THE EFFECTS OF ACUTE, LOW-DOSE CARBON MONOXIDE INHALATION ON OXYGEN UPTAKE AND ENERGY EXPENDITURE DURING SUBMAXIMAL GRADED EXERCISE

by

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B.A., University of Colorado, 2013

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Master of Science

Department of Integrative Physiology

2013

This thesis entitled: "The effects of acute, low-dose carbon monoxide inhalation on oxygen uptake and energy expenditure during submaximal graded exercise"
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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

IRB protocol # 12-0546

Abstract

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The effects of acute, low-dose carbon monoxide inhalation on oxygen uptake and energy expenditure during submaximal graded exercise

Thesis directed by Associate Professor William C. Byrnes

Carbon monoxide (CO) interacts with many physiological systems, similar in effect to nitric oxide (NO). Manipulating the NO synthesis pathway through dietary nitrate supplementation decreases oxygen consumption and energy expenditure during submaximal exercise in moderately trained individuals, but the adaptations and acute effects from low-dose CO administration have not been described in full. Our purpose was to quantify any changes to oxidative metabolism, measured by oxygen consumption and energy expenditure during submaximal exercise. Nine recreationally active individuals (ages 20–32 years) familiar with cycling exercise completed four graded submaximal exercise tests, with each test occurring during a separate visit. Subjects received a low dose (1.2 $mL \cdot kg^{-1}$ body mass) of CO or room-air placebo in a randomized, subject-blind fashion prior to exercise during the first visit in order to study their responses to acute administration. This exercise task was repeated 24 hours later (visit 2) to assess any lasting effects. Subjects returned to the lab after a washout period (2–10 days) to repeat the study procedures with the alternate dose (visits 3 and 4). Acute CO administration did not affect oxygen consumption or energy expenditure during submaximal exercise. However, significant increases in heart rate ($P = 0.028$; ~4 bpm) and perceived exertion ($P = 0.036$; 0.36 units) were observed across workloads after acute CO inhalation. Further, the increase in blood lactate concentration from rest after acute CO inhalation was higher than after the placebo intervention at the two highest work rates. No changes to the respiratory exchange ratio or

ventilation were observed between visits. In conclusion, acute low-dose CO inhalation temporarily increased heart rate, blood lactate, and perceived effort during submaximal exercise. However, a low-dose of CO had no effects on the energetics of submaximal exercise immediately or 24 hours following its administration.

Acknowledgements

Major thanks are in order for the members of my thesis committee, who graciously donated their time and energy into reviewing this thesis. I would particularly like to thank Dr. Byrnes for agreeing to be my advisor and for showing me how to develop and complete this project. His excellent academic guidance and caring nature shepherded me through this graduate experience. I would be nowhere without his efforts.

I would also like to thank everyone who helped this project come to fruition. To Ben Ryan, thank you for all the time you put in to this protocol; your comments—every last one of them—and ability to run the show in my absence saved this ship. You make me think harder than I'd like most times, but it has always paid off. Thank you to Jim Peterman and Eric Homestead for showing me the ropes in the AESL, to Caitlin Oriel and Ryan Harned for assisting in data collection, and Dave Caha for helping to initiate this study. This data would not exist without subjects who generously volunteered their time and effort and obeyed the study guidelines, and to them I am forever indebted. Finally, thank you Sierra, Mom, Dad, and 'Thaniel for motivating me in the first place, supporting me through my education, and inspiring me when I needed it most.

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Chapter I
Introduction

Carbon monoxide (CO) binds to the blood protein hemoglobin and lowers the ability of circulating red blood cells to carry and deliver oxygen to systemic tissues. Despite its toxicity in high doses, CO's hemoglobin-binding capability was being utilized in research even before the first full characterization of this interaction by Douglas in 1910 (12). Haldane and Smith (23) first developed a model to tag hemoglobin with CO gas (elevating the percent of carboxyhemoglobin, HbCO%) and accurately estimate the total mass of circulating hemoglobin (Hb_{mass}). This procedure has since been fine-tuned into the optimized carbon monoxide-rebreathing (oCOR) method by Schmidt and Prommer (60). The applications of this method span clinical and research arenas, from measuring the *in vivo* viability of blood transfusions (4) to assessing hematological changes from various interventions (reviewed in (60)). Most early research (and even recent analyses) tracked changes in hemoglobin concentration as a marker for hemoglobin mass, but changes to fluid balance make this parameter subject to large variability. Being able to assess changes in the total amount of hemoglobin in circulation in tandem with the small measurement error typically reported for the oCOR method make it a potentially useful tool as an anti-doping control in sport (2, 14, 16, 51), although many researchers remain hesitant about this prospect (36, 48). Low-dose CO inhalation is a frequent tool and has the potential to become more widespread. However, scientists have not completely evaluated the physiological effects of low-dose CO administration.

Alterations in blood oxygen content such as those seen after CO administration may alter the physiology of submaximal exercise by increasing relative workload intensity. Many studies that reduced subjects' arterial oxygen content by low-dose CO inhalation have recorded decreases in the maximal rate of oxygen consumption ($\dot{V}O_{2max}$). Schmidt and Prommer (60)

showed a 3.0% reduction in $\dot{V}O_{2max}$ with CO onboard in their proposal of the oCOR method, and this slight reduction is supported by previous literature (26, 31). Acute elevations in HbCO cause tissue hypoxia and may have effects similar to altitude exposure. Altitude shifts substrate utilization toward carbohydrate (in men), increases heart rate, and magnifies the sympathetic nervous response during light and moderate workloads (38). This is in addition to other large, often sustained changes at altitude such as significant reductions in plasma volume (to concentrate O_2 content per unit blood) (46). Since CO lowers maximal work capacity, the same absolute workload becomes a higher relative percentage of one's $\dot{V}O_{2max}$ when CO is onboard, and therefore its administration would likely signal physiological changes during submaximal work rates.

CO is chemically and physiologically similar to nitric oxide (NO), a molecule that has stimulated significant research in the scientific community. However, research on CO gas stagnated from the time of its discovery circa 1865 until the late twentieth century, when breakthroughs in NO physiology exploded from the scientific literature (reviewed in (59)). The ability of CO to reversibly occupy oxygen binding sites on hemoglobin and inactivate mitochondrial enzymes led to its toxic reputation and no doubt discouraged initial research into this molecule (13, 64). Insights into NO signaling pathways, realization of its inherent ties to mammalian physiology, and optimization of the CO-rebreathing procedure have brought CO into the scientific forefront over the past two decades.

Research manipulating the nitrate-nitrite-NO pathway has shown reduced oxygen cost of exercise (improving economy) and raises the possibility that low-dose CO administration may similarly influence exercise energetics. Dietary nitrate is reduced by the oral microbiota and acidic gut environment to nitrite and NO, along with other nitrogen species (27). In the exercise

context, acute dietary nitrate increases economy (5, 6, 34), efficiency (6, 35), and endurance performance (33), although these results have not yet been replicated in elite athletes (6, 7, 10, 41, 70). CO and NO bind many of the same hemoproteins and demonstrate similar effects *in vitro*, although CO is far less effective (59). Although previous researchers have reported no changes in oxygen consumption ($\dot{V}O_2$) during CO exposure, closer examination of the data collected by Vogel et al. (67) indicate a possible reduction at light and moderate workloads. In addition, Klausen et al. (30) found "hardly any changes" in $\dot{V}O_2$ at rest, and these changes appear to reflect reduced oxygen use (no mean values or statistics were reported). With the whole-body effects documented from nitrate supplementation, the possibility of CO administration similarly influencing the energetics of endurance exercise needs to be determined.

Recently, there have been several research studies exploring potentially beneficial outcomes from low-dose CO inhalation. Most of these interventions have been aimed at medical outcomes (reviewed in (39)), but some evidence does exist for interactions with systems involved in exercise. For example, Richardson et al. (55) administered a relatively large dose of CO (≈ 20% HbCO) in humans and found reduced a-v O_2 difference in exercising limbs at submaximal workloads, along with a matched increase in muscle blood flow to maintain net $\dot{V}O_2$. On the other hand, CO has also been found to uncouple mitochondrial respiration, therefore altering efficiency and reactive oxygen species generation (29). CO stimulates mitochondrial biogenesis in human skeletal muscle even at doses lower than those prescribed in the oCOR method (53). These researchers also found increased expression of MHC class I protein in skeletal muscle after CO exposure, suggesting a shift to more efficient muscular phenotype. The interactions mentioned here may play an important role in submaximal exercise performance immediately following CO exposure and might additionally have lasting effects.

With the current application of CO inhalation in clinical, research, and applied settings, it is imperative to determine how low doses of this gas affect physiological responses to physical activity. Athletes who undergo Hb_{mass} measurements and the individuals who implement these tests should be informed of all physiological changes that result from low-dose CO administration. Although CO and NO have the potential to exert similar effects, CO is less potent in its signal. Therefore, despite the improvements documented in moderately trained individuals after nitrate supplementation, examining the CO literature led us to hypothesize that there would be no changes to our primary variables of interest—$\dot{V}O_2$ and whole-body energy expenditure (EE)—with CO onboard. Though CO exposure has been shown to modify mRNA transcription and protein expression within 24 hours (63), we expected this potential effect to be so minor as to change neither $\dot{V}O_2$ nor EE during submaximal exercise 24 hours after low-dose CO inhalation. As CO exposure transiently reduces $\dot{V}O_{2max}$, we expected acute CO inhalation to cause responses consistent with hypoxic exposure during submaximal exercise—that is, increases in heart rate, ventilation, blood lactate concentration, carbohydrate utilization, and perceived effort.

