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CO and NO pulmonary diffusing capacity during pregnancy: Safety and diagnostic potential

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Abstract

This paper reviews the scientific evidence for the safety of carbon monoxide (CO) and nitric oxide (NO) inhalation to measure pulmonary diffusing capacity (DL_{CO} and DL_{NO}) in pregnant women and their fetuses. In eight earlier studies, 650 pregnant women had DL_{CO} measurements performed at various times during pregnancy, with a minimum of two to four tests per session. Both pregnant subjects that were healthy and those with medical complications were tested. No study reported adverse maternal, fetal, or neonatal outcomes from the CO inhalation in association with measuring DL_{CO}. Eleven pregnant women, chiefly with pulmonary hypertension, and 1105 pre-term neonates, mostly with respiratory failure, were administered various dosages of NO (5–80 ppm for 4 weeks continuously in pregnant women, and 1–20 ppm for 15 min to 3 weeks for the neonates). NO treatment was found to be an effective therapy for pregnant women with pulmonary hypertension. In neonates with respiratory failure and pulmonary hypertension, NO therapy improved oxygenation and survival and has been associated with only minor, transient adverse effects. In conclusion, maternal carboxyhemoglobin ($[Hb_{CO}]$) levels can safely increase to 5% per testing session when the dose-exposure limit is 0.3% CO inhalation for ≤3 min, and for NO, 80 ppm for ≤ 3 min. The risk of late fetal or neonatal death from increased Hb_{CO} from diffusion testing is considerably less than the risk of death from all causes reported by the Centers for Disease Control, and is therefore considered "minimal risk".

Keywords

Carbon monoxide; Nitric oxide; Guidelines; Recommendations; Pregnancy

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Conflicts of interest

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1. Introduction

The 2005 guidelines for standardizing pulmonary function tests have been jointly updated, approved, and published by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) (Macintyre et al., 2005; Miller et al., 2005a,b; Pellegrino et al., 2005; Wanger et al., 2005). These guidelines reflect the current knowledge in the field, and establish measures of safe clinical practice in quantifying pulmonary function. According to the ATS/ERS guidelines, only a few circumstances exist in which lung function testing is contraindicated (Miller et al., 2005b). For lung function testing that includes measurements of pulmonary diffusing capacity, there are only four contraindications: (1) chest or abdominal pain, (2) oral or facial pain, (3) stress incontinence, (4) and dementia. Pregnancy is not considered a contraindication for the measurement of pulmonary diffusing capacity (Miller et al., 2005b).

Despite the ATS/ERS guidelines, the question of whether measurement of pulmonary diffusing capacity in pregnant women is “safe” and “warranted” for themselves and their fetuses has not been studied adequately. Concern with performing diffusing capacity measurements during pregnancy stems from carbon monoxide (CO) exposure, resulting in increases in carboxyhemoglobin (Hb_{CO}) in the blood of the mother and fetus with each test. Accumulation of Hb_{CO} in excess can diminish the oxygen-carrying capacity of maternal and fetal blood to unsafe levels.

Nonetheless, determination of pulmonary diffusing capacity can be an excellent prognostic indicator for maternal and fetal outcomes, fitness, and mortality. Since impaired lung function is associated with an increase and recurrence of cardiovascular disease (Coburn et al., 1963), measuring pulmonary function in a pregnant woman may be a valuable prognostic indicator of adverse maternal (e.g. preeclampsia, gestational diabetes, cesarean section) and fetal outcomes (e.g. large for gestational age, small for gestational age infants). In fact, a forced expiratory volume in 1 s that is <80% of predicted in women who are pregnant is related to higher incidence of pre-term births (<37 weeks gestation), a higher prevalence of gestational hypertension, and a higher risk for low birth weight babies (<2500 g) (Getahun et al., 2006, 2007; Schatz et al., 2006). Most recently, a low maternal hemoglobin concentration (<10 g/dl) has been related to a higher risk for stillbirths, pre-term births, and small for gestational age babies (Gonzales et al., 2009). Because hemoglobin concentration is a determinant of pulmonary diffusing capacity, measurement of pulmonary diffusion may also relate to maternal and fetal outcomes, a possibility that has not yet been tested. Pulmonary diffusing capacity for carbon monoxide (DL_{CO}) and nitric oxide (DL_{NO}) at rest is also related to aerobic capacity (Zavorsky et al., 2009), a strong independent predictor of death in women (Gulati et al., 2003) and men (Myers et al., 2002). Thus, a measurement of pulmonary diffusion could be a prognostic marker for mortality in pregnancy. Low pulmonary diffusion in a pregnant woman may prompt a physician to recommend regular aerobic exercise to improve her fitness. Finally, DL_{CO} and DL_{NO} are sensitive indicators of the morphological changes assessed with computed tomography to detect emphysema and cystic fibrosis (Dressel et al., 2009; van der Lee et al., 2009).

