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"Model-Based Estimate of Carbon Monoxide Uptake by Heart Muscle During Exercise".
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List of Terms and Abbreviations

CO = carbon monoxide
Hb = hemoglobin
Mb = myoglobin
ECG = electrocardiogram
HbCO = carboxyhemoglobin
MbCO = carboxymyoglobin
PO2 = partial pressure of oxygen

Abstract

Exposure to carbon monoxide (CO) concentrations that exceed the Permissible Exposure Level (PEL) (50 ppm averaged over 8 hr) is the most common cause of work-related inhalation fatalities. The morbidity and mortality resulting from these CO exposures are due primarily to the effects of severe hypoxia on the heart and central nervous system. The long-standing practice of focusing on the carboxyhemoglobin (HbCO) level as the primary indicator of the severity of a CO exposure essentially ignores the CO load that accumulates in the extravascular tissues that are most vulnerable to a diminished oxygen supply such as the myocardium and brain. We propose that a predictive measure of the CO burden in these tissues during acute or chronic CO exposures in an occupational setting would provide a more accurate assessment of the risk associated with these exposures.

The first aim of this proposal was to use existing data sets from human CO exposure studies to develop and enhance our mathematical model of whole-body CO uptake and distribution. We will use this model to predict CO and oxygen levels in blood and tissues of workers engaged in physical activity who are exposed to CO, both during the poisoning event and during therapy under room air or hyperoxia.

All of our findings are based on simulation studies after developing mathematical models of whole-body CO uptake and distribution during CO exposures and therapies. Our modeling studies have shown repeatedly that therapies involving the breathing of 100% oxygen do lead to more rapid removal of CO from blood (than breathing air) but some of that CO diffuses into muscle and combines with myoglobin (Mb), where it may remain for hours. In rest and in situations of moderate physical activity, the myocardium is predicted to be at greater risk for hypoxic injury than skeletal muscle during the course of CO exposure and washout (therapy). The time course and depth of hypoxia in cardiac muscle during CO poisoning and therapy differs greatly from that in skeletal (exercising) muscles. Furthermore, the severity of tissue hypoxia in the myocardium is influenced by many factors. Consequently, the usual clinical index of severity of CO poisoning, the carboxyhemoglobin (HbCO) level, does not adequately reflect the potential for injury to the myocardium. We suggest that clinicians should be encouraged to obtain multi-lead electrocardiograms from all CO poisoning victims.

Whole-body CO uptake and distribution during CO exposures and therapies occurs much more slowly than commonly assumed from the rate of uptake in the blood. Our findings strongly suggest that clinicians should not use HbCO level alone as a predictor of hypoxic injury of the myocardium (or of the brain), nor should they consider a fall in HbCO below some arbitrary level (e. g., 4%) as an indication that therapy for CO poisoning is no longer necessary. Further studies should be done to determine the optimal levels and durations of hyperoxic exposures given to CO poisoning victims to achieve washout of CO from the entire body (and not just from the blood).

Our findings also indicate the importance of an awareness of the hemoglobin concentration in any worker likely to be exposed to ~ 30 ppm CO or more over an 8-hr day. As anemia is often amenable to therapeutic intervention, this should be the first consideration. If the worker's anemia is not correctable, it may be advisable to remove him/her from a setting in which exposure to CO is likely to occur. Removal from CO exposure would be even more important if the job also entailed exposure to particulates

unless it could be demonstrated that the worker's minute ventilation was within the normal range.

Our second objective was to determine the extent to which the predicted CO dose to the myocardium correlates with subtle changes in continuously-recorded electrocardiograms (ECGs) that are suggestive of hypoxia or ischemia. Despite having identified two possible sources of data for this aim, we were unsuccessful in acquiring appropriate data. The proposed data were inadequate because our simulations (discussed above) predicted the maximum impact of CO on the heart would occur at a time after the exposure ended, and recordings from this time period were unavailable.