Chapter II
Methods

This study was designed to examine the effects of a low, exogenous dose of carbon monoxide on oxygen consumption and energy expenditure during submaximal cycling. These metabolic parameters were determined at various workloads with a graded cycling protocol immediately following the administration of a small dose of carbon monoxide (CO condition). Subjects were also given room air as a placebo gas during a separate session (PLA condition) to serve as a control. To examine the possibility of a delayed effect from carbon monoxide, subjects repeated the cycling test without any intervention 24 hours following the administration of each gas (CO24 and PLA24, respectively). Subjects were randomized and blinded to their treatment order, and a 2–10 day washout period was enforced between test sets. For each subject, all four sessions were completed within a 2-week timespan. A study diagram is provided in **Figure 1**. All data were collected between December 2012 and March 2013.

Subjects

Nine recreationally active males volunteered for this study. All subjects met our lab's definition of being recreationally active (>1 time per week of planned exercise) and at the onset of the study were using a bicycle for exercise or transportation a minimum of twice per week. Subjects were required to be acclimatized to the Boulder, Colorado area for at least 8 weeks and self-reported as non-smokers for at least 6 months prior to beginning their first study session. Volunteers were screened for their ability to safely engage in the exercise tasks with health questions taken from the Physical Activity Readiness Questionnaire (PAR-Q) (15). Descriptive data for the subjects in this study are listed in **Table 1**. Before testing, all subjects gave informed,

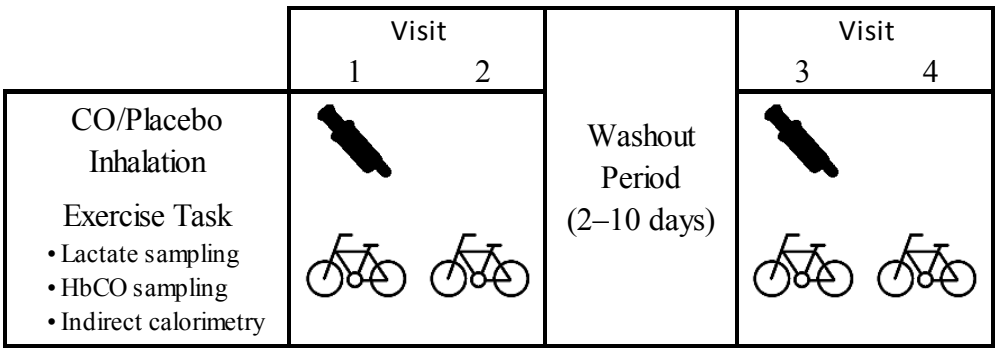

Figure 1. Overview of the study design. Exercise Task (🚲) indicates the sub-maximal graded cycling protocol. Subjects performed the inhalation procedure (🚬) immediately before exercise on visits 1 and 3, and returned to the lab 24 hours later for visits 2 and 4, respectively.

Table 1. Descriptive Data for the Nine Subjects in this Study.

Physical Characteristics	**Mean (n = 9)**
Age (yrs)	26.4 ± 4.8
Height (cm)	181.8 ± 4.4
Mass (kg)	79.0 ± 8.7
BMI (kg/m^2)	24.0 ± 2.4
Gross Mechanical Efficiency, average (%)	20.5 ± 0.8

BMI, body mass index. Gross mechanical efficiency for each subject was averaged across completed workloads. Data are from the PLA visit and presented as mean ± SD.

written consent as approved by the University of Colorado at Boulder Institutional Review Board (IRB) to the risks involved with participation in the experimental protocol for this study.

Preliminary Procedures

Prior to visiting the lab for their first experimental session, subjects were asked to record in detail their eating habits for the preceding day in a 24-hour diet log. Following the first session, a photocopy of this log was returned to subjects, and they were asked to maintain the same eating routine for each of the remaining sessions. In addition, subjects were instructed to avoid caffeine for at least 6 hours and any high-intensity exercise for 24 hours before all study procedures.

Experimental Procedures

Upon signing the Informed Consent document, subjects changed into their exercise clothing, and a researcher recorded their height and weight. They then sat for 10 minutes while their right hand was warmed with a heating pad to arterialize capillary blood. The inhalation protocol was again explained to participants during this time. An arterialized finger was cleaned with an alcohol swab after 10 minutes' rest, and a fingerstick was performed on this finger using a Unistik 2 device (Owen Mumford Ltd. Medical Division, Oxford, England). Two samples were obtained from this fingerstick, one to determine resting blood lactate concentration and one to determine baseline capillary percent carboxyhemoglobin (HbCO%). The lactate sample (approximately 50 μL capillary blood) was drawn into a 75 μL microhematocrit tube (Fisher Scientific, USA). Twenty-five μL from this sample were then pipetted into a 150 μL microcentrifuge tube containing 50 μL of a "cocktail"—a solution consisting of a buffer, Triton

XL-100 as a lysing agent, and sodium fluoride as an anti-glycolytic agent. These samples were mixed by vortex immediately to ensure proper combination and were analyzed in duplicate for blood lactate using a YSI 2300 Blood Glucose/Lactate analyzer (YSI, USA). This analysis occurred during the subject's visit by another trained researcher when feasible; otherwise samples were refrigerated for later analysis. Before sampling, the lactate analyzer was calibrated against a known standard and automatically re-calibrated during sampling after either 15 minutes or five samples (whichever came first). The remaining sample (approximately 75 μL capillary blood) was collected into a 100 μL micro-capillary tube (Clintubes, Radiometer, Denmark), mixed immediately to prevent coagulation, and analyzed in triplicate during the subject's visit for baseline capillary HbCO% (OSM3 Hemoximeter, Radiometer, Denmark). Our laboratory has generated an arterial HbCO% standard, and samples from this were used prior to data collection to ensure accurate HbCO%.

Subjects then inhaled a small, quantified volume of 99.5% CO (Airgas, Colorado, USA) (1.2 $mL \cdot kg^{-1}$ body mass) or room-air placebo (matched volume) consistent with their treatment order from a pre-calibrated syringe. We used a dose slightly higher than that recommended by Schmidt and Prommer (60) due to the decreased partial pressure of the bolus at our lab's altitude of 1,625 meters (5,330 feet). Subjects wore nose clips and, following a maximal exhalation, held a syringe with its measured volume of gas to their lips, formed a tight seal, and inhaled its contents fully. Subjects then removed the syringe from their lips and continued inhaling room air until they had inhaled completely. Subjects were instructed to hold this breath for a minimum of 30 seconds (or as long as comfortable) to facilitate CO diffusion. This breath was followed 6–8 minutes later by a second fingerstick to determine the amount of CO absorbed and bound to hemoglobin. Pilot work conducted by researchers in our lab showed that this modified inhalation

method causes a similar change in HbCO% as that seen with the oCOR procedure.

A submaximal graded cycling task was used to determine oxygen consumption and energy expenditure after each intervention. Subjects fit themselves on a stationary bicycle (Lode ergometer, Netherlands) and warmed up at a preferred cadence until they were ready to begin the test. Bike fit and cadence (within ± 5 rpm) were maintained for all return visits. A commercially available heating pad (Little Hotties Hand Warmers, Implus Footcare LLC, USA) was placed inside a nitrile glove with the fingertips removed, and the glove was placed on subjects' right hands to improve hand blood flow during the exercise task. The protocol began at a power output of 90 watts, increasing by 30 watts every 4 minutes until subjects reported a Rating of Perceived Exertion (RPE; Borg's 6–20 scale) greater than or equal to 15. Data resulting from previous experiments in our lab indicated that this value is a reliable estimate of the lactate threshold. These same workloads were repeated for all experimental sessions. Tests were conducted with an average barometric pressure and temperature of 624.9 ± 4.5 mmHg and 20.6 ± 1.7 ˚C, respectively. Subjects were cooled with a standing fan during each of the tests.

During the exercise tasks, rate of oxygen consumption ($\dot{V}O_2$), rate of carbon dioxide production ($\dot{V}CO_2$), heart rate (HR), respiratory exchange ratio (RER), expired pulmonary ventilation rate ($\dot{V}_E$), rate of energy expenditure (EE), respiratory rate (RR), and tidal volume (V_T) were recorded every 15 seconds through a computer-assisted indirect calorimetry metabolic cart system (Parvomedics, Sandy, UT). Expired gases were collected through a high-flow Daniels' valve and were in turn fed through the Parvo system for mixing and analysis. Prior to each test, gas fractions were calibrated with a primary standard gas mixture within the physiological range (16.01% O_2 and 4.01% CO_2). The pneumotach was calibrated using a 3-liter syringe at five distinct flow rates (≈ 75 $L \cdot min^{-1}$ to 275 $L \cdot min^{-1}$ APTS). Our calibration criteria

were within 3% of the calibration volumes, and gas fractions within 0.02% of calibration values (e.g. 16.01 ± 0.02%). Average typical error of oxygen consumption during this study was 0.052 $L \cdot min^{-1}$, and was calculated using data from PLA and PLA24 sessions. $\dot{V}O_2$ measurement error was independent of work rate, so percent error of the measurement reached a minimum at the highest rates of oxygen consumption.

Indirect calorimetry and HR data were averaged over the last 2 minutes of each 4-minute stage to ensure steady state values had been reached. Correlations between oxygen consumption and power output were linear ($r > 0.99$) for all subjects. Gross mechanical efficiency (GE) was calculated as follows:

GE (%) = mechanical power ($kcal \cdot min^{-1}$)/metabolic power ($kcal \cdot min^{-1}$) x 100%

Mechanical power was converted from Watts at each stage and metabolic power was estimated using $\dot{V}O_2$ and the energetic equivalent of O_2, accounting for average RER at a given stage (42). HR was measured using radio telemetry (Polar Electro, Finland) and also integrated into the metabolic cart system. Subjective effort (RPE) and blood lactate measures were taken in the fourth minute of each stage. In the final stage of the exercise task (RPE ≥ 15), an additional blood sample was collected to analyze HbCO% at the end of exercise and to calculate the half-time of HbCO during a submaximal graded exercise task.

