjects were randomly assigned to exposures of either fresh air or one of the two levels of CO. During each test visit, each subject twice performed a symptom-limited standardized exercise treadmill test, with a recovery and exposure period between the two tests. These exercise tests used a modified Naughton treadmill exercise test procedure (Raider 1973).

The subject's dose of CO was monitored by blood samples taken at predetermined intervals throughout the course of the laboratory visit. Carboxyhemoglobin levels were routinely monitored at the three test centers using the CO-Oximeter. The COHb levels in the key blood samples were also measured by gas chromatography at the Reference Laboratory in St. Louis, and the gas chromatography values were used in the analyses to test the major hypotheses of the study.

The two primary health endpoints used in this study were (1) the time to onset of exercise-induced angina, and (2) the time to a predefined ST-segment change. Unprocessed and computer-processed ECG records were analyzed and evaluated by two cardiologists who were blinded to the subject's identification and exposure level. The ST-segment change provided an objective measure of cardiac ischemia, whereas the angina endpoint was necessarily subjective. The total duration of exercise, the duration of ST-segment change, the amplitude of maximum ST change, and the double products (heart rate multiplied by blood pressure) at the time of the ST change and the angina were also examined as secondary endpoints.

DATA ANALYSIS

Specific plans for the primary analysis of data were developed by the investigators before the study began. The approach was as follows:

Let:

x = time to endpoint during the preexposure exercise test, and

y = time to endpoint during the postexposure exercise test

Then:

$x - y$ = apparent effect of exposure, and

$r = 100\,(x - y)/x$ = relative (percent) effect of exposure

It was decided that r should be the primary indicator of effect, and that separate analyses should be performed comparing exposure to air with exposure to each level of COHb for each endpoint. In order to protect the analyses from the substantial influence of outlying observations, the investigators decided in advance to use trimmed means, in which the largest two and the smallest two measurements would be excluded. The investigators also decided to use one-sided tests of significance when reporting the primary analyses, on the ground that the only effects of CO exposure that were of interest were those related to decreased myocardial function.

SUMMARY OF INVESTIGATORS' CONCLUSIONS

Based upon an analysis of the 63 subjects who met all the protocol criteria, the investigators concluded that at both the 2%- and 4%-COHb-target levels, there was a statistically significant decrement in the time to onset of ST-segment change, as well as the time to onset of angina, compared to air exposure. At an average COHb level of 2.0% (which represents an increment of 1.4 from the average base-line level of 0.6% COHb), there was an average 5.1% reduction in the time to reach the ST endpoint; at an average COHb level of 3.9% (which represents an increment of 3.3 from the average base-line level of 0.6% COHb) there was an average 12.1% reduction. The reductions in time to onset of angina at the same levels of COHb were 4.2% and 7.1%, respectively. The linear regression analyses presented in the final report indicate that there is roughly a 3.9% decrease in the time to onset of ST changes, and a 1.9% decrease in the time to onset of angina, for each 1% elevation in blood COHb. The results exhibit more variability for the angina endpoint, which is not surprising because they depend on the subjective reporting of symptoms.

The positive findings from the primary endpoint analyses are further supported by significant differences in some of the secondary endpoints. Maximum ST amplitude and the summed ST score increased at both levels of CO exposure, while the heart rate-blood pressure double product at the time of ST change, and the total exercise duration, were significantly different only at the 4%-COHb-target level.

TECHNICAL EVALUATION

Several aspects of the study design, study conduct, and data analysis are discussed below. This discussion is intended as a guide both for understanding better the findings of the study and for planning future investigations.

STUDY DESIGN

This was a carefully planned, well-organized, and generally well-executed study. The multicenter nature of the study gave the investigators an opportunity to study the effect of CO in a far greater number of subjects than had been studied previously by other investigators. The protocol combined several well-known approaches, and was appropriate for meeting the objective of the study. The use of ST-segment changes as an objective measure of ischemia was one of the strengths of this study. Information on angina, a subjective symptom of myocardial ischemia, is also useful because it allows comparison of the results from this study to results from earlier studies.

Another strength of this investigation was the meticulous monitoring of the exposure and dose of CO and blood COHb levels. In all other cardiovascular studies, the level of CO in the exposure chamber was the same for each subject. However, in this study, a major effort was made to achieve the same COHb levels in all subjects by predetermining each individual's CO-uptake constant and by varying the chamber CO concentration and exposure time. This issue is further discussed below.

STUDY EXECUTION

In reviewing the results from large studies, such as the HEI study, an important distinction should be made between major errors of performance that might seriously compromise the credibility of the results of the study and those almost unavoidable minor shortcomings that, although they should be guarded against, are not likely to have serious consequences. The Review Committee has not found any major errors in the HEI study. However, as is typical of such complex studies, a number of minor shortcomings are evident. Although the shortcomings are mentioned at various places in the Investigators' Report, the report would have been improved if they had been clearly discussed in a separate section, and their effects evaluated, to the extent possible.

Assurance of the Quality of the Data

Two approaches were used to assure the quality of data in this study. First, standard operating procedures were adopted that addressed all aspects of the study to ensure that the collected data were accurate and precise; this was primarily a quality control effort. Second, an external contractor, Arthur D. Little, Inc., was given the specific responsibility for the overall quality assurance.[1] The representatives of Arthur D. Little were involved in the review of the standard operating procedures, and they periodically audited the test centers and the Statistical and Data Management Center to assure that the investigators were adhering to the protocol and the procedures for the study. These two approaches are closely related, and both affect the quality and reliability of the data.

The protocol details were decided upon by the investigators before the study began. These included standard operating procedures for the cross-checking of the CO-Oximeters and the ambient CO analyzers at the three centers. From the data presented in the appendices, it is clear that a substantial effort was made in these areas; consequently, the data on CO exposure and dose are detailed, accurate, and reliable. An effort was made to confirm the COHb gas chromatography results with some investigators at Stanford University, but these efforts were not successful.