What is the clinical significance of measuring alveolar membrane diffusing capacity (DM) in the pregnant female “diseased” lung? Well, the global measurement of DL_{CO} only provides a global indication of whether gas exchange is normal or not. It does not specify where the abnormal physiology lies, whether the issue is low (or high) hemoglobin concentration, or low (or high) pulmonary capillary blood volume, or whether the problem lies solely within the alveolar-capillary membrane. A measurement of both DL_{CO} and DL_{NO} together allows partitioning to obtain pulmonary capillary blood volume (Vc) and DM (DM is essentially DL_{NO}) so that there is a more precise determination of the location of the

pathophysiology. Pregnant women who have a high DM/Vc ratio (which is equal to the DL_{NO}/DL_{CO} ratio) should be evaluated for high pulmonary artery pressure compatible with pulmonary hypertension (Bonay et al., 2004; van der Lee et al., 2006). Therefore, a disproportionate reduction in DM relative to Vc would decrease the DM/Vc ratio (and thus decrease the DL_{NO}/DL_{CO} ratio), which is related to a wide spectrum of pulmonary vascular diseases (Oppenheimer et al., 2006) and could apply to pregnant women. Diabetes, which can cause pulmonary microangiopathy, lowers DL_{NO}/DL_{CO} compared to controls (Chance et al., 2008). Thus, a DL_{NO} and DL_{CO} measurement could be a prognostic indicator for gestational diabetes.

Therefore, future direction of the measurements of DL_{CO} and DL_{NO} in pregnancy has promise as a screening tool for predicting aerobic capacity (as a determinant of mortality), and to help identify pulmonary hypertension, pulmonary vascular diseases, and gestational diabetes (including type II and possibly type I diabetes). With the goals of advancing knowledge of alveolar gas exchange during pregnancy and ensuring safe testing of lung function, the guidelines proposed herein should help to facilitate the use of pulmonary diffusing capacity measurement in the pregnant woman.

2. Carbon monoxide

Pulmonary diffusing capacity for carbon monoxide is a standard function test that measures alveolar-capillary diffusion. Because the measurement of oxygen transfer through the lung is technically difficult, and may be limited by blood flow and pulmonary tissue O_2 consumption, carbon monoxide (CO) has been used as an indirect index of oxygen transfer, due to its high affinity to bonding with hemoglobin (Forster, 1957).

The standard DL_{CO} protocol is to use the single-breath method. Following a few normal breaths, a subject first exhales to residual volume, and then inhales a standard concentration of gases (0.3% CO, 10% Helium, 21% O_2, balance N_2) to total lung capacity. As total lung capacity and vital capacity are minimally affected regardless of the stage of pregnancy (Alaily and Carrol, 1978; Baldwin et al., 1977; McAuliffe et al., 2002; Milne, 1979) and regardless of obesity (Eng et al., 1975), inhalation to total lung capacity is possible during all three trimesters. After a 10-s breath-hold, the patient (subject) exhales in a smooth, unforced manner, without hesitation or interruption all the way to residual volume. The first 0.75–1.0 L of expired air is discarded, and only the second liter of air (which reflects alveolar gas) is analyzed. The remainder of the expired air is discarded as well. DL_{CO} is calculated as the total CO uptake over time divided by the alveolar partial pressure of CO (Macintyre et al., 2005).