Data were included in the final analysis if the blood sample collected in that stage showed that the lactate threshold had not been surpassed. Lactate threshold was defined as a blood lactate concentration 1 mM above a baseline, which included the resting sample (11). Three independent, trained researchers traced a line 1 mM above where the lactate profile inflected from that baseline, and the average lactate concentration from these three determinations was used as the cutoff value. No significant differences in threshold values were

found between observers.

Subjects returned to the lab 24 hours after their first visit (range: 23–25 hr). Body mass data and resting lactate and HbCO% samples were collected prior to repeating the same submaximal graded cycling protocol from the previous day.

Following a washout period of 2–10 days, subjects returned to the lab and repeated the cycling task with the other gas (for example, with CO if the placebo was given during visit 1). They then returned to the lab 24 hours following this test to repeat the exercise test.

Statistical Analysis

Data were analyzed using SPSS software (SPSS Inc., Chicago, IL). Mean values across workloads for $\dot{V}O_2$, EE, HR, $\dot{V}_E$, RER, blood lactate, and RPE, as well as interactions and possible carryover effects, were tested with linear mixed-effects model analyses with PLA as the default reference group in all models. We used the same approach to compare baseline HbCO% and the slopes and intercepts of $\dot{V}O_2$, EE, and HR for differences between treatment days. For HbCO%, the linear mixed model analysis was also used to test for changes within sessions. Post-hoc inter-treatment contrasts were adjusted for multiple comparisons using the Bonferroni correction. The linear oxygen consumption–power output relation for each exercise session was confirmed using Pearson's correlation coefficient. Differences in lactate threshold values between researchers were compared using one-way ANOVA. Statistical significance was set a priori as $\alpha = 0.05$. All values are expressed as mean ± standard deviation (SD).

Chapter III
Results

Carboxyhemoglobin Levels

Our inhalation method elevated HbCO by 4.2 ± 1.0% immediately following the CO treatment. Using the method described by Weaver et al. (69), HbCO half-time was calculated as 51.3 ± 23.5 min during the graded exercise task. Although HbCO saturation significantly dropped during the exercise bout, this parameter remained significantly elevated from baseline during the final stage (**Table 2**). Controlling for visit, there were no differences in baseline HbCO% values across the treatment days. Holding the effects of visit and treatment constant, however, a significant ($P = 0.026$) carryover effect existed following CO treatment (CO24 condition) and accounted for its increased mean.

Oxygen Uptake and Energy Expenditure

Data from the submaximal graded cycling tasks are listed in **Tables 3a & 3b**. Steady state oxygen uptake and whole-body EE were not significantly different between CO, CO24, PLA, and PLA24 conditions across all power outputs (**Figures 2** and **3**, respectively). These results are reflected in slope and intercept data for $\dot{V}O_2$ and EE, which also showed no differences between conditions. Most subjects had exceeded their lactate threshold at 240 W in each condition (remaining n = 3, 2, 2, and 2, respectively), so this stage was excluded from $\dot{V}O_2$, EE, and RER analysis due to insufficient data points.

Hypoxic Response

Controlling for power, HR (**Figure 4**) was elevated by 4.2 bpm during CO compared to PLA ($P = 0.028$). Post-hoc comparisons also revealed a significant difference from PLA24

Table 2. Carboxyhemoglobin Levels (%) at Baseline during Acute (PLA, CO) and 24hr Post (CO24, PLA24) Conditions, and Post-Dose and Post-Exercise during PLA and CO.

	Baseline	**Post-Dose**	**Post-Exercise**
PLA	0.47 ± 0.20	0.49 ± 0.22	0.41 ± 0.19
PLA24	0.36 ± 0.17		
CO	0.55 ± 0.22	4.78 ± 0.85 *	3.01 ± 0.63 *‡
CO24	0.66 ± 0.36 †		

Values are means ± SD.
* Significant difference from Baseline ($P < 0.001$).
‡ Significant difference from Post-Dose ($P < 0.001$).
† Indicates carryover effect from previous visit ($P < 0.05$).

Table 3a. Steady State Data Collected at Each Workload during Acute (CO, PLA) and 24hr Post (CO24, PLA24) Conditions.

	90 W		120 W		150 W		180 W	
	Acute	**24hr Post**	**Acute**	**24hr Post**	**Acute**	**24hr Post**	**Acute**	**24hr Post**
Oxygen uptake ($L \cdot min^{-1}$)								
CO	1.54 ± 0.13	1.56 ± 0.10	1.81 ± 0.13	1.82 ± 0.13	2.11 ± 0.12	2.11 ± 0.12	2.41 ± 0.11	2.41 ± 0.12
PLA	1.58 ± 0.11	1.55 ± 0.10	1.82 ± 0.13	1.78 ± 0.08	2.11 ± 0.11	2.09 ± 0.12	2.42 ± 0.12	2.40 ± 0.12
Energy expenditure ($kcal \cdot min^{-1}$)								
CO	7.38 ± 0.63	7.51 ± 0.50	8.76 ± 0.62	8.85 ± 0.61	10.25 ± 0.59	10.25 ± 0.58	11.81 ± 0.63	11.77 ± 0.55
PLA	7.59 ± 0.56	7.49 ± 0.49	8.86 ± 0.67	8.66 ± 0.43	10.30 ± 0.55	10.19 ± 0.57	11.88 ± 0.51	11.66 ± 0.55
Heart rate ($beats \cdot min^{-1}$)								
CO	103.2 ± 8.6	101.5 ± 9.2	113.1 ± 9.8	111.4 ± 11.3	123.7 ± 11.0	120.0 ± 10.8	134.7 ± 11.9	130.0 ± 12.4
PLA	100.3 ± 9.0	98.6 ± 6.6	109.5 ± 9.3	107.9 ± 6.4	120.4 ± 10.5	116.4 ± 7.1	130.2 ± 10.3	128.3 ± 9.5
Ventilation ($L \cdot min^{-1}$)								
CO	38.36 ± 6.17	39.00 ± 5.06	45.51 ± 6.02	46.95 ± 5.93	54.52 ± 7.17	53.21 ± 7.50	63.08 ± 8.12	62.16 ± 8.21
PLA	38.71 ± 6.54	39.19 ± 6.21	46.54 ± 6.77	46.21 ± 6.18	53.38 ± 6.74	54.33 ± 7.13	62.70 ± 7.61	62.88 ± 7.93
RER								
CO	0.844 ± 0.053	0.836 ± 0.049	0.872 ± 0.051	0.876 ± 0.044	0.889 ± 0.049	0.879 ± 0.045	0.903 ± 0.047	0.896 ± 0.044
PLA	0.832 ± 0.042	0.841 ± 0.041	0.878 ± 0.036	0.884 ± 0.029	0.893 ± 0.036	0.895 ± 0.029	0.906 ± 0.040	0.904 ± 0.029
Blood lactate ($mmol \cdot L^{-1}$)								
CO	0.93 ± 0.34	0.94 ± 0.41	0.97 ± 0.38	0.88 ± 0.38	1.01 ± 0.39	0.93 ± 0.36	1.16 ± 0.45	1.14 ± 0.32
PLA	1.05 ± 0.28	1.07 ± 0.46	1.09 ± 0.41	1.21 ± 0.58	0.97 ± 0.24	1.05 ± 0.35	1.22 ± 0.23	1.31 ± 0.34
RPE								
CO	8.2 ± 1.0	8.1 ± 0.8	9.7 ± 1.0	9.3 ± 0.7	11.3 ± 0.5	10.9 ± 1.1	12.6 ± 0.5	12.0 ± 0.7
PLA	8.1 ± 0.9	8.2 ± 0.8	9.6 ± 1.1	9.3 ± 1.1	10.8 ± 1.2	10.6 ± 1.3	12.0 ± 0.7	11.7 ± 1.1

RER, respiratory exchange ratio; RPE, rating of perceived exertion. Values are presented as means ± SD of the means (n = 9).

Table 3b. Steady State Data Collected at Each Workload during Acute (CO, PLA) and 24hr Post (CO24, PLA24) Conditions.

	210 W		240 W		Slope		Intercept	
	Acute	24hr Post	Acute	24hr Post	Acute	24hr Post	Acute	24hr Post
Oxygen uptake ($L \cdot min^{-1}$)					($L \cdot min^{-1} \cdot W^{-1}$)		($L \cdot min^{-1}$)	
CO	2.75 ± 0.14	2.73 ± 0.13			0.0100 ± 0.0004	0.0099 ± 0.0004	0.623 ± 0.142	0.641 ± 0.108
PLA	2.73 ± 0.11	2.72 ± 0.09			0.0098 ± 0.0006	0.0100 ± 0.0005	0.667 ± 0.140	0.615 ± 0.118
Energy expenditure ($kcal \cdot min^{-1}$)					($kcal \cdot min^{-1} \cdot W^{-1}$)		($kcal \cdot min^{-1}$)	
CO	13.42 ± 0.71	13.41 ± 0.60			0.0502 ± 0.0027	0.0658 ± 0.0482	2.78 ± 0.71	2.89 ± 0.59
PLA	13.39 ± 0.53	13.35 ± 0.35			0.0492 ± 0.0026	0.0494 ± 0.0033	3.04 ± 0.72	2.87 ± 0.74
Heart rate ($beats \cdot min^{-1}$)					($beats \cdot min^{-1} \cdot W^{-1}$)		($beats \cdot min^{-1}$)	
CO	146.7 ± 11.3	141.3 ± 11.5	158.4 ± 10.7	152.3 ± 11.0	0.365 ± 0.067	0.337 ± 0.049	69.6 ± 12.6	70.7 ± 12.2
PLA	141.4 ± 10.3	140.0 ± 9.9	152.7 ± 10.3	152.8 ± 10.3	0.352 ± 0.059	0.358 ± 0.065	67.7 ± 13.0	65.1 ± 10.0
Ventilation ($L \cdot min^{-1}$)								
CO	73.37 ± 10.06	73.66 ± 8.43	87.29 ± 14.64	86.90 ± 12.10				
PLA	71.39 ± 9.86	73.78 ± 8.49	84.05 ± 12.41	85.55 ± 10.95				
RER								
CO	0.915 ± 0.048	0.917 ± 0.041						
PLA	0.915 ± 0.035	0.918 ± 0.028						
Blood lactate ($mmol \cdot L^{-1}$)								
CO	1.91 ± 0.65	1.61 ± 0.37	2.94 ± 1.15	2.32 ± 0.53				
PLA	1.65 ± 0.26	1.68 ± 0.47	2.52 ± 0.51	2.69 ± 0.45				
RPE								
CO	13.8 ± 0.8	13.4 ± 0.9	15.1 ± 1.0	14.5 ± 0.5				
PLA	13.6 ± 0.9	13.2 ± 1.1	14.8 ± 0.9	14.4 ± 1.2				

RER, respiratory exchange ratio; RPE, rating of perceived exertion. Values are presented as means ± SD of the means. n = 9 until 240 W (n = 8), except for oxygen uptake, energy expenditure, and RER at 210W (CO n = 6, CO24 n = 9, PLA n = 9, PLA24 n = 8).