[1] We use the term "quality assurance" here to refer to assurance of the quality of the data during the planning, execution, and analysis phases of the study.

The ECGs recorded during the exercise tests were blind-coded, and were interpreted by at least two cardiologists during periodic cardiology consensus meetings. The details of the ECG issue are discussed in the Evaluation of Health Endpoints section of this report.

During the periodic visits to the test centers, the Arthur D. Little audit team focused on ensuring that the previously agreed-upon procedures were being adhered to, and checked whether or not the data were being recorded accurately and appropriately on standardized forms. Reports from these periodic visits were sent to the HEI executive director, and any necessary corrective action was requested by the HEI project manager.

A confidential report summarizing a number of potentially significant protocol violations was prepared for the Health Effects Institute by Arthur D. Little, Inc. at the end of the study. This report was reviewed by all nine members of the Technical Review Panel, and by two members of the Review Committee. The detailed reports from the individual quality assurance audits, which resulted from visits to the test and data management centers, were considered confidential. Therefore, the Review Committee did not review the detailed reports, and its ability to comment on the effectiveness of these efforts is limited.

The Investigators' Report includes references to certain departures from the protocol that occurred during the course of this study. One of these departures resulted in the elimination of four subjects (202, 203, 208, and 211) from the main data analysis because "their ST changes on the visit-1 exercise test were not sufficient"(as defined in the study protocol; see the Subject Enrollment and Exclusion section of the Investigators' Report). The investigators decided to eliminate these subjects at an early time during the course of the study, long before the data were analyzed. The Investigators' Report documents this departure from the protocol, and includes these subjects in the data and analyses presented in Appendices B1 and C.

Other departures from the protocol include the use of an incorrect randomization scheme for some subjects (see Appendix D) and, on three occasions, failure to reschedule visits when the subject arrived at the laboratory with a COHb level above that specified in the protocol (see the Subject Enrollment and Exclusion section of the Investigators' Report).

Some of the minor shortcomings discussed above, and elsewhere in this report, might have been avoided if the study had included a brief initial pilot study to test the practical problems in implementation of the protocol and the operating procedures. It should be stressed, however, that none of these minor shortcomings appears to represent serious errors that compromise the overall conclusions of the study. Such minor shortcomings are typically found in clinical studies subjected to detailed review and, in the Review Committee's opinion, their presence here should not detract from the usefulness of this study for scientific or public policy purposes.

Subject Recruitment and Selection

The original goal of the investigators was to enroll 75 subjects. Seventy-six subjects were initially enrolled and randomized; of these, only three dropped out. Four others were excluded part way through the test sequence because they did not meet the protocol requirements at test visits. Thus, 69 subjects remained eligible and completed all the laboratory visits. Among these 69, six subjects, who failed to meet all the protocol requirements, were excluded from the main analysis by the investigators. The data, as well as the supplementary analyses, on all the 69 subjects are, however, reported in the report appendices.

The HEI investigators studied the largest number of subjects of any single CO-cardiovascular study to date—this number is more than twice the number of subjects in the second largest study. The subjects for the study were recruited from various outpatient clinics at the test centers. The stringent entry requirements ensured that all subjects enrolled in this study had clinically verified coronary artery disease with reproducible indicators of myocardial ischemia.

Blinding

The Investigators' Report properly emphasizes the importance of maintaining "double-blind"conditions, that is, making sure that neither the subject nor the laboratory personnel who interacted with the subject knew whether a particular exposure was to air or CO. These efforts at maintaining blind conditions were largely successful. However, the Investigators' Report indicates that, while the "exercise-cardiology"personnel were always blinded, the "exposure" personnel, who could not be blinded, sometimes had contact, albeit limited, with subjects. Specifically, it appears that in some instances the individual who controlled the exposure conditions also drew blood (this never happened at Rancho Los Amigos, occurred a few times at St. Louis, and always happened at Johns Hopkins).

On the whole, the blinding conditions were well maintained, and it does not appear likely that the breach in double-blind conditions noted above had any effect on the results of the study, particularly those based on the ST endpoint.

Randomization

In view of the many influences, both on the subject and on the laboratory, that vary with time, the decision to randomize order of assignment to treatment group was both sound and necessary. Because there are six orders in which the three distinct treatments (air and the two doses of CO) can be applied, any one of those six orders can be chosen at random

for each subject. In this study, it was deemed undesirable to allow order effects to be entirely random, and therefore it was decided that subjects should be randomly allocated in blocks. In addition, it was decided to stratify also by history of previous myocardial infarction. Such grouping, or blocking, was done separately for each center. In the end, with a little over 20 subjects per center, each center would appear to have had only one or two complete blocks, and a final incomplete block, in each stratum.

As is typical of most clinical studies, not all the blocks were completely filled, and analyses of results did not take blocking into account. Given the number of subjects at each of the centers, it might have been better not to have attempted such extensive blocking. However, the incomplete filling of all the blocks, and the failure to account for the block effect in the analyses are not likely to have had an appreciable effect on the results.

CARBON MONOXIDE EXPOSURE AND CARBOXYHEMOGLOBIN DETERMINATION

In order to establish the relationship between exposure to CO and the results of that exposure, careful measurements were made of blood COHb levels, CO levels in air, and rates of uptake of CO for each individual. Performance of the CO-Oximeter and the ambient CO analyzer used for measurements was routinely checked; this is another of the major strengths of this study. The series of calibrations and cross-checks have resulted in accurate and reliable measurements of CO and COHb levels far surpassing the accuracy and precision achieved in earlier studies. The description of such monitoring and quality control procedures are documented in appendices E through Q of the Investigators' Report.