We have accomplished additional work not discussed in the original proposal. We modified one of our published models in order to examine the susceptibility of anemic women to CO hypoxia at rest and during elevated metabolism. Our model predictions confirmed the widely-held opinion that the severity of symptoms in anemic women does not correlate closely with Hb level. Workers with anemia may be somewhat more susceptible to CO poisoning; however, hyperventilation (which accompanies anemia) and the resulting exacerbation of particulate exposures that may occur in parallel with CO poisoning, may be a more significant concern.

In other studies in collaboration with investigators from the Australian Institute of Sport, we have used simulations to validate two common clinical methods for estimating Hb mass and have characterized their limitations in much greater detail than can be done experimentally.

Despite the fact that CO toxicity has been studied for many years, we have demonstrated in the studies supported by this grant that mathematical modeling can be used successfully to better understand the uptake and distribution of CO in exposed workers and to predict outcomes in individuals with anemia.

Highlights/Significant Findings

All of our findings are based on simulation studies after developing various mathematical models of whole-body CO uptake and distribution during CO exposures and therapies. Our modeling studies have shown repeatedly that therapies involving the breathing of 100% oxygen do lead to more rapid removal of CO from blood but some of that CO diffuses into muscle and combines with myoglobin (Mb), where it may remain for hours.

In rest and moderate exercise, the myocardium is predicted to be at greater risk for hypoxic injury than skeletal muscle during the course of CO exposure and washout (therapy). Furthermore, the usual clinical index of severity of CO poisoning, the carboxyhemoglobin (HbCO) level, did not reflect the severity of tissue hypoxia in the myocardium.

Other model predictions confirmed the widely-held opinion that the severity of symptoms in anemic women does not correlate closely with Hb level. Although HbCO levels tended to be higher in the anemic women compared to controls, this was not always the case. Increases in cardiac output and minute ventilation in anemia mitigate against hypoxia in Mb-containing tissues.

Two clinical methods for determining Hb mass and blood volume from CO uptake were tested in simulations and found to be capable of determining these physiological parameters to within 5% of their true values. A method based on rebreathing of a CO mixture for only 2 minutes may be accurate over a wider variety of subjects if only capillary blood samples are available.

Translation of Findings

We have demonstrated conclusively that whole-body CO uptake and distribution during CO exposures and therapies occurs much more slowly than was reported by Coburn's laboratory. Further studies should be done to determine the optimal levels and durations of hyperoxic exposures given to CO poisoning victims to achieve washout of CO from the entire body (and not just from the blood).

Our findings also indicate the importance of an awareness of the hemoglobin concentration in any worker likely to be exposed to ~ 30 ppm CO over an 8-hr day. As anemia is often amenable to therapeutic intervention, this should be the first consideration. If the worker's anemia is not correctable, it may be advisable to remove him/her from a setting in which exposure to CO is likely to occur. This would be particularly important if the job also entailed exposure to particulates unless it was possible to demonstrate that the worker's minute ventilation was within the normal range.

Because the myocardium is predicted to be more susceptible to CO hypoxia than skeletal muscle, clinicians should be encouraged to obtain a multi-lead electrocardiogram from all CO poisoning victims.

Our findings also validate two common methods for clinical evaluation of Hb mass and blood volume based on uptake of CO but also indicate that one method may be preferred when only capillary blood samples are available.

Outcomes/Relevance/Impact

1. Potential outcomes

Our findings strongly suggest that clinicians should not use HbCO level alone as a predictor of hypoxic injury of the myocardium (or of the brain), nor should they consider a fall in HbCO below some arbitrary level (e. g., 4%) as an indication that therapy for CO poisoning is no longer necessary.

A multi-lead electrocardiogram should be acquired from all CO poisoning victims, and even subtle abnormalities should motivate clinical follow-up.

Workers with anemia may be somewhat more susceptible to CO poisoning; however, hyperventilation (which accompanies anemia) and the resulting exacerbation of particulate exposures that may occur in parallel with CO poisoning, may be a more significant concern. Such workers should be removed from environments where particulate exposures are possible.