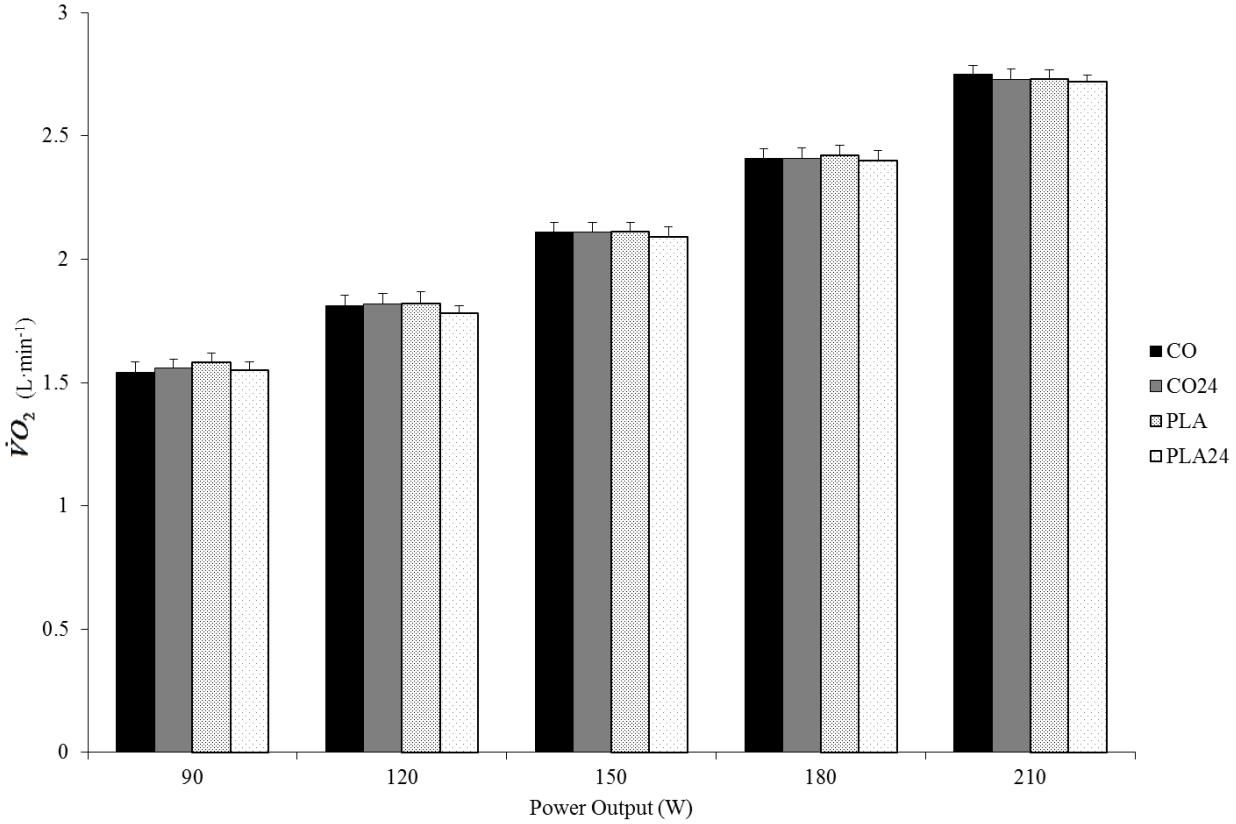

Figure 2. Mean oxygen uptake ($\dot{V}O_2$) below the lactate threshold for each workload during Acute (CO, PLA) and 24hr Post (CO24, PLA24) conditions. No differences were observed for $\dot{V}O_2$ between treatment groups at any workload. $\dot{V}O_2$ increased significantly ($P < 0.001$) at each stage. Values represent mean ± standard error of the mean (SEM) in nine subjects, except at 210 W (CO n = 6, CO24 n = 9, PLA n = 9, PLA24 n = 8).

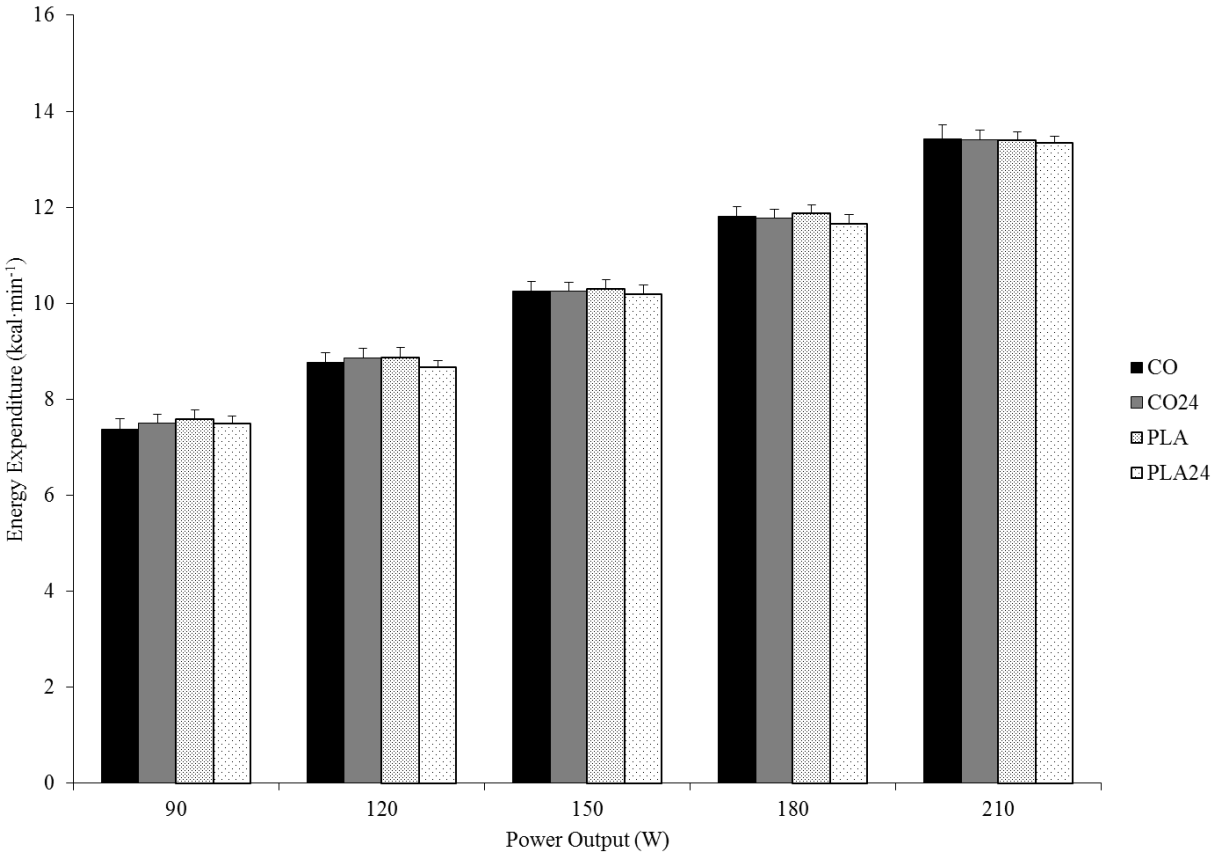

Figure 3. Mean energy expenditure (EE) below the lactate threshold for each workload during Acute (CO, PLA) and 24hr Post (CO24, PLA24) conditions. No differences were observed for EE between treatment groups at any workload. EE increased significantly ($P < 0.001$) at each stage. Values represent mean ± SEM in nine subjects, except at 210 W (CO n = 6, CO24 n = 9, PLA n = 9, PLA24 n = 8).