Blood Carboxyhemoglobin Versus Ambient Carbon Monoxide

The exposure protocol in this study was designed to achieve predefined levels of blood COHb (3.2% and 5.7% by CO-Oximeter, corresponding to 2.0% and 4.0% by gas chromatography). The CO-uptake rate determined during each subject's first laboratory visit was used to calculate the exposure concentration of CO. The end-of-exposure value of COHb was further fine-tuned by measuring the COHb values at several points during the exposure period and accordingly varying the exposure period between 50 and 70 minutes. The mean CO concentrations in the exposure chamber were 117 (range 42 to 202) and 253 (range 143 to 357) ppm. Thus, whereas previous studies on the effects of CO on exercise tolerance used a fixed ambient concentration of CO for a fixed time period for all subjects, this study varied both the CO concentration and the time of exposure. A more thorough discussion in the Investigators' Report of the rationale for the use of blood COHb values rather than ambient CO levels, and an evaluation of the consequences of this choice, would have been useful.

The exposure strategy adopted in the HEI study is, however, justified for several reasons. Because COHb is believed to be the predominant mediator of CO toxicity, the broad regulatory question "Does CO exposure have an effect on individuals with coronary artery disease?" can be divided into two parts: What is the relation between CO exposure and COHb levels, and what are the health effects of COHb in individuals with coronary artery disease?

Regarding the first issue, the relation between CO exposure and blood COHb levels (that is, external exposure and internal dose) has been extensively studied in the laboratory in human subjects (see, for example, Forbes et al. 1945; Coburn et al. 1965; Peterson and Stewart 1970, 1975; Joumard et al. 1981). However, we have only a limited understanding of the factors that influence the COHb distribution in the population (U.S. Environmental Protection Agency 1984; Wallace and Ziegenfus 1985). It is generally believed that COHb levels in individuals are influenced by time-activity patterns, and by spatial and temporal variations in ambient CO levels in microenvironments (Akland et al. 1985). A resolution of these issues, while very important, requires empirical methods that are very different from those employed in the HEI study. The planners of the HEI study appropriately focused attention on the second aspect of the regulatory issue; namely, the health effects of elevated COHb levels in individuals with coronary artery disease. This essentially was the unresolved question that arose from the Aronow and Anderson studies (Anderson et al. 1973; Aronow and Isbell 1973; Aronow et al. 1974; Aronow 1981), and that was readily amenable to a clinical study.

The exposure strategy used in the HEI study is also commendable because it employed a biomarker for CO exposure. Blood COHb provides a link between the external CO exposure and the internal dose; it is an ideal biomarker because it serves both as a marker of the integrated internal dose of CO and as an endpoint directly related to adverse health effects.

Adoption of the exposure strategy, however, added to the complexity of the testing protocol: each subject's CO-uptake rate had to be predetermined, and blood samples had to be collected and immediately analyzed during the exposure period. The data in the report suggest that, *on the average*, the investigators were successful in achieving the target COHb levels (see Tables 8, 11a, 14a, and Appendix M). On the other hand, the COHb levels for the *individual* subjects were variable around the target levels (see Figures 10 and 12, and Appendix B; also note the differences in center average COHb levels [Table 9]). The investigators have estimated (Appendix O) that the use of this exposure strategy, rather than exposure

of all subjects to the same concentration and time, reduced the standard deviation of postexposure COHb levels by about 50%, thus improving the precision of the independent variable.

Two other aspects of the exposure strategy should be noted. First, each subject was exposed to CO while at rest, and then was exercised while breathing room air. During exercise, a modest amount of the CO was lost (which resulted in average lowering of the blood COHb levels by 0.4% on the 2%-COHb-target day and 0.8% on the 4%-COHb-target day). In analyzing the results of the study, the investigators used the postexercise COHb levels, arguing that this specifies the subject's COHb level at the onset of symptoms. However, since CO loss during exercise appears to be proportional to the total duration of exercise (Appendix P), it could be argued that the postexercise COHb value is not the true independent variable, and its use in the analysis would be expected to somewhat weaken the dose-response relationship.

Second, does the rate of attainment of COHb level have any impact on the results of the study? The answer to this question, at relatively low levels of COHb, appears to be negative. Horvath and coworkers (1975) have noted that a decrease in maximum aerobic capacity occurred at 4.3% COHb, regardless of whether the COHb level was reached by constant exposure to 100 ppm, or by exposure to a high initial CO concentration followed by a maintenance level during exercise.

Gas Chromatography Versus CO-Oximetry

The CO-Oximeter is known to give results of limited accuracy at low-COHb values (under 5%) (Guillot et al. 1981; Kane 1985). The current study provides very extensive data on the measurements of low-COHb levels using both the CO-Oximeter and gas chromatography. As expected, the gas chromatography values were found to be lower than the CO-Oximeter values. The difference between the two values is termed the "offset."

Supplementary experiments conducted as a part of this work revealed that the offset depends upon a multiplicity of factors, including the subject's oxyhemoglobin level and the test center's CO-Oximeter instrument (Appendix I). Much of this information became available only during the course of the study, and could not be integrated in the protocol. Therefore, during most of the study, the investigators utilized two fixed values for the offset (1.0 and 1.3 at the lower and higher doses, respectively); 56 subjects were tested using these offset values.

Because the statistical analyses and conclusions of the study are based on the more reliable COHb values determined by gas chromatography, the impact of the offset on the results of the study is minor and probably insignificant. However, the results described here and by other investigators (Guillot et al. 1981; Kane 1985) raise questions regarding the reliability of CO-Oximeter readings at low-COHb levels. Inasmuch as the gas chromatograph is a complex piece of equipment that is more resource-intensive to operate and does not produce rapid readings, most clinical and research laboratories will continue to use the CO-Oximeter for routine purposes. A resolution of the factors that affect CO-Oximeter readings was not one of the objectives of this study. However, for future research on the health effects of CO, a better understanding of the limitations of the CO-Oximeter, and ways of dealing with the offset, are clearly very important.