DL_{CO} can also be measured by rebreathing a standard concentration of gases (0.3% CO, 10% Helium, 35% O_2, balance N_2), with a bag volume that ranges from tidal volume (Snyder et al., 2006) to 60% of vital capacity (or 500 ml to 3.5 L) (Takahashi et al., 1995). Gases are inhaled from a closed circuit anesthesia bag, and rebreathed for 15 s at a frequency of about 30 breaths/min. The calculation of DL_{CO} using the rebreathing technique is essentially the same as that with breath-holding. This method for DL_{CO} is less commonly used, as it requires more expensive equipment, such as a respiratory mass spectrometer, and more complex calculations. However, because with rebreathing the CO gas is distributed in the lung more evenly, an advantage of this method, as opposed to the single-breath, is its relative insensitivity to unequal distribution of ventilation (Jansons et al., 1998; Roberts et al., 1990) and diffusion (Jansons et al., 1998; Kreukniet, 1970). Furthermore, rebreathing is preferred when subjects cannot hold their breath for long periods or, if for some reason, vital capacity is too small.

The primary safety concern with inhaling CO is the increase in fetal and maternal carboxyhemoglobin concentration ([Hb_{CO}]) resulting in diminished blood oxygen-carrying capacity. Carbon monoxide is a naturally occurring gas that is produced endogenously by catabolism of hemoglobin and other heme pigments, chiefly in the liver and spleen. Endogenous CO production is approximately 0.001–0.007 ml/min (Coburn et al., 1963, 1965). In a non-smoking individual this results in a normal carboxy-hemoglobin level of about 0.7–1.1% (Coburn et al., 1965), which increases in pregnancy (Delivoria-Papadopoulos et al., 1974). In non-smoking pregnant mothers, maternal [Hb_{CO}] is reported to be 1.1 ± 0.2% (Longo, 1976), but the range is wide, at 0.4–2.6% (Longo, 1977). Fetal [Hb_{CO}] is about 1.8 ± 0.3% (Longo, 1976), with a range of 0.4–2.8% (Longo, 1977).

Tests of pulmonary diffusing capacity increase [Hb_{CO}] by approximately 0.7–0.8% per 10-s breath-hold maneuver (Forster et al., 1954; Frey et al., 1987). The standard mixture of gases in a medical grade gas tank for testing is 0.3% CO, 10% He, 21% O_2, with a balance of N_2. Therefore, whether the diffusing capacity measurement involves single breath-hold or rebreathing maneuvers, the increase in [Hb_{CO}] is about 0.7% per test. The ATS/ERS guidelines suggest that no more than five measurements of diffusing capacity tests be performed in one testing session for all subjects, including pregnant women (Macintyre et al., 2005). The limit of five diffusing capacity tests is not related to safety concerns with [Hb_{CO}] accumulation, but rather because each maneuver increases [Hb_{CO}] by about 0.7% and may reduce the DL_{CO} measured in subsequent tests due to a back pressure of CO in lung capillaries. Five diffusing capacity tests in a session reduces DL_{CO} by 1.5 ml/min/Torr, which equates to a reduction of about 5% from the first to the fifth determination (Zavorsky and Murias, 2006). These guidelines are based on DL_{CO} measurement at rest, however. With exercise, additional testing may be warranted to match the various levels of oxygen consumption to DL_{CO}. In that manner, the slope of the relation between oxygen consumption and DL_{CO} would provide an indication of pulmonary microvascular regulation.