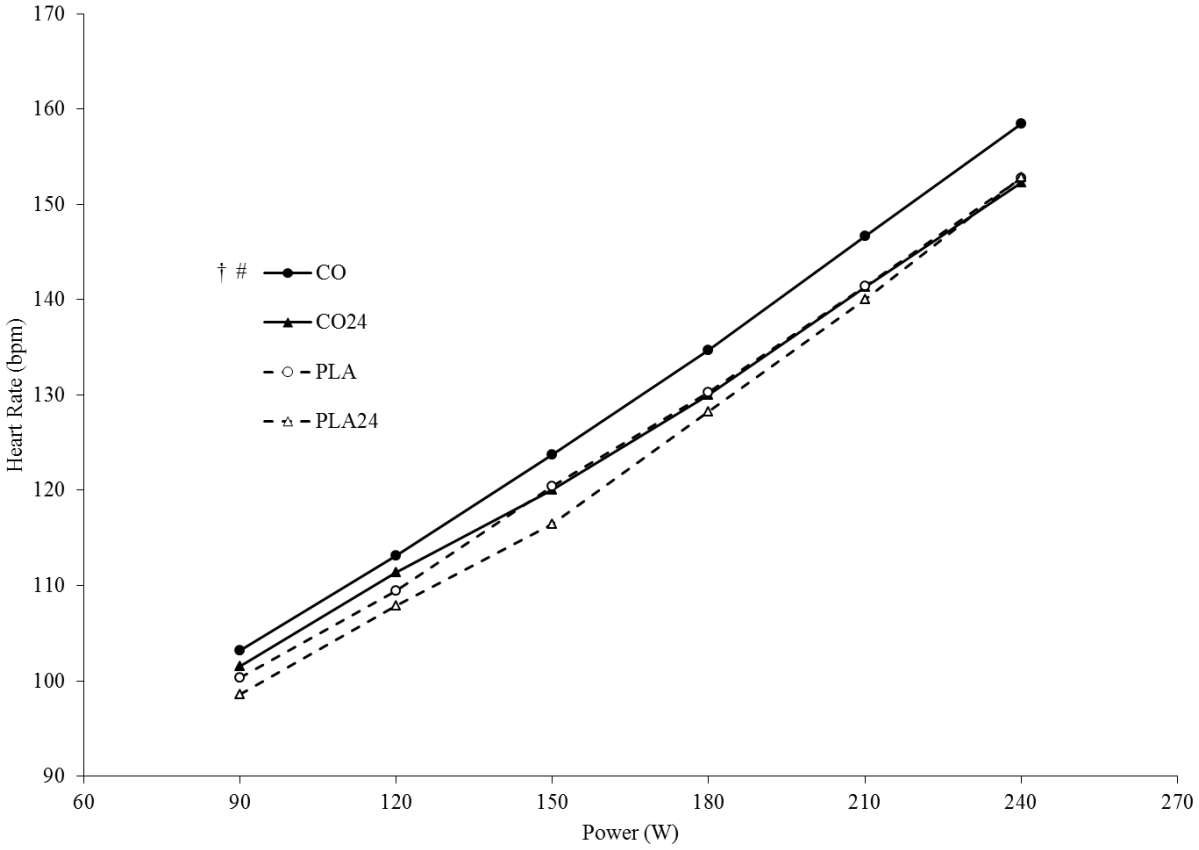

Figure 4. Mean heart rate (HR) for each workload during Acute (CO, PLA) and 24hr Post (CO24, PLA24) conditions. HR increased significantly ($P < 0.001$) at each stage. Values represent group means for nine subjects, except at 210 W (n = 8). † Significant treatment effect compared to PLA ($P < 0.05$). # Significant treatment effect compared to PLA24 ($P < 0.05$).

across all workloads. No differences in RER or $\dot{V}_E$ were observed between conditions during the submaximal graded cycling task. Subjects' RPE scores decreased in each visit, but remained significantly elevated in CO compared to PLA (estimate = 0.36, $P = 0.036$) after controlling for the effects of visit and workload. Power was a significant predictor for each of the above variables at all workloads when compared to 90 W ($P < 0.001$).

No overall effects were observed in absolute blood lactate concentrations between treatments (**Figure 5**). Controlling for condition, workload significantly increased lactate concentration from rest at 210 and 240 W. A significant interaction existed at 240 W for the CO condition. To minimize day-to-day variations in resting lactate, we expressed lactate as a percent increase from rest **(Figure 6)**, and relative lactate increases during CO were significantly higher than PLA at 210 and 240 W (202 ± 146% versus 79 ± 27% at 210 W, and 370 ± 272% versus 187 ± 90% at 240 W).

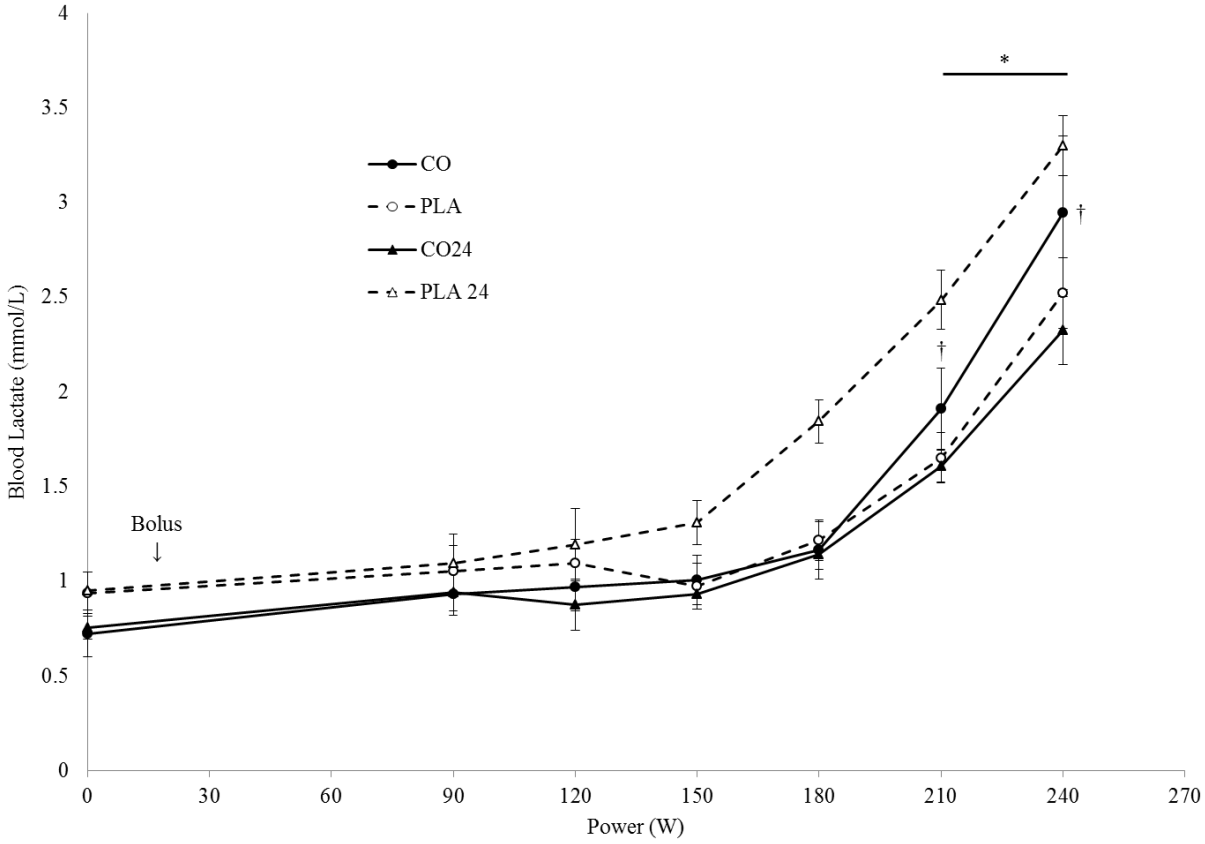

Figure 5. Mean blood lactate concentration for each workload during Acute (CO, PLA) and 24hr Post (CO24, PLA24) conditions. Values were significantly ($P < 0.05$) elevated from rest at 210 and 240 W. No overall differences were observed for blood lactate concentration between treatment groups, but CO was significantly different than PLA at 240 W ($P = 0.037$). Values represent group means for nine subjects, except at 210 W (n = 8). * Workload effect ($P < 0.05$). † Treatment effect ($P < 0.05$).

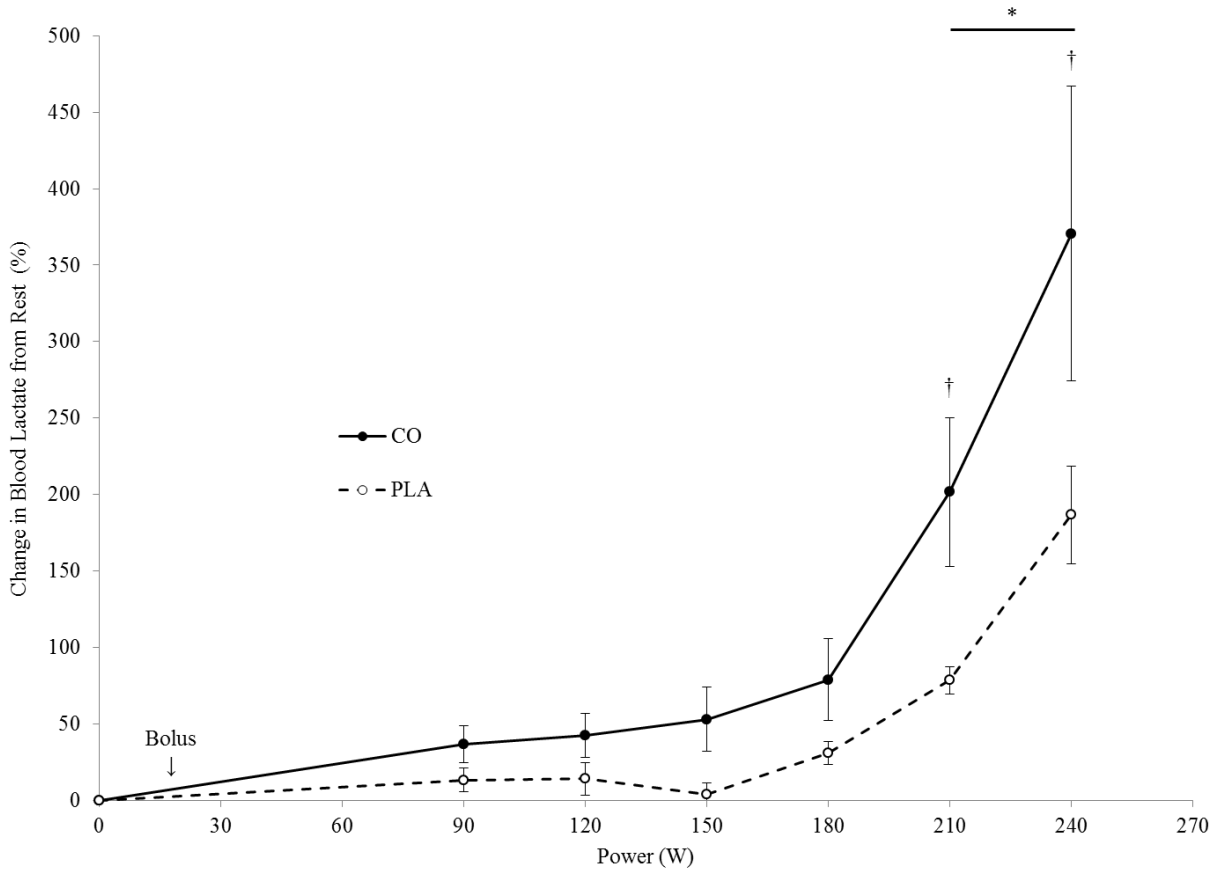

Figure 6. Mean relative changes in blood lactate concentration for each workload during Acute (CO, PLA) conditions. Relative blood lactate appears to inflect at a lower workload during CO compared to PLA. CO24 and PLA24 conditions are not significantly different from PLA ($P \geq 0.05$) and have been removed for clarity. Values represent group means for nine subjects, except at 210 W (n = 8). * Workload effect ($P < 0.05$). † Treatment effect ($P < 0.05$).