2. Intermediate outcomes

Some of our studies have been discussed in the 2007 *Yearbook of Emergency Medicine*, and the Principal Investigator was invited to evaluate proposed revisions of the W. H. O. Indoor Air Quality Guidelines related to CO.

3. End outcomes

Although the results of our studies have been published in peer-reviewed journals, no documented reductions in workplace morbidity, mortality, or exposure have been identified.

Scientific Report

Specific Aim 1. Using data sets from two human carbon monoxide (CO) exposure studies, we will develop methods to fit our model to actual HbCO values at rest and in response to three levels of exercise and then, using the fitted model, we will estimate the time course of myocardial carboxymyoglobin (MbCOmc) during exposure and post-exposure periods. To achieve this aim, we will first expand the structure of our published whole-body model of CO (and oxygen) uptake and distribution to include a separate myocardial compartment, and extend the behavior of the model to include responses to exercise; then we will develop a method for fitting the model to data from individual subjects and estimate MbCOmc in these subjects at rest and during exercise followed by hyperoxic washout of CO.

Before adding a myocardial compartment to the model, we evaluated the limitations of representing muscle tissue as a homogeneous, uniformly-mixed compartment by comparing the compartmental tissue oxygen partial pressure (PO2) of the model to the distribution of PO2 values reported in experimental studies of skeletal muscles. It was apparent that the single-compartment model was inadequate to represent regions of muscle tissue of greatest concern – i. e., where PO2 would be below average. An additional problem is that there is no accounting for the effects of gaseous diffusion within the tissue in a uniform compartment. Consequently, we subdivided the original muscle tissue compartment in the model into two subcompartments while allowing diffusion between the subcompartments; one subcompartment represents tissue perfused primarily by arterioles and the second represents tissue perfused mainly by capillaries.

Each tissue subcompartment has an associated vascular subcompartment with which it exchanges gases, and the first tissue subcompartment also communicates with a third vascular subcompartment (comprising venules) which permits tissue to venous gas exchange. These seemingly small enhancements actually triple the computational complexity of the muscle tissue part of our model. Now, in comparison to experimental data of skeletal muscle PO2 distributions, we are able to predict the 25th and 75th percentiles of these distributions as well as the average tissue PO2s. Our ability to predict tissue PO2 in relatively underperfused portions of skeletal muscle during CO exposure was greatly improved. This improved model was described in a recent publication [1].

Subsequently we added a myocardial tissue compartment to the whole-body model. This myocardial compartment also comprises two tissue subcompartments with three vascular subcompartments. To determine parameter values for this model we utilized literature reports as much as possible, then simulated various experimental procedures from the literature and adjusted the remaining parameter values to obtain the best matches to the experimental data. This work on enhancing our model was undertaken by a graduate student as part of her M. S. thesis [3, 4].

Another aspect of Specific Aim 1 was to modify the whole-body model to simulate CO uptake during exercise. It is assumed that an increase in metabolic rate of oxygen consumption (MRO2) is the defining characteristic of exercise. To model the interaction of CO exposure and exercise, we used extensive literature reviews to establish quantitative formulas for the following relationships: (i) increase of heart rate as a function of whole-body MRO2; (ii) cardiac output as a function of whole-body MRO2; (iii) cardiac output and heart rates as functions of %HbCO; (iv) myocardial MRO2 and myocardial blood flow as functions of heart rate in exercise. We also collaborated with Dr. Marko Laaksonen (Mid-Sweden University) to obtain his data on myocardial perfusion in humans during exercise. In addition, skeletal muscle blood flow and MRO2 are now calculated separately for arm, trunk, and leg muscles so that exercise can be apportioned to the appropriate muscle group. The model was validated for exercise and CO exposures by comparing it to the experimental data from Kizakevitch, et al. (Eur J Appl Physiol. 2000, 83:7-16). This final version of the model, and its ability to predict experimental findings during CO exposures at rest and during exercise, was described in a recent publication [2].