Table 1 presents the eight published studies in which DL_{CO} measurements were performed in pregnant women using the standard inspiratory CO concentration of 0.3% CO per test (Boggess et al., 1995; Garcia-Rio et al., 1996; Gazioglu et al., 1970; Lalli and Raju, 1981; Lehmann, 1975; McAuliffe et al., 2003, 2002; Milne et al., 1977; Norregaard et al., 1989). These tests were performed at rest, and some of these women had lung and/or cardiopulmonary disease (Boggess et al., 1995; Gazioglu et al., 1970; Lalli and Raju, 1981). In each study, two to four diffusion tests were performed per session. DL_{CO} decreased by 10–15% in the second and third trimester in both singleton and twin pregnancies compared to the first trimester and post-partum (Gazioglu et al., 1970; McAuliffe et al., 2002; Norregaard et al., 1989). Greater resistance to diffusion through the alveolar membrane (DM) and not a decrease in pulmonary capillary blood volume (Vc) accounted for the decrease in DL_{CO} (Gazioglu et al., 1970). In pregnant women with emphysema, DL_{CO} increases throughout pregnancy, with increased Vc being the likely cause. In pregnant women with pulmonary sarcoidosis, DL_{CO}, alveolar membrane diffusing capacity for CO (DM_{CO}), and Vc remain unaltered throughout pregnancy (Gazioglu et al., 1970). In the testing of 650 pregnant women and their fetuses no adverse events have been reported (Table 1).

The current Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for carbon monoxide for all workers, including pregnant women, is 50 parts per million (ppm) (55 mg/m^3) with an 8-h time-weighted average (TWA) concentration (28,800 s) (National Institute for Occupational Safety and Health, 2005). Thus, data presented in Table 1 demonstrate that CO inhalation at concentrations recommended by the ATS/ERS for DL_{CO} testing is within acceptable limits of the U.S. government regulations and has been demonstrated to be safe in a number of published reports.

Overall pulmonary CO diffusion can be partitioned into the subcomponents of alveolar DM and pulmonary capillary blood volume. The equation for DL_{CO} has been described by Roughton and Forster (1957):

$$\frac{1}{DL_{CO}} = \frac{1}{DM_{CO}} + \frac{1}{\Theta_{CO} \cdot Vc}$$

where DM_{CO} is the alveolar membrane diffusing capacity for carbon monoxide, Θ_{CO} is the specific blood transfer conductance for CO, and Vc is the pulmonary capillary blood volume (Roughton and Forster, 1957). To obtain DM_{CO} and Vc, DL_{CO} has been traditionally measured at two different levels of alveolar P_{O_2} (P_{AO_2}), e.g., at about 100–120 mmHg and about 600 mmHg. For each alveolar P_{O_2} level, $1/DL_{CO}$ is plotted on the *y*-axis and $1/\Theta_{CO}$ is plotted on the *x*-axis. A line is drawn through the two points and the *y*-intercept ($1/DM_{CO}$) and slope (1/Vc) can be solved.

3. Nitric oxide

During the past 15 years, the measurement of diffusion capacity of the lung using the transfer gases CO and nitric oxide (NO) together has been developed to obtain DM_{CO} and Vc in a single-breath maneuver. The advantage of adding NO is that its rate of combination with hemoglobin is many-fold faster than that for CO (Meyer and Piiper, 1989), and the specific blood transfer conductance for NO (Θ_{NO}) is so great that the red cell resistance to NO ($1/\Theta_{NO}$) approaches zero (Johnson et al., 1996; Manier et al., 1993, 1991; Phansalkar et al., 2004; Tamhane et al., 2001; Zavorsky and Lands, 2005; Zavorsky et al., 2004). Therefore, DL_{NO} equals membrane diffusion capacity for NO (DM_{NO}) and is independent of either pulmonary capillary blood volume or hemoglobin concentration (van der Lee et al., 2005). The ratio of DL_{NO} to DM_{CO} is about 2.4 (Phansalkar et al., 2004; Tamhane et al., 2001); therefore, $DM_{CO} = DL_{NO}/2.4$. Because exposures of ≤60 ppm NO of a few seconds to a few minutes do not interfere with various cardiopulmonary parameters or DL_{CO} in adults (Brett et al., 1998; Phansalkar et al., 2004; Sheel et al., 2001; Tamhane et al., 2001; Zavorsky and Murias, 2006), it is useful to use NO along with CO to assess alveolar-membrane function and Vc.