Chapter IV
Discussion

As expected, our CO inhalation method increased average HbCO by 4.2% before the exercise task. This value is similar to those recommended for the oCOR procedure (60). The graded cycling task used in this study significantly decreased HbCO (from 4.8 to 3.1%), and individual subject data revealed the half-time of HbCO during exercise to be 51.3 ± 23.5 min. Recently, other researchers have reported comparable reductions in HbCO half-time during exercise (60, 71), which is entirely consistent with the idea that increased ventilation hastens CO clearance.

A novel finding of this study was the elevation in resting HbCO% 1 day following administration of a low dose of CO. Previously reported half-times of HbCO cannot explain this increase. Classic data held that HbCO has a mean half-time of 320 min during rest in humans (44). However, more recent reports have shown significantly shorter values (132 min (60); 131 min (69)). Nevertheless, assuming half-time increased to 320 min immediately following completion of exercise, baseline HbCO levels should have been reached before the 24-hour follow-up session. In fact, if we use the elimination equation reported by Peterson and Stewart (44), resting HbCO would have been only 0.16% at CO24. This calculation assumes a mean half-time of 320 min and that the follow-up blood sample was collected roughly 23 hours following exercise completion. However, this estimation has limitations; it is not necessarily correct to assume a constant half-time for HbCO (25), and the calculation does not take into account changes in the endogenous production of CO. Our subjects also lived at moderate altitude (~1625 m), and the drive for oxygen to displace CO from hemoglobin is therefore reduced from sea level (Boulder PO_2 = 139 mmHg, compared to 159 mmHg at sea level). It should be noted that many of our subjects engaged in planned exercise following their CO session and also that,

to our knowledge, all were active commuters. It is probable that HbCO half-time outside of the lab for our subjects was considerably shorter than 320 min. The only explanation we can offer for the consistent increase in baseline HbCO% at CO24 is induction of heme oxygenase-1 (HO-1), one of the enzymes responsible for endogenous CO production. It is thought that changes in the supply of endogenous CO are due to this inducible protein (59). Increases in reactive oxygen species and soluble guanylyl cyclase activity augment HO-1 expression, and both of these are possible outcomes from CO exposure (reviewed in (59)). To support this idea, Rhodes et al. (53) demonstrated an increase in HO-1 expression after 5 days of low-dose CO exposure. If this occurred in our subjects, we do not know which proteins are being catabolized by HO-1 to yield higher endogenous CO levels. As no differences in our metabolic variables were seen during exercise at CO24, it is unlikely that the slight elevation in baseline HbCO% had an effect on the relative intensity of exercise. We are also unsure how long this elevation persisted, and whether continued increases in endogenous CO production have additional consequences. We recognize that these conclusions are outside the scope of this project, but their implications for athletes warrant further investigation.

In testing our primary and secondary hypotheses, this study concluded that 1) acute elevation of HbCO by 4.2% causes no changes in $\dot{V}O_2$ or EE during submaximal exercise, 2) exercising $\dot{V}O_2$ and EE remain unchanged 1 day following low-dose CO exposure, and 3) low-dose CO inhalation causes some exercise responses similar to acute exposure to moderate altitude. From our results, we therefore conclude that the only effect from this low-exposure protocol was a temporary decrease in $\dot{V}O_{2\max}$, reflecting an increase in the relative intensity of exercise while CO was onboard.

Although enhanced exercise economy has been documented in the NO literature, oxygen

uptake data from this study (**Tables 3a & 3b**) confirm the results from several other groups that there is no effect on submaximal $\dot{V}O_2$ following CO exposure (21, 30, 43, 55, 67, 68). It should be noted that many of these previous studies elevated HbCO (18–20%) far past what we achieved with our low dose, and thus could have masked any pharmacologic effects of CO by inducing mild toxicity. Additionally, some investigations used isolated limb exercise models, leaving unclear what the effects of activating larger muscle masses would be (21, 55). Our data support these previous findings that $\dot{V}O_2$ is unchanged in skeletal muscle during CO-induced hypoxic exercise and add that no significant changes occur within the next 24 hours. The disparity in oxygen uptake after nitrate supplementation and CO inhalation may lie in the lesser potency of CO compared to NO.

The absence of change in EE during submaximal exercise with CO onboard was expected and supports our primary hypothesis. It seems clear that $\dot{V}O_2$ remains unchanged compared to control data, and no studies have documented a shift in substrate selection during CO exposure. We conclude that the mitochondrial uncoupling caused by CO (29) is either insufficient to change whole-body energetics during exercise or does not occur with our single, low dose. If this effect were present, data should have opposed those from dietary nitrate supplementation, which show decreases in proton leak across the inner mitochondrial membrane (35). Although the possible shift in fiber type suggested by Rhodes et al. (53) is appealing to consider, our data indicate no significant alteration in submaximal exercise efficiency either during acute exposure or 1 day thereafter. We also assume that there was no substantial remodeling within skeletal muscle during the following week (on average), since there were no carryover effects on $\dot{V}O_2$ and EE in the four subjects who received CO during their first laboratory visit.

Although our graded cycling task did not continue to subjects' $\dot{V}O_{2\text{max}}$, we can estimate

the reduction in exercise capacity caused by our protocol. Using the relationship between HbCO and percent impairment in work capacity determined by Horvath et al. (28), our subject-specific reductions in $\dot{V}O_{2\max}$ likely ranged from 5.3–7.7%, with a mean of 6.1%. These calculations yield results that are similar to those found in other low-dose CO protocols (reviewed in (60)).

Absolute blood lactate data did not support our hypothesis, but there appeared to be an interaction based on resting (pre-dose) levels (see CO and PLA in **Figure 5**). We therefore expressed lactate as a relative change from baseline to control for day-to-day variability. Relative increases in blood lactate were higher at every workload, although these changes were only significant at 210 and 240 W. This result is consistent with effects seen during acute altitude exposure, and is related to the increase in relative work intensity caused by impaired work capacity (38). CO inhalation caused the mean lactate profile to inflect at an earlier absolute workload when compared to room-air inhalation (PLA). Others have shown no differences when blood lactate is standardized to relative work intensity (67), indicating that CO per se does not alter lactate production or disappearance.

Previous studies using an acute dose of CO have yielded mixed results for the HR response post-exposure, but data from the present study support our hypothesis that exercising HR would be highest during the CO condition. Compared to exercising controls, no changes in HR were reported by González-Alonso et al. (21) or Hanada et al. (24) even with HbCO levels raised to 18–20%. Vogel and Gleser (66) also saw no changes in HR during resting conditions, but differences appeared during cycling exercise. The exercise modes used in the previous two investigations (single-leg knee extension and hand-grip exercise, respectively) likely did not elevate HR high enough from rest to see any differences (their data range from 60 to 90 bpm). Similarly, although we found a significant main effect of 4.2 bpm during the CO condition, the

mean difference from PLA appears to trend upward with exercise intensity (2.9 bpm at 90 W vs. 5.7 bpm at 240 W). However, no significant interactions were found in our HR data. Impaired oxygen delivery per unit blood during exercise with CO onboard most likely caused an increase in cardiac output and hence muscle blood flow via elevated HR. Many other researchers have documented this same phenomenon following CO administration (26, 30, 43, 55, 66, 67), and the cause is apparently that the same absolute workload represents a larger relative percentage of one's $\dot{V}O_{2\max}$ while CO is onboard.

We reason that relative exercise intensity increased following acute CO inhalation, but we did not observe the expected shift in substrate utilization toward carbohydrate. True changes in RER during the CO condition may have been masked by the typical error of our metabolic cart. However, this is likely not the case since visual inspection of the data (**Table 3**) revealed no obvious trends according to treatment. The slight increase in relative exercise intensity with CO onboard must therefore not be great enough to yield a significant change in substrate utilization.

We did not observe the typical increase in $\dot{V}_E$ seen as an early response to altitude exposure, suggesting that separate mechanisms are responsible for pulmonary homeostasis during normobaric CO-induced hypoxia and the hypobaric hypoxia of altitude. Whereas hypobaric hypoxia reduces the partial pressure of arterial oxygen (PaO_2), CO inhalation during normoxia only reduces CaO_2. The peripheral chemoreceptors appear to be insensitive to this change and do not stimulate hyperventilation, as is expected during rest and submaximal exercise during acute altitude exposure. Hanada et al. (24) elevated subjects' HbCO to 20.3% and still found no change from controls in $\dot{V}_E$ during handgrip exercise, indicating that CO has no stimulatory effect on this system. The implications of this fact are worth considering for moderate to severe CO poisoning, since ventilation is the primary mode of CO elimination.