EVALUATION OF HEALTH ENDPOINTS

The study used a well-defined population of males with reproducible, stable, exertional angina and ECG evidence of ischemia secondary to coronary artery disease. The stringent enrollment criteria, although affecting the ability to directly generalize the results, ensured that virtually 100% of the subjects studied had well-recognized and reproducible indicators of coronary artery disease; consequently, useful information was obtained from a very high proportion of the subjects and tests.

The study used the preexposure time to endpoint as the base line on each day that the subject was tested. Although this approach increased the amount of effort needed on the part of the subjects and the investigators and added some additional measurement error to the estimates, it eliminated the effect of day-to-day variability on the results (Starling et al. 1984). The Investigators' Report does not directly assess the net gain or loss in precision resulting from this procedure, a matter of considerable interest for the design of future studies.

The duration of exercise on the treadmill was used as a surrogate for exercise capacity. Although it would have been ideal to have data on oxygen consumption ($\dot{V}_{O_2}$), obtaining such data would have added greatly to the complexity of the protocol. The Naughton exercise protocol, which was used in this study, was an appropriate choice; this is a well-standardized protocol, designed so that the duration of exercise (time on treadmill) is nearly proportional to exercise capacity (oxygen consumption) (see Table 6 in the Investigators' Report) (Raider 1973). Thus, the subject exercised with increasing work load (speed and grade) until he complained of fatigue or moderately severe chest pain, or until he reached any of the other criteria for stopping exercise listed in Table 7 of the Investigators' Report.

The criteria used for stopping exercise appear to be appropriate. It should be pointed out that while moderately severe chest pain was often a reason for stopping exercise (see Table 7), it was the time to mild chest pain (Level 1) that was defined as the angina endpoint; also, while major ST-segment

change (greater than 3 mm) was occasionally a reason for stopping exercise, the ST endpoint (as defined and discussed in the Specific Definitions and Exercise Testing sections of the Investigators' Report) was evaluated from the recorded ECGs subsequent to the test visits.

The time to the onset of angina and to ST-segment change were the two primary endpoints in this study. The investigators also used an appropriate format for assessing angina (Levels 1 through 4 angina, according to the severity of the chest pain). The angina endpoint is a standard, albeit somewhat imperfect, method (largely due to its subjective nature) long used in clinical studies.

The use of the ST-segment change by ECG as an indicator of ischemia of the ventricular myocardium is commendable. In contrast to angina, which has been used as the endpoint in previous studies, the ST-segment change provides an objective endpoint. Recording of the ECG was continuous during the course of the exercise test, and ST-segment changes were evaluated by two cardiologists who were unaware of the subject's exposure. Although Type-2 ST changes (see the Specific Definitions section under Methods in the Investigators' Report) were the most frequent (93%) ST changes found among the subjects, it appears that the three types of ST changes were combined in the analysis. Although this procedure is appropriate for the purposes of a large-scale study such as this, upsloping ST segments and ST elevations (Type-1 and -3 changes) are less specific indicators of myocardial ischemia than are ST depressions (Type-2 change).

In a few cases, the subject reached one endpoint but not the other. Although such missing data are unavoidable in a study of this type, they were relatively rare in this study (six ST and one angina endpoints in 376 tests). It would have been more appropriate to decide how such data would be treated before the study began. Nevertheless, the investigators' post hoc approach to this issue, namely, using the total duration of exercise in place of an absent ST or anginal endpoint, is justified; and, as they note, this appears to be a conservative approach (that is, towards accepting the null hypothesis).

The ECG readings (unprocessed as well as computer-processed) were analyzed by the principal cardiologist from each center and by at least one other cardiologist from one of the other two centers during periodic meetings of the study cardiologists. The cardiologists were blinded to the subjects' identity and exposure. In view of the central importance of the ECG readings for this investigation, it would have been ideal if the investigators had collected some information on the inter- and intraobserver variability of the ECG interpretations, and if the interpretations of the study cardiologists had been further verified by having a small sample of the ECGs read independently by a group of cardiologists who were not associated with the study. However, the cardiologists involved in the study are experienced and competent researchers, and although the above-mentioned effort would have added credibility to the ECG data, the Review Committee believes that the blind ECG readings were reliable and objective.

It should also be noted that a demonstrable training effect, comparable in magnitude to the effect at 2% COHb, was observed in this study: On average, the subjects were able to exercise for longer times at each succeeding visit (Table 15a). Such learning effects are not uncommon in clinical studies of this kind. Fortunately, the study was designed so that the order of exposure to air and the two doses of CO for each subject was randomized. It thus appears that training did not affect the overall results of the study.

ANALYSIS OF RESULTS

Basic Data Presentation and Analysis

One of the elements of credibility in clinical studies is the establishment, before data are collected, of a primary endpoint(s) and the basic methods of analysis. When this is not done, questions may be raised as to whether or not the report emphasizes a particular endpoint and a particular form of analysis because it gives the most favorable results. The investigators in the present study were commendably explicit, before data collection, about their primary endpoints and analysis.

Since the study was planned as a multicenter study and identical standard operating procedures were used at the three centers, the results from the three centers could be combined and analyzed together. The results of analyses of the primary endpoints are presented in Tables 11a and 14a of the Investigators' Report. Also presented in the report are the results of analyses of several secondary endpoints. In addition, several supplementary analyses are presented in Appendix C. These include: conventional means; data that include the six subjects who were later judged not to meet the protocol; and analysis of the absolute, rather than relative, time to endpoint. In several cases, the results from each of the centers are also presented and analyzed separately. Finally, though not itself an analysis, the credibility of the report is greatly strengthened by the inclusion of detailed data for each of the 69 subjects.