We used this new model to predict carboxymyoglobin (MbCO) levels and oxygen tensions (PO2) in the myocardium for several CO exposure regimens at rest and during exercise. Model predictions were validated with experimental data in normoxia, hypoxia, and hyperoxia. We simulated exposures at rest that produced peak %HbCO ~30% (i. e., 6462 ppm CO for 10 min and to 265 ppm CO for 480 min), and during three levels of exercise at 20% HbCO. We compared responses of carboxyhemoglobin (HbCO), MbCO, and myocardial PO2 to estimate the potential for myocardial injury due to CO hypoxia. Simulation results predict that during CO exposures and subsequent therapies, cardiac tissue has higher MbCO levels and lower myocardial PO2's than skeletal muscle. CO exposure during exercise further decreases myocardial PO2 from resting levels. We conclude that in rest and moderate exercise, the myocardium is at greater risk for hypoxic injury than skeletal muscle during the course of CO exposure and washout. Due to the relatively high myocardial perfusion, blood-borne oxygen reaches the heart quickly during hyperoxic therapy and the minimum tissue oxygen tension occurs shortly after the end of CO exposure whereas PO2 in muscle has a nadir about 20 minutes after starting hyperoxic therapy. The usual clinical index of severity of CO poisoning, the carboxyhemoglobin (HbCO) level, does not correlate with the severity of tissue hypoxia in the myocardium. Because the model can predict CO uptake and distribution in human myocardium, it could be a tool to estimate the potential for hypoxic myocardial injury and facilitate therapeutic intervention. These findings were also described in a recent publication [2].

A limitation of the aformentioned model is that it assumes that ventilation is constant (or changes only occasionally at discrete times). A more comprehensive model requires that physiological mechanisms which control ventilation be added to the model. To address this issue, these mechanisms have been added and ventilation now is predicted by the model for situations of normoxia, hyperoxia, hypoxia, and hypercapnia. This range of situations is far more extensive than presented in other models of CO uptake and distribution. Also incorporated into the model are relationships for CO2 mass balance in every compartment and prediction of H+ and lactate concentrations. Thus, this version of the model should be useful for investigating "typical" responses to CO exposures in a large variety of workplace scenarios, including situations comprising both mild and moderate exercise levels. Development of this model has been the central focus of a doctoral dissertation research program. The model will be used to simulate both intermittent and sustained CO exposures that might occur in a workplace in which workers are not "at rest". The model and simulation results will be described in a manuscript to be submitted in the near future.

<u>Specific Aim 2</u>. Using the predicted time course of myocardial MbCO for each subject from two data sets, we will test whether model predictions of the timing and magnitude of peak myocardial MbCO values correlate with premature atrial (PAC), or premature ventricular (PVC), contractions or with sensitive measures of S-T disturbances, T-wave morphology, and heart rate variability.

Despite several attempts, including visiting his laboratory, we were not successful in acquiring the proposed data set from Dr. Weaver. We did collaborate with Dr. Paul Kizekevitch to acquire copies of all of his data files from his studies of CO exposure of 16 male subjects at rest and during exercise, including about 75 minutes of ECG recordings from each study. After close inspection of these data, we recognized that they do not include the time period during which our model predicts the highest probability of cardiac electrophysiological abnormalities because the data stop at the cessation of CO exposure. Consequently, although we prepared software for such analyses [5], we were unable to address this aspect of Specific Aim 2.

Additional Accomplishments.