The ability to estimate DM_{CO} and pulmonary capillary blood volume from the one-step simultaneous measurement of DL_{NO} and DL_{CO} has at least four advantages over the traditional two-step method. (1) With the standard method, cardiac output may vary between measurements of DL_{CO} at different oxygen tensions, which then have to be interpolated to obtain DL_{CO} at the two O_2 tensions at the same cardiac output (Phansalkar et al., 2004). With the DL_{NO}–DL_{CO} method, all measurements are obtained at the same cardiac output and O_2 tension, thus no interpolation is necessary. (2) With the traditional method, the CO gas distribution in the lungs may be different at two different oxygen tensions, but with the DL_{NO}–DL_{CO} method only one inspiration is required which results in a similar distribution of NO and CO throughout the lung. (3) With the traditional two-step method, there is systematic underestimation of Vc and an overestimation of DM_{CO}, as the inspiration at two different oxygen tensions affects alveolar-capillary membrane diffusion (Hsia et al., 1995). The DL_{NO}–DL_{CO} method avoids this error and improves the accuracy of estimated DM_{CO} and Vc. (4) The DL_{NO}–DL_{CO} method reduces the testing time and number of measurements by half, allowing for easier data collection and half the CO exposure. This is a significant advantage for investigation as well as patient care.

Like CO, NO is produced by the body and can be measured in exhaled breath. The amount of exhaled NO per breath is about 2–22 parts per billion in women (Olivieri et al., 2006). As

with CO, safety is a concern when using NO in pregnant women. Nitric oxide reacts at a nearly diffusion-limited rate with oxyhemoglobin to produce methemoglobin, and reacts with deoxyhemoglobin to produce iron nitrosyl hemoglobin (Hb_{NO}). Because neither methemoglobin nor Hb_{NO} can bind oxygen, accumulation of either species can become a safety concern if it occurs to an extent that it impairs significantly the oxygen-carrying capacity of the blood. Small accumulations of methemoglobin and Hb_{NO} are of minimal safety concern, because methemoglobin (Fe^{3+}) is reduced to ferrous hemoglobin (Fe^{2+}) by endogenous methemoglobin reductase enzymes, with a methemoglobin half-life of 2–4 h (Blood and Power, 2007), and Hb_{NO} is eliminated with a half-life of 15–45 min (Takahashi et al., 1998). Due to the rapid reaction of NO with pulmonary capillary hemoglobin, no free NO reaches the fetus. In fact, the half-life of NO in blood is only milliseconds (Eich et al., 1996) and any free NO diffusing into pulmonary capillary blood is metabolized before reaching the systemic circulation.

In the presence of oxygen, NO converts to nitrogen dioxide (NO_2), a potent oxidant that can result in pulmonary edema and lung injury. However, with the addition of 60 ppm NO in the presence of 35% oxygen in an anesthesia bag for 30 s, only about 1 ppm of NO_2 will be produced (Fine, 1972; Sokol et al., 1999). Therefore, NO_2 production is negligible provided care is taken to prevent the mixture of NO with air until immediately prior to inhalation.

4. Evaluating the risk of CO and NO inhalation

In several research studies, [Hb_{CO}] has been increased experimentally, providing information regarding the toxicity of inhaled CO. One study increased [Hb_{CO}] in ten healthy subjects to 15% over a period of 30 min to test a new monitoring device (Barker et al., 2006). No adverse outcomes were reported. Despite a reduction in aerobic capacity ($\dot{V}_{O_2}$ peak), which decreased by 6–9% when [Hb_{CO}] was 4–7% (Ekblom and Huot, 1972; Horvath et al., 1975; Raven et al., 1974), and decreased by 15–24% when [Hb_{CO}] was 15–20% (Vogel and Gleser, 1972; Vogel et al., 1972), no adverse outcomes were reported, and [Hb_{CO}] returned to normal within 24 h.