On the whole, the data are completely presented, and several analyses of the data are offered. It would have been better if, like all other results presented in the report, the results of the primary analysis had also been presented with two-sided tests of significance, as is customary, although the reader can accomplish this by doubling the reported p-values of the one-sided tests.

For this study, the relative decrease in time to endpoint was chosen as the primary indicator of effect. It should be noted that the discrimination between the effects seen at various

levels of exposure proved to be no better when the ratios $(x - y)/x$ were compared than when the differences $(x - y)$ were compared (see Tables C.3 and C.4).

Regression Analysis

The basic analyses presented in the report are paired comparisons of the effect of CO exposure with the effect of air exposure; the analyses are included both for combined data from all the centers, and for data from each of the centers. Because there is evidence of some learning effect and of differences between the centers, it would have been desirable to have presented these group comparisons with adjustment for center and sequence. (Such adjustment would not have large effects, however, because center and sequence effects are so nearly balanced.) Instead, the report emphasizes the variations in postexposure COHb values at each of the centers, and undertakes to allow for these differences by concentrating on regression analysis of the time to endpoint on the *attained* COHb value (postexercise). With allowance for the postexercise COHb value incorporated, evidently, the effects of most other covariates (in particular, the centers) are no longer statistically significant. Accordingly, the report suggests that the equations derived from Figures 10 and 14 may be an efficient summary of the findings on time to the two endpoints. However, as noted earlier, the postexercise COHb level is clearly related to the dependent variable (the COHb level declines by about 20% during exercise due to exercise-induced hyperventilation) and thus may not be an entirely suitable choice for a regressor.

The Review Committee believes that the statistical significance of the results is best assessed from the group comparisons, which are firmly based on the initial randomization.

Adjustment For Preexposure Exercise Tolerance

Based upon a report by Starling and coworkers (1984), it was assumed in designing the HEI study that it would be advantageous to obtain preexposure measurements of the time to endpoint to calibrate the outcome at each visit. Thus, in terms of the notation developed in the Data Analysis section in this report, it was assumed that the *adjusted* difference, say $\{(y_2 - x_2) - (y_0 - x_0)\}$, would be less variable than the *unadjusted* difference, say $(y_2 - y_0)$. For the adjusted differences to be more precise, the correlation between $(y_2 - y_0)$ and $(x_2 - x_0)$ would have to be greater than 0.5. In fact, the correlation is near 0.5, and it is not clear that the substantial effort required to get the preexposure measurement was rewarded by improvement in precision. The question has considerable importance for the planning of future studies, and deserves further exploration.

RESULTS OF THE STUDY

The results of this study confirm the hypothesis that elevation in blood COHb levels accompanying exposure to CO decreases the time to the onset of myocardial ischemia in exercising males with coronary artery disease. The decrements in the time to onset of electrocardiographic ST-segment changes provide the best evidence in support of this hypothesis. The time to the onset of angina is also decreased in response to elevations in COHb levels. The results are convincing, even though the magnitude of the effect is small and is superimposed on considerable biological variability.

A 5% decrement in the time to onset of ST-segment changes was seen for the average blood COHb value of 2% (which represents a 1.4% increment from average base-line %COHb), and a 12% decrement was seen for 3.9% COHb (which represents a 3.3% increment from average base-line %COHb) (Table 11a of the Investigators' Report). The results of changes in ST segment are statistically significant at the conventional two-sided 5% level whether the results are analyzed as absolute or relative changes, trimmed means or nontrimmed means, and whether the six subjects who did not meet the protocol criteria are included in the analysis or not (see Table 11a and Appendix C). A linear dose-response relationship, with an estimated slope of 3.85% decrease in time to onset of ST change for each 1% increase in COHb (intercept 8%), was also reported.

For the onset of angina, decrements of 4.2% and 7.1% were observed at blood COHb levels of 2% (which represents a 1.4% increment from average base-line %COHb) and 3.9% (which represents a 3.3% increment from average base-line %COHb), respectively (Table 14a of the Investigators' Report). Again, as in the case of ST-segment changes, the results of the angina endpoint were statistically significant by all but one of the different approaches to data analysis (Table 14a and Appendix C); the only exception was the result at 2% COHb, where for the 62 subjects, by trimmed-means analysis, the one-sided p-value was 0.027 (see Table 14a); if analyzed two-sided, this would fall at 0.054, barely not significant at 0.05. A linear dose-response relationship was also reported for the angina results (estimated slope of 1.9% decrease in time to angina for each 1% change in COHb; intercept -1%).

The results of the analyses of several secondary endpoints were also consistent with those of the primary endpoints. The maximum ST amplitude and the summed ST score increased at both levels of CO exposure. The heart rate-blood pressure double product at ST change and the total exercise duration were significantly different at the 4%-COHb-target level but not at the 2%-COHb-target level. The duration of ST change, the double product at angina, and the double product at peak exercise were not affected at either the high- or low-exposure level.

INTERPRETATION OF RESULTS AND CONCLUSIONS FROM THE STUDY

The results of this study demonstrate that exposure to levels of CO that are sufficient to elevate blood COHb to 2% and 4% potentiates symptoms of myocardial ischemia in patients with

well-recognized indicators of coronary artery disease. Evidence in support of this hypothesis is provided by the reduction in time to onset of ST-segment change and angina, as well as by effects on several secondary endpoints. The results of the various statistical analyses are in good agreement with each other. The magnitude of the effect is modest (5% and 12% reduction in time to ST change at 2%- and 4%-COHb-target levels) compared to between-subject variability (estimated standard deviation is approximately 20%, reading from Figure 10), but such effects may well have important biological and public health significance.