Administration of a low dose of CO caused a slight increase in individuals' perceived exertion during exercise after accounting for the significant effect of visit number. This main effect was independent of workload and indicates that perceived effort was significantly higher at low workloads, even when no significant differences were observed between treatments in blood lactate. HR was elevated at these low workloads during CO compared to PLA, alluding to a link between subjective effort and HR. This finding is not consistent with other data, which reported no differences in perceived effort (26), and the difference is probably due to the confounding effect of treatment order (visit number during CO exposure). This may have implications for laborers exposed to CO (62), especially for those with physically demanding occupations.

We cannot offer evidence regarding the effects of either repeated or prolonged exposure as this study administered only a single, low dose of CO, but data from Klausen et al. (30) suggest that prolonged CO exposure has a similar influence (or lack thereof) on submaximal exercise energetics. Similarly, it remains unknown whether repeated CO exposure could stimulate other physiological mechanisms and influence maximal exercise capacity. Several studies assessing the stability of Hb_{mass} in athletes have performed frequent measures over various time periods, and such a scenario could have separate physiological outcomes not covered in this study. In addition, many medical investigations have used extended CO gas exposure or CO-releasing molecules to increase *in vivo* CO levels for many hours and with repeated doses. Pilot data collected by researchers in our lab show that blood erythropoietin fluctuates alongside repeated increases in HbCO% (unpublished observations, 2012). A current project in our lab is investigating the effects of chronic low-dose CO inhalation; we expect repeated elevations of erythropoietin to improve selected markers of endurance performance.

The intervention in this study was acute—the initial mean value of 4.8% HbCO began decreasing immediately as a result of ventilation during the exercise task—and different data may result from a more chronic intervention.

In conclusion, the results of this study suggest that low-dose CO administration does not offer the acute benefits to submaximal exercise seen with dietary nitrate supplementation, and that no significant adaptations to change this are apparent the following day. The indication of elevated endogenous CO production during CO24 seems to have no effect on exercise energetics, but the implications and true mechanism for this phenomenon need to be explored in further studies. Our protocol targeted oxidative metabolism, and no studies have examined the role of CO in other energetic pathways such as the phosphagen system. Also, just as exercise improvements following nitrate supplementation are dependent on training status, CO may exert different effects in athletes of different calibers. Additional investigations are needed to determine the effects of low-dose CO inhalation in these other populations (e.g., elite athletes who may participate in Hb_{mass} procedures) and types of exercise (e.g., sprinting), as well as any outcomes from repeated HbCO elevation.

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Appendix A
Literature Review

Although high doses of carbon monoxide (CO) are considered poisonous, the utility of this gas in clinical, research, and therapeutic applications is gaining recognition. Low doses of CO have been used for more than 100 years to measure hematological parameters (as reviewed in (9)), with a special emphasis on the total circulating mass of hemoglobin (Hb_{mass}) around the turn of the last century. CO is routinely used to assess total blood volume, plasma volume, and Hb_{mass} due to its extreme affinity for hemoglobin, and in addition is used as a tool for assessing lung function. CO has also been documented to bind to other heme-containing proteins (59), and in the past couple of decades interest has shifted toward a potential therapeutic application in animals and humans (reviewed in (39) and (52)). Many physiological systems involved in exercise interact with CO, and this fact may have application in modifying the energetics of exercise. However, little research has examined whether low-dose CO administration induces acute or rapid changes to $\dot{V}O_2$ and energy expenditure in humans.

Interactions with Hemoglobin

CO is most recognized by its potentially lethal ability to bind hemoglobin, the oxygen-carrying protein within red blood cells. Gas exchange at the lungs normally results in dissolved carbon dioxide (CO_2) being traded for oxygen (O_2), which binds to a ferrous (Fe^{2+}) iron buried in the hemoglobin protein. Each hemoglobin tetramer houses four iron atoms, which are stabilized by porphyrin rings (termed heme groups when bound to iron) that effectively prevent rusting when O_2 is bound. Hemoglobin demonstrates a special ability known as cooperative binding: the phenomenon of one ligand increasing the affinity of subsequent ligand binding. This trait allows for hemoglobin's unique ability to reliably transport O_2 through the blood and offload it at active

tissues and is illustrated by sigmoidal oxyhemoglobin dissociation curve. CO, however, has an affinity approximately 240 times that of O_2 for heme groups in hemoglobin (59). When dissolved in the blood, this gas outcompetes oxygen for the heme binding sites, and at half-saturation (two binding sites occupied) causes a leftward shift in the dissociation curve (25, 55, 58). This prevention of O_2 offloading causes tissue hypoxia and is the reason CO exposure—even at normal atmospheric P_{O_2}—is lethal starting at carboxyhemoglobin (hemoglobin bound to CO; HbCO) levels around 50%. At sub-lethal doses, CO reduces the maximal rate of oxygen consumption ($\dot{V}O_{2max}$), limiting the amount of external work that can be accomplished (1, 53, 55, 60).

CO in Hb_{mass} Literature

In the early 1900s, Haldane and Smith developed a procedure that utilized CO inhalation to determine Hb_{mass} (23). Modifications to this procedure have occurred over the years, and a recent modification by Schmidt and Prommer (60), which facilitates CO administration and requires only capillary blood sampling, has increased the use of this technique in the scientific literature. The optimized CO rebreathing method (oCOR method) has become commonplace for measuring hematological responses to stimuli such as endurance training and altitude exposure. Briefly, subjects inhale a known amount of CO and rebreathe this gas along with O_2 for two minutes to ensure adequate uptake into the blood. Capillary blood samples are taken before this procedure as well as when venous and arterial HbCO concentrations have stabilized. The total volume of CO administered depends on subjects' training status and the altitude at which the test is conducted, but typically elevates HbCO by 3–8%. By making minor assumptions, this method reliably estimates Hb_{mass}. Although a meta-analysis by Gore et al. (19) documented the mean

typical error in laboratories currently using this method to be 2.2%, values much lower than this have recently been reported (14, 17, 18, 20, 51, 56, 68).

The low typical error associated with the oCOR method has given researchers the ability to confidently track Hb_{mass} under various circumstances. Wachsmuth et al. (68) recently investigated the hypothesis of increased Hb_{mass} during a classic altitude training program in elite swimmers. The CO administration protocol was conducted six times on average per participant in the 2-year data collection period, and found an average 7.2% increase in athletes' Hb_{mass} in response to training at 2320 m. Administering the oCOR test at various time points after altitude training camps also allowed them to determine of the time course of Hb_{mass} loss after returning to normoxic conditions. Another study which used the oCOR method also found significantly increased Hb_{mass} following intermittent altitude exposure (50). However, this group saw a negative trend in $\dot{V}O_{2max}$ upon return to sea level, fueling the debate on what governs O_2-carrying capacity. Frequent administration of the oCOR method is now common for recording the response of hematological variables over time.

The requirement for an accurate test for total hemoglobin mass lies in the unreliable nature of other blood parameters. Hemoglobin concentration ([Hb]) and hematocrit (Hct) have been the classic measures to monitor the stability of hemoglobin mass, but these measures are subject to multiple sources of error. For example, decreased [Hb] and Hct result from both plasma volume expansion and erythrolysis—two vastly different phenomena. Considering the variability in [Hb] and Hct measures, Garvican et al. (16) measured Hb_{mass} over the course of a 6-day ProTour cycling race and found little deviation in Hb_{mass} (± 1.9% of baseline, given a 1.3% typical error). The same group performed a similar study over the course of six months to document Hb_{mass}'s seasonal variation in cyclists (17). This repeated measures design showed an

overall stability in Hb_{mass} (± 3.3%), which is notable considering the deviations in training load and environmental conditions over the course of the racing season. [Hb] and Hct are subject to fluid shifts and variations in hydration status associated with stage races, and in these study designs would have masked the true nature of the Hb_{mass} parameter.

The ease and precision of Hb_{mass} measurements—as well as the variability in other blood parameters—support its potential as a tool to detect blood doping in sport. While tests exist to detect doping practices such as exogenous EPO administration and homologous blood transfusions, no test is currently accepted to discover autologous transfusions. Potential of the oCOR method as tool to detect doping was first voiced by Pottgiesser et al. (47), who detected significant increases in Hb_{mass} after reinfusion of just one bag of previously-withdrawn packed red blood cells. Other groups are similarly able to detect autologous transfusion, although after larger changes in Hb_{mass} (4). As this method detects variations in the total amount of one's own red blood cells, it is necessary to know what proportion of the changes in that value represents normal physiological adaptation. The reports mentioned earlier demonstrate that mean Hb_{mass} is a stable parameter, and deviations from that value are smallest when measurements are made often. However, we must acknowledge that the oCOR technique is not perfect, and cited fluctuations—1.9% in one week (16), 3.3% over the course of a competitive season (17), and 5.6% over the course of one year (51)—are predicated on a measurement subject to error (1.3%, 1.8%, and 0.8–3.1% typical error, respectively). Many authors have backed the oCOR method as a realistic method to detect autologous blood doping, whereas others have pointed to the wide variation in subject-specific Hb_{mass} that depends on training status, altitude exposure, and injury. Significant debate presently exists regarding the sensitivity and specificity of the oCOR method as a future anti-doping measure given biological and analytical oscillations in Hb_{mass} (a current

overview is provided in (65)). However, there is the potential that CO will soon enter the athletic arena, exposing a higher population of athletes to CO both in- and out-of-competition.

Half-Time of HbCO

Due to its high affinity for hemoproteins and limited modes of elimination, CO tends to remain *in vivo* for extended periods. The initial study which outlined the oCOR method documented a 132-minute half-time ($t_{½}$) of blood HbCO during resting conditions (60). Although other reports have found similar data (69), classic data held that $t_{½}$ averages 320 minutes in inactive individuals (44).