A reduction in arterial oxyhemoglobin concentration [Hb_{O_2}] (as from CO exposure) with reduction of arterial oxygen content induces systemic compensatory mechanisms. These include an increase in the fraction of oxygen extracted by the tissues, and increased blood flow, so that oxygen consumption is maintained (Vogel and Gleser, 1972). During moderate exercise, cardiac output increases (Pirnay et al., 1971; Vogel and Gleser, 1972; Vogel et al., 1972), while pH, arterial P_{CO_2} and P_{O_2}, the alveolar-to-arterial P_{O_2} difference and the respiratory exchange ratio are not affected, even when [Hb_{CO}] equals 18–20% (Brody and Coburn, 1970; Vogel and Gleser, 1972). Thus, appropriate compensatory mechanisms are in place when [Hb_{CO}] is increased, as high as up to levels of 20% [Hb_{CO}].

When arterial P_{O_2} is normal (95–100 Torr), as in breathing room air, the increase in [Hb_{CO}] changes the shape of the oxyhemoglobin dissociation curve similarly as if there were only a reduction in hemoglobin concentration (Brody and Coburn, 1969). For example, a [Hb_{CO}] of 10% reduces the arterial oxygen content by the same amount as a reduction in hemoglobin concentration by 1.5 g/dl (Brody and Coburn, 1969). Therefore, a female with a hemoglobin concentration of 10.5 g/dl and 98% arterial oxyhemoglobin saturation [Hb_{O_2}] would have the same arterial oxygen content as a female with a hemoglobin concentration of 12 g/dl and an arterial [Hb_{O_2}] of 88% (Brody and Coburn, 1969).

The use of inhaled NO gas has been routine in the treatment of pulmonary hypertension in both newborn and adult patients for more than 10 years, providing useful information in evaluating the safety of NO for diffusion testing in pregnant women. As detailed in Table 2, a number of reports have described the use of inhaled NO in pregnant women suffering from

pulmonary hypertension (Bonnin et al., 2005; Decoene et al., 2001; Goodwin et al., 1999; Lam et al., 2001; Lust et al., 1999; McMillan et al., 2002; Robinson et al., 1999). In these reports, NO was administered at delivery rates of 5–80 ppm for minutes to hours, with no reported elevations of maternal methemoglobin levels. In term and pre-term infants, inhaled NO has been given at concentrations as high as 20 ppm and for up to a week at a time (Table 3). The administration of 1–20 ppm NO to infants for days at a time was well-tolerated with the only noticeable effect being increased concentrations of methemoglobin. Even so, the methemoglobin concentrations were not high enough to warrant discontinuation of inhaled NO treatment. The production of methemoglobin is a result of the direct reaction between NO and oxyhemoglobin in the lungs. Because NO in the blood is metabolized too rapidly for it to travel from the maternal lung to the fetal circulation, there should be no concern that inhaling NO will result in fetal methemoglobinemia. Large multi-center studies with thousands of enrolled patients have demonstrated no adverse effects, including methemoglobinemia, of inhaled NO administered for days or even weeks at 20 ppm (Kinsella and Abman, 2007). In fact, studies demonstrate that inhaled NO in pre-term infants decreases the risk of cognitive impairment and abnormal neurodevelopmental outcomes (i.e. cerebral palsy, bilateral blindness, bilateral hearing loss, or development delay) 2 years later by about half, compared to a placebo group (Mestan et al., 2005). Other studies have revealed no adverse NO attributable neurodevelopmental outcomes by 2 years of age (Hintz et al., 2007; Konduri et al., 2007) in NO-exposed infants.