For any study, the exact magnitude of the investigated effect is highly dependent on various features of study design and data analysis. It is important that such sources of variability be kept in mind so that the study results are appropriately interpreted. One of the sources of variability in the current study may be related to the fact that most subjects in the study were on medication; this was a reasonable choice made by the investigators, and it is unlikely that it had any qualitative effects on the outcome of the study. The investigators report that, from covariate analysis, the effect of the use of beta-blockers, or of other therapeutic drugs, was not significant. It is reassuring to note that none of the drugs appeared to make the subjects especially vulnerable to the effects of CO in this study. However, in the larger population of coronary artery disease patients, drugs other than those examined in the HEI study may also be in use. In addition, coronary artery disease in some patients may not be completely manageable with drugs. Some of these patients may be at increased risk from CO exposure.

Another source of variability, as pointed out earlier, is the differences in the results from the three centers. This kind of variability is not uncommon in multicenter studies; in the present study, it is likely to be related to the relatively small numbers of subjects enrolled at each of the centers, and to the relatively small magnitude of the effect being studied. However, other factors may also have contributed to the inter-center differences. For example, the socioeconomic status of the subjects enrolled at the three centers was different (see Characteristics of Study Population section in the Investigators' Report), and the relative humidity in the environmental chambers at the three centers varied between 45% and 80% (Appendix Q). It also became clear during the course of the study that there were appreciable differences in the CO-Oximeter calibrations at the three centers (see Appendix K).

The treatment of missing data on a few of the subjects in the study is another factor affecting the quantitative results. The choice made by the investigators, to substitute the total exercise time for ST-change time when the latter information was missing, is justifiable and conservative, and does not vitiate the qualitative conclusions. It should be pointed out, however, that other, equally reasonable approaches to treating the missing data are also possible. For example, the slope of ST-segment-heart-rate could have been determined, and the ST-segment variations not reaching the 0.1-mm level by the end of exercise could have been extrapolated to the time when 0.1 mm would have been expected.

In the discussion of implications of the findings, the Investigators' Report states that their data do not provide "evidence of a measurable threshold effect." This conclusion is correct, but it should be emphasized that this study was not designed to look for a threshold and, in light of the considerable interday variability in the same subject as well as intersubject variability, it would be quite difficult to detect a threshold, even if there were one.

Finally, the subjects in this study were exposed to CO while at rest. In the everyday exposure to CO, it would be more typical for an individual to encounter CO while engaged in outdoor activity, so that exposure to CO and exertion are simultaneous. Because blood COHb is believed to be the sole mediator of CO-induced toxicity, this issue is not likely to be important. If other heme-containing proteins were also to influence CO-induced toxicity, then the combined effect of exertion (tissue hypoxia) and CO-mediated ischemia might have an effect on the outcome.

How do the results of this study compare with the results of other studies on the effects of CO on the heart? As pointed out in an extensive discussion in the Investigators' Report (see Comparison with Other Studies section), the results of the HEI study, and four (Anderson et al. 1973; Aronow and Isbell 1973; Kleinman et al. 1973; Aronow 1981) out of five studies are in general agreement; the estimated effects in the other study was not statistically significant (Sheps et al. 1987), but was not necessarily inconsistent with the results of those studies in which statistical significance was demonstrated. The investigators have also discussed the similarities and the differences among these studies, and we will only supplement their discussion with three additional points here.

First, one of the merits of the HEI study is that it included careful and thorough measurements of the COHb levels; other investigators have spent much less effort in this area. Because the CO-Oximeter readings are not very accurate at low levels of COHb, it is almost certain that the COHb values reported in the other studies are too high. In the absence of simultaneous COHb values determined with an accurate procedure such as the gas chromatograph, there is no way to reliably estimate the appropriate offset value for the other studies. Also, it was learned during the course of the HEI study that the difference between the CO-Oximeter and gas chromatography values for blood COHb depends on, among other things, the particular instrument that is used (Appendix M). Therefore, use of the HEI offset values for correcting the COHb values from the other studies may give erroneous results.

Second, one of the most recent studies (Sheps et al. 1987) did not report statistically significant effects on angina,

maximum ST-segment depression, and a number of other endpoints at 4.1% COHb (as measured by CO-Oximeter). However, these authors did observe a statistically significant effect of CO exposure on the maximum change in left ventricular ejection fraction, which is perhaps the most sensitive indicator of functional impairment of the myocardium (Table 3 of Sheps et al. 1987). Because of the lack of a statistically significant effect on the other endpoints examined in their study, Sheps and coworkers did not give much credence to this observation; however, in view of the results of the HEI study, it would appear that the effect of CO on maximum cardiac ejection fraction may be physiologically relevant.

Third, Aronow and Isbell (1973) and Anderson and coworkers (1973) were the first to report an effect of CO exposure on exertional angina. Subsequent review of Aronow's work raised questions about the quality, and therefore the reliability, of the data (Horvath et al. 1983). The results of the HEI study are in general agreement with the hypothesis generated by the early studies by Aronow and by Anderson.

As the investigators bave discussed in their report, detailed comparison of the HEI study with the other studies is fraught with difficulties because of factors such as differences in study design, exposure and exercise protocols, endpoint evaluation, rigor in conduct, and number of subjects. As stressed in other parts of this report, the HEI study was far more carefully planned and executed, and has been much more fully analyzed and reported, than the other studies. The HEI study is also based on a considerably larger number of subjects than all the other studies. Therefore, the Review Committee considers the results of the HEI study to be far more reliable than those of the other studies on this topic.

PUBLIC HEALTH IMPLICATIONS

The findings of this study clearly indicate that small increases (of the order of 1.4%) in blood COHb levels resulting from transitory exposure to CO produce evidence of myocardial ischemia in males who have stable coronary artery disease with reproducible exertional angina pectoris.