We have been in contact with Drs. Caroline Burge and Chris Gore of the Australian Institute of Sport, who have been conducting human trials of CO exposures. In particular, we participated in the design of their most recent experiments to maximize our ability to simulate their findings in order to validate our model and apply it to a range of subjects. The objective of these experimental studies, which were conducted over the past year, was to evaluate two different methods of estimating hemgoglobin (Hb) mass (and blood volume) based on measuring the amount of CO taken up during a brief exposure. These methods are important for assessing training effects in sports medicine and for determining oxygen delivery capacity in, for example, anemic subjects. These studies also allow us to better test the ability of our model to predict the uptake of CO by Mb (which has been said previously to be both negligibly slow and rapid; our model predicts slow but significant uptake even during brief CO exposures). We have received their data and will use our published model [2] to evaluate the two methods of estimating Hb mass. (The experimental studies did not utilize a "gold standard" of measurement, so our determination of possible errors using the model will enhance the value of those studies.) We have already determined that our model can reproduce their experimental data with a high degree of accuracy, and that both methods typically produce less than a 5% error in estimated Hb mass. These simulation studies will be reported in a manuscript [7] in the near future.

We have modified one of our published models [1] in order to examine the susceptibility of anemic women to CO hypoxia at rest and during elevated metabolism. The model was modified by including the effects of anemia on ventilation, cardiac output, and pulmonary (physiological) dead space. Although the effects of chronic exposure to CO on individuals with cardiovascular disease are well-studied, the potential for adverse effects in those with anemia (Hb levels <13 for women and < 15 for men) has received little attention. We have substantially modified our model to take into account the parameter values likely to change with anemia and have validated the model with data from anemic women (Brannon et al., J Clin Invest 24:332-6, 1945). This endeavor has greatly facilitated the ability to predict the range of responses that might be expected to occur in anemic women exposed to currently-acceptable levels of CO in a work place setting. The model predictions confirmed the widely-held opinion that the severity of symptoms does not correlate closely with Hb level. Although HbCO levels tended to be higher in the anemic women vs. the control, this was not always the case. Cardiac output (ml/min/kg), which was higher in anemic women prior to the CO exposure, was further elevated during the CO exposure. In one of the anemic women the predicted cardiac output was almost 2 times greater than that of the control. Another finding relevant to the work place was that minute ventilation was much higher in the anemic subjects, greatly increasing their risk for inhalation, deposition, and retention of particulates to which they are exposed on the job.

Publications

1. Bruce E.N., M. C. Bruce, and K. Erupaka: [2008] Prediction of the Rate of Uptake of Carbon Monoxide From Blood by Extravascular Tissues. Respir Physiol Neurobiol 161: 142-159. (PMID: 18313993).
2. Erupaka K., M. C. Bruce, and E. N. Bruce: [2010] Prediction of Extravascular Burden of Carbon Monoxide (CO) in the Human Heart. Annals Biomed. Eng. 38(2): 403-438. (PMID: 19834811).
3. Erupaka K., M. C. Bruce, and E. N. Bruce: [2006] Extravascular Burden of Carbon Monoxide (CO) During CO Exposure and Washout, Society for Mathematical Biology, Raleigh, NC; Aug. 1-4.
4. Erupaka, K: [2008] Determination of the Extravascular Burden of Carbon Monoxide (CO) on Human Heart. M. S. Thesis, University of Kentucky.
5. Duddekunta, K: [2006] A Program for Calculating Various Parameters from an ECG Recording. Project submitted as part of non-thesis M. S. program, University of Kentucky.
6. Bruce. M. C., and E. N. Bruce. Modeling Study of CO Exposures in Anemic Women (in preparation).
7. Chada, K., M. C. Bruce, and E. N. Bruce. Validation and Limitations of Methods for Estimating Hemoglobin Mass Via CO Uptake: A Modeling Study. (in preparation).

Inclusion of gender and minority subjects

Since this study involves mathematical modeling only, no subjects were recruited for the study. The model, however, does include variations in parameters related to gender.

Inclusion of children

No subjects, and thus no children, were recruited for this study.

Materials available for other investigators

An annotated list of the equations of the model of reference #2 is available as an appendix of that publication. The software code for implementing this model using the simulation package Advanced Continuous Simulation Language (ACSL™) is available on the web site http://www.uky.edu/~ebruce.