While a minute portion of CO is oxidized to carbon dioxide within mitochondria, the overwhelming majority of CO gas is removed from the circulation through ventilation (22). Hyperbaria and hyperoxia (either used singularly or in combination) are classic methods for increasing the rate of excretion during cases of CO toxicity, as the higher drive for O_2 binding displaces CO from hemoglobin. However, recent evidence suggests the possibility of a more practical intervention when these approaches are unavailable. Schmidt and Prommer (60) found HbCO $t_{½}$ after 25 minutes of heavy exercise in normoxic normobaria to be 40% shorter than in sedentary controls. Another study took a comparative approach and assessed CO clearance under normoxic conditions during various cycling workloads as well as during light exercise in hyperoxia (71). Data showed significantly reduced $t_{½}$ of HbCO in all conditions compared to rest, and concluded that even light exercise (40% of a subject's oxygen uptake reserve) was sufficient to enhance CO clearance. The mechanism underlying these results is simply an increase in ventilation. The absence of environmental CO drives its offloading from hemoglobin at the lungs; therefore, increased air across the lungs hastens its removal. However, these data

should be taken prudently, as hyperoxic conditions are still far more effective at clearing CO. Exercise interventions could be considered for mild CO poisoning when systemic tissue hypoxia is not a serious concern.

Effects of Exogenous CO Administration

Studies implementing multiple Hb_{mass} measurements raise the issue of "how much is too much?" In their revival of the CO rebreathing technique, Schmidt and Prommer (60) stated, "it must not be harmful or dangerous and should have minimal effects on physical performance and well being" (p. 486). These researchers defined a somewhat minimal decline in $\dot{V}O_{2max}$ (3.0%) following the oCOR method, and reasoned with their observed 132-minute $t_{½}$ that all effects should dissipate within 24 hours. While the 3% reduction in $\dot{V}O_{2max}$ seems somewhat conservative, it is reasonably similar to other reports of impaired oxygen uptake due to a ~5% elevation in blood HbCO (26, 31). Schmidt and Prommer (60) reckoned that HbCO would return to basal levels by 6–12 hours, suggesting that the oCOR method could be applied in athletic populations without concern for impaired performance the following day. As previously mentioned, the presumption of no alterations to performance was at the foundation of a study monitoring Hb_{mass} in cyclists for seven consecutive days (16). These studies, however, only consider immediate physiological changes, disregarding any possibility of long-term adaptation to the HbCO stimulus.

We must consider the possibility that CO has effects resulting from its ability to reduce $\dot{V}O_{2max}$. As noted by several researchers, HbCO-induced impaired $\dot{V}O_{2max}$ from CO inhalation is similar to arterial hypoxemia experienced at altitude (55, 60). The hypoxic stimulus of altitude enhances erythropoietin (EPO) release (37), which in turn increases erythrocyte precursor

survival and the rate of erythrocyte production (3). Under certain circumstances, EPO also extends the normal lifespan of red blood cells (54). Extended altitude exposure therefore theoretically increases overall O_2-carrying capacity upon return to sea level. One study inadvertently examined the long-term effect of intermittent HbCO elevations (14). Subjects performed the oCOR procedure 42 times within a 100-day period, and showed a stable Hb_{mass} throughout. However, this is in contrast to correlational data suggesting increased hemoglobin mass (measured as [Hb]) as a result of chronic HbCO elevation in smokers compared to nonsmokers (40). Other effects to consider from CO administration are the acute effects of hypoxic exposure. Altitude shifts substrate utilization toward carbohydrate (in men), increases heart rate, and magnifies the sympathetic nervous response during light and moderate workloads (38). This is in addition to other large, often sustained effects such as significant reductions in plasma volume (to concentrate O_2 content per unit blood) (46). The hypoxic stimulus of CO inhalation has some similarities to altitude exposure, such as impaired $\dot{V}O_{2max}$ and increased HR during submaximal exercise (26, 30, 43, 55, 66, 67), and may induce comparable adaptations if administered frequently.

Although CO clearly binds to heme groups in hemoglobin and affects O_2 binding in this protein, this is not the only protein with which CO is known to combine—nor is this its only downstream effect. As mentioned earlier, CO diffuses to tissues outside the blood to occupy heme groups in other proteins. CO unquestionably enters skeletal muscle; Prommer and Schmidt (49) estimated 0.32% of inhaled CO to leak to myoglobin in skeletal muscle per minute during the oCOR method. While a simple correction factor accounts for this loss when calculating Hb_{mass}, it does consider the effect that intramuscular CO may exert. Suggestive of an ergogenic effect, three days of low-dose CO were shown to independently elevate transcription factors

responsible for mitochondrial biogenesis in human skeletal muscle (53). Although their protein expression data implied muscular adaptation to low-dose CO administration, the researchers failed to document any significant whole-body performance benefits in retest conditions. This report illustrates that CO has the potential to elicit physiological changes, but indicates that these modifications may be too slight or temporary to yield observable effects on exercise performance.

Endogenous CO

It has been known for more than four decades that CO has an intimate tie with mammalian metabolism. First described by Tenhunen et al. (64), heme groups are catabolized by the microsomal heme oxygenase (HO) enzyme, which produces biliverdin and bilirubin as waste products. While the researchers proved the enzymatic nature of HO through its inhibition by CO in the presence of oxygen, they also derived a mechanism in which carbon monoxide is *produced* by the enzyme itself. This is one reason that baseline HbCO% in humans is typically reported within 0.2–2.8% (9, 25, 26, 30, 32, 36, 44, 45, 53, 60, 62, 71). Data by the Tenhunen group can be taken to show a classic negative feedback loop; higher HO activity will cause CO accumulation and therefore self-inhibition. Previous evidence also gave evidence that endogenous CO production is a dynamic system; certain disease states, such as hemolytic anemia, can cause high resting values of HbCO due to a rise in heme catabolism (61).

CO in Pharmacology

The explosion of recent research on CO was spurred after the 1980s discovery that a separate gaseous molecule had potent vasodilating properties. In truth, the effects of this

endothelial cell-derived relaxing factor (EDRF) were known before it was recognized to be nitric oxide (NO) (a brief history is provided by (59)). This finding nonetheless renewed curiosity in any potential effects due to CO other than the tissue hypoxia induced from high doses. CO is chemically and biologically similar to NO, as both bind heme-containing proteins with high affinity. Furthermore, in the past few decades, the proteins known to bind CO have been associated with oxidative stress, hypoxia, and apoptotic pathways (reviewed in (39, 52, 59). The similarity in downstream results from CO and NO administration is bolstered by the fact that CO even activates nitric oxide's synthetic enzyme (NOS). However, dose must be considered as the two gases are competitors for the same binding sites. For example, both CO and NO can independently relax the arterial vasculature, but NO is roughly 1,000 times more potent. Therefore, high concentrations of CO actually inhibit NO-dependent vasodilation by occupying binding sites in the endothelium. Competition between the two gases depends on the system in question though, because CO displays higher selectivity than NO; the latter is capable of binding to both ferrous (Fe^{2+}) and ferric (Fe^{3+}) hemoproteins, whereas CO only binds Fe^{2+} centers (57, 59).

The physiological role of CO is actively being explored in human clinical trials (39). The focus of these investigations includes a range of outcomes, including improved function in the cardiovascular, pulmonary, and visceral organ systems. Another avenue of current research in CO therapy is investigating the potential of various CO-releasing molecules (CORMs, reviewed in (39)), drugs which are able to maintain tissue O_2 delivery while allowing CO to interact with cellular mechanisms outside of the blood. Briefly, these preparations show the same modulation of pro- and anti-inflammatory genes and redox, vasoactive, and cytoprotective processes as exogenous CO gas, although some are more effective *in vivo* than others. CORMs are still under

preclinical development, whereas some clinical trials using CO gas have already been completed.

While most interest is in its medical potential as a disease intervention, a gap remains in the literature regarding the effects of CO during exercise. To be sure, insight has been offered indicating limitations during maximal exercise (1, 53, 60). However, data regarding submaximal exercise is weak and incomplete. Indirect evidence indicates elevated skeletal muscle mitochondrial biogenesis in humans following CO administration (53), and this observation is consistent with effects seen in other tissues (52). These reports point to potential changes in oxygen consumption as a result of CO-induced shifts in muscle fiber and mitochondrial metabolism. The hole concerning submaximal exercise is especially apparent considering the recent literature on nitrate supplementation and endurance exercise.

Dietary nitrates (popularly obtained from beetroot juice) enhance economy during submaximal exercise. Economy, expressed as the ratio of oxygen uptake to power output (L $O_2 \cdot W^{-1} \cdot min^{-1}$), is one contributor to exercise performance, and has traditionally been viewed as a stable parameter per athlete. The oral microbiota are thought to catalyze reduction of nitrate to nitrite, which systemic enzymes can convert to circulating NO (27). Dietary nitrate supplementation has been shown to increase blood flow to exercising skeletal muscle, which is most likely due to NO-induced vasodilation (5). Other reports point to increased skeletal muscle efficiency (6, 35), as well as enhanced endurance exercise performance (33). Economy during light and severe exercise following nitrate supplementation improves by 13.7% and 25.2%, respectively (6). Acute hypoxia also triggers vasodilation in skeletal muscle, and similar increases in blood flow have been documented in skeletal muscle following CO administration (55). Considering the similar biological activities of NO and CO, the lack of research concerning sustained exercise after CO exposure should not be overlooked.

Conclusions

Low-dose CO administration is becoming more commonplace as a scientific intervention. Regarded by most people as a toxic gas, CO was largely ignored for the better part of a century after its initial discovery. However, insights into NO signaling pathways, realization of CO's inherent tie to mammalian physiology, and optimization of the CO-rebreathing procedure have brought CO back to the scientific forefront. Research on CO's therapeutic potential is widespread, ranging from ischemia/reperfusion injuries to rheumatoid arthritis, and synthetic molecules which release targeted CO are being actively pursued. The long half-time of HbCO under normal conditions offers a sustained stimulus from CO signaling, but the exhaustive list of this gas's downstream effects has yet to be determined. Bearing in mind the increased prevalence with which CO is administered in the athletic performance literature, it is imperative to fully evaluate any beneficial or detrimental side-effects.