Decrements in the time to onset of the electrocardiographic ST-segment change and angina were used as the indicators of myocardial ischemia in this study. There is sufficient evidence in the literature to show that these changes are reliable indicators of ischemia (Braunwald and Sobel 1984; Fisch 1984). Since myocardial ischemia is considered to be one of the most important prognostic factors in heart disease (Epstein et al. 1988), the results on ST-segment and angina change represent physiologically and clinically relevant endpoints.

It could be argued that angina pectoris is a transient, reversible process and that no, or relatively little, harm results from a small but finite decrease (5% to 15% decrease, representing 30 seconds to one minute) in time to ST-segment change. However, as the investigators have persuasively argued, the levels of CO and exertion used in this study may be easily encountered in the everyday life of these subjects; the health effects reported here, therefore, would be expected to limit the activity of the subjects and affect their quality of life.

Further evidence of the health significance of the findings of this study is provided by evidence in the literature that recurrent episodes of myocardial ischemia may predispose a person to myocardial cell loss. Myocardial fibrosis has been found on biopsy in patients with a history of angina pectoris but without a history of infarction; this fibrosis has been correlated with a decrease in cardiac function, and ultimately, congestive heart failure (Allison et al. 1963). Repetitive bouts of myocardial ischemia are also thought to predispose an individual to ventricular arrhythmias and the risk of sudden death (Wiggers 1940; Herman 1967; Levites et al. 1975; Kerin 1979; Carboni et al. 1987). There is also a suggestion that repetitive bouts of ischemia migbt predispose one to further progression of atherosclerosis, catecholamine release, platelet activation, plaque rupture, and possibly acute thrombosis, although this latter sequence remains speculative (Maseri et al. 1978; Robbins and Coltran 1979; Braunwald and Alpert 1984). Thus, although the effects reported in this study are modest, such ischemic episodes may represent steps in a sequence of potentially serious pathophysiological changes in the myocardium and, therefore, should be considered important from a clinical perspective.

What are the implications of the findings from this study for the general population of coronary artery disease patients? It is rarely possible that a group studied in a clinical trial can be regarded as a random sample of the target population to which the findings of the study are intended to be applied. Several factors, such as informed consent, age, medical condition, and presence of other diseases, as well as motivation to tolerate the demands of the protocol affect the selection process. The subjects recruited for the HEI study were ambulatory and were enrolled from outpatient clinics. The selection process was further restrictive because the entry criteria for this study were appropriately exacting (documented coronary artery disease, with mild, stable exertional angina, and with reproducible ST-segment change).

It would have been desirable if the details regarding recruitment, that is, how many patients were screened and approached, and how many elected to enter the study but did not qualify, had been collected as a part of this study. In the absence of such sampling information, the investigators have argued that the subjects in the HEI study appear to be similar to other angina populations that have been studied (see Demographic Perspective section of the Investigators' Report). However, given the exigencies of the selection process, it would appear that there is a large fraction of individuals in

the general population of patients with ischemic heart disease who are less stable and manageable or have a lower level of exercise tolerance than the subjects in the HEI study. Such patients would also be expected to be prone to CO-induced myocardial ischemia, and would be at risk from CO exposure.

Are there other groups of patients for whom the results of this study have significant implications? Based upon our understanding of the mechanism of coronary artery disease, it would appear that perhaps foremost among such groups are individuals with so-called silent ischemia (Cohn 1988; Epstein et al. 1988). These individuals do not have overt symptoms of disease, such as chest pain, but show the typical transient ST-segment changes, as well as other hemodynamic, metabolic, and functional abnormalities that are representative of myocardial ischemia. Recent studies suggest that risk factors and prognosis of the subjects with classical and silent ischemia are identical (Amsterdam et al. 1986; Falcone 1987). In patients who report angina, the episodes of silent ischemia may be threefold more prevalent than are episodes of painful ischemia; the prognosis for these patients appears to be closely related to the electrical evidence of ischemia, rather than to the presence or absence of chest pain (Schang and Pepine 1977; Deanfield et al. 1983; Quyyumi et al. 1985). It also appears that 10% to 15% of acute myocardial infarctions are silent (Kannel and Abbott 1984). The prevalence of silent ischemia in the general population, or in people with other risk factors, is not known. Thus, given the emerging knowledge regarding the effects of myocardial ischemia, and the apparently frequent occurrence of silent ischemia, it would appear that the results of the HEI study are potentially relevant for any individual with ischemia (painful or painless). Exposure to CO in these individuals, who are often unaware of their predisposition to myocardial ischemia, might represent a hitherto unrecognized risk.

An equally important group of patients for whom the results of this study may be relevant are those who have congestive heart failure. Congestive heart failure is a major public health problem. It has been estimated that approximately two million Americans suffer from heart failure; about 250,000 new cases of congestive heart failure are diagnosed each year in the United States. The mortality among these patients is high, about 10% to 20% each year. The patients with congestive heart failure have a markedly reduced circulatory capacity and, therefore, may be very sensitive to any further limitations in oxygen-carrying capacity. Thus, exposure to CO is likely to further reduce their exercise tolerance. In addition, patients with congestive heart failure have a high incidence of serious arrhythmias, which are often the cause of death. Since CO exposure has been reported to induce arrhythmias (Davies and Smith 1980), CO exposure could be a risk factor in patients with congestive heart failure. Unfortunately, no scientific evidence is available today on the effect of CO exposure in congestive heart failure patients.

Does this study have any implications for any individuals who are free of overt heart disease? Clearly, any condition in which either the oxygen-carrying capacity of the blood or the compensatory mechanisms that respond to CO are compromised would be of concern. Examples include patients with occlusive vascular disease (such as stroke patients), and pregnant women. In addition, individuals with impairment in tissue oxygen delivery, such as those who live at high altitudes, or who have significant anemia, may also be at risk.

Cigarette smoke contains high levels of CO; consequently, smokers have high levels of blood COHb—typically around 4.5% (Radford 1983). Although COHb at such levels would be expected to affect the oxygen-carrying capacity of blood, the effects of prolonged high-level CO exposure from cigarette smoke on the body's compensatory mechanisms have not been fully investigated, and we do not know whether smokers are more or less sensitive to the adverse effects of incremental CO exposure from ambient air. Another issue related to smoking concerns environmental tobacco smoke (or passive smoking). Carboxyhemoglobin levels in individuals exposed to environmental tobacco smoke are mildly elevated for a short duration (National Research Council 1986). In view of the results of the HEI study, exposure to environmental tobacco smoke, either alone or in combination with ambient CO exposure, may represent a risk for individuals with coronary artery disease.

It should also be noted that only male subjects were studied in this investigation. The exclusion of women is justified in view of the reports of a greater frequency of false-positive exercise tests among them (Pasternak et al. 1980; Ilsley et al. 1982; Val et al. 1982). Although coronary artery disease occurs later in life in women than in men, its anatomical distribution and clinical consequences are the same. In both men and women, ischemic artery disease is the leading cause of death in the United States (537,000 total deaths in 1985) (U.S. Department of Health and Human Services 1988). Hence, although the effects of CO in women have not been specifically documented, in the absence of any contradictory information it would be reasonable to assume that women with coronary artery disease are also at increased risk from exposure to CO.

REMAINING UNCERTAINTIES AND AREAS FOR FUTURE RESEARCH

This study focused on the health effects of relatively low levels of CO in a highly selected group of subjects with coronary artery disease. Although the results of this study are con-

vincing, they suggest several areas where further research on cardiovascular effects of CO would be fruitful. This discussion is intended only to highlight such areas; specific recommendations on how such research should be approached, planned, designed, or executed are beyond the scope of this report.

1. Mechanism of Action. In order to understand fully the implications of the HEI study, and similar studies, research on the mechanisms by which CO produces adverse effects is important. Mechanism-oriented research will not only increase our understanding of the effects of CO, but will also allow a more reliable extrapolation of this knowledge to conditions in which the effects of CO have not been studied.
2. Clinical Effects of CO. Scientific evidence summarized earlier suggests that CO-induced ischemia may be a risk factor in individuals with various cardiovascular diseases (such as silent ischemia, congestive heart failure, intermittent claudication). Carbon monoxide is also likely to adversely affect women with coronary artery disease. Carefully planned and conducted studies in such groups should be considered. It is also important that laboratory research be conducted on the effects of CO on heart function, heart rhythm, and isolated heart cell metabolism and function.
3. Adaptation. The delivery of oxygen to tissues is a finely tuned process, and there are a number of compensatory mechanisms available to overcome limitations in oxygen delivery. There is very little information in the literature regarding whether or not there are any differences in the body's compensatory mechanisms under conditions of short-term versus long-term exposure, and whether or not the human body can adapt to long-term exposures to CO. Research is also important on the effects of ambient CO exposure in smokers, who have chronically elevated levels of blood COHb.
4. Exposure. As discussed briefly in this report, and in more detail in the Investigators' Report, our knowledge of CO exposure for the general population is quite poor. Ambient CO is known to show wide temporal and spatial variations, and is a poor predictor of individual COHb levels (Akland et al. 1985; Wallace and Ziegenfus 1985). There is practically no information on the time-activity patterns of coronary artery disease patients, or of other potentially sensitive groups. Furthermore, in order to make the best use of the results of this study, it is important that the relationship between ambient CO and COHb levels be more fully understood.

A related issue is the difficulty of reliably determining low levels of COHb. Because of the convenience, simplicity, and cost, optical methods (such as the CO-Oximeter) are likely to be the method of choice for routine determination of COHb levels; however, such methods are inaccurate at low levels of COHb. A resolution of the difficulties with the optical methods, or the development of other simple and reliable methods, is very important for determining the population burdens of COHb.

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Title	Publication Date
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research	September 1985
Automotive Methanol Vapors and Human Health: An Evaluation of Existing Scientific Information and Issues for Future Research	May 1987
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Report No.	Title	Principal Investigator	Publication Date
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2	Disposition and Metabolism of Free and Particle-Associated Nitropyrenes After Inhalation	J. Bond	February 1986
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4	The Metabolic Activation and DNA Adducts of Dinitropyrenes	F.A. Beland	August 1986
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When a study is completed, a draft final report authored by the investigator(s) is reviewed by the Health Review Committee. The Health Review Committee has no role either in the review of applications or in the selection of projects and investigators for funding. Members are also expert scientists representing a broad range of experience in environmental health sciences. The Committee assesses the scientific quality of each study and evaluates its contribution to unresolved scientific questions.

Each Investigator's Report is peer-reviewed, generally by a biostatistician and three outside, independent reviewers chosen by the Review Committee. At one of its regularly scheduled meetings, the Review Committee discusses the Investigator's Report. The comments of the committee and the peer reviewers are sent to the investigator, and he or she is asked to respond to the comments and, if necessary, revise the report. The Review Committee then prepares its own report, which includes a general background on the study, a technical evaluation of the work, a discussion of the remaining uncertainties and areas for future research, and implications of the findings for public health. The HEI Research Report, which includes the Investigator's Report and the Review Committee Report, is then published in monograph form after evaluation by the HEI Board of Directors. The Research Reports are made available to the sponsors, the public, and many scientific and medical libraries, and are registered with NTIS.

All HEI investigators are urged to publish the results of their work in the peer-reviewed literature. The timing and nature of HEI report releases are tailored to ensure that the Health Review Committee's report does not interfere with the journal publication process.

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