To adequately assess the risks of CO and NO inhalation, it is useful to draw comparisons with exposure to these gases as a result of smoking tobacco. Each cigarette produces about 4% CO by volume (40,000 ppm), resulting in an average alveolar CO concentration of about 450 ppm (Osborne et al., 1956). The NO concentration in cigarette smoke is as much as 500 ppm (Eiserich et al., 1994; Pryor and Stone, 1993). Therefore, cigarette smoke contains many-fold higher concentrations of CO and NO than employed in a diffusing capacity test. Each cigarette smoked may increase the [Hb_{CO}] by about 1% (Russell et al., 1973a,b). One pack per day smokers have an average [Hb_{CO}] of 5–6% (Hampson and Scott, 2006; Light et al., 2007; Reddy et al., 2007; Russell et al., 1973a), and for some 10% of smokers [Hb_{CO}] are ≥7.5% (Radford and Drizd, 1982). For those that regularly smoke about 20 cigarettes during the course of a day, mean [Hb_{CO}] levels are 8.2%, with some subjects as high as 14.2% and a day-to-day variation of 0.94% (Smith et al., 1998). Therefore, smokers have high levels of [Hb_{CO}] that persist day-to-day.

Maternal Hb_{CO} levels are 4–5% in pregnant mothers who smoke regularly (Cole et al., 1972; Davies et al., 1979) and [Hb_{CO}] is linearly related to the number of cigarettes smoked per day (Cole et al., 1972). It is not possible, however to estimate fetal [Hb_{CO}] on the basis of a single maternal blood sample without knowledge of exposure pattern. At steady state, fetal [Hb_{CO}] is higher than maternal due to a greater affinity of fetal hemoglobin for the CO and because average fetal oxygen saturations are less than maternal (Longo, 1977). The elimination half-life of CO from the fetal circulation is longer than from maternal circulation (Hill et al., 1977; Longo and Hill, 1977). When maternal and fetal [Hb_{CO}] levels are equilibrated, the mean fetal to maternal ratio for Hb_{CO} is 1.84 giving the relation: [Fetal [Hb_{CO}]% = 1.27 × maternal [Hb_{CO}]% + 0.76] (Cole et al., 1972). Fig. 1 shows the predicted maternal and fetal Hb_{CO} levels equivalent to smoking 1–1.5 packs of cigarettes per day, followed by an 8-h sleep period. Note that peak fetal Hb_{CO} levels are higher than maternal Hb_{CO}, and that, fetal Hb_{CO} lags substantially behind maternal Hb_{CO} levels until 12 h into the day (Longo, 1977). Mathematical models predict a 4-h exposure to 300 ppm CO will produce peak maternal Hb_{CO} levels of about 25% after 4 h, while fetal [Hb_{CO}] will be only 12% (Longo, 1977) (Fig. 2). Thereafter maternal [Hb_{CO}] is predicted to fall, while fetal [Hb_{CO}] peaks at 15% (Longo, 1977) (Fig. 2). From this background it is concluded that a diffusing capacity test that lasts 10–15 s per test (with exposure to 3000 ppm CO) would not

appreciably increase fetal [Hb_{CO}] levels, and maternal levels of [Hb_{CO}] would increase by about 0.7% per test (Forster et al., 1954; Frey et al., 1987). Ten tests (100–150 s of total CO exposure) would increase maternal [Hb_{CO}] to about 7%. Even with more prolonged maternal exposures lasting up to 2 min fetal [Hb_{CO}] is anticipated to increase only slightly from pre-exposure levels reaching a peak about 2 h after testing. It is concluded that routine diffusing capacity testing at rest and during exercise would not increase fetal [Hb_{CO}] appreciably or to a level that would adversely affect fetal physiology.

Given the NO and CO content in cigarettes, it may be useful to extrapolate available data regarding the effects of smoking on pregnancy outcomes to evaluate the risks of inhaling NO and CO gas for lung diffusing capacity testing in pregnancy. Pregnant smokers have a late fetal plus neonatal death rate that is higher by 10 per 1000 births (to a 4.2% absolute death rate) compared to pregnant non-smokers (3.2% absolute death rate) (Butler et al., 1972). Such smoking throughout pregnancy may be compared to a testing session in which maternal [Hb_{CO}] increases to 5% and persists for about 4 h, or 0.12% of the last 20 weeks of pregnancy (4/3360 ×100 = 0.12% per testing session). Assuming a linear time dependency for adverse effects, this would increase the death rate by about 0.0012%. The Centers for Disease Control (CDC) death rate for accidental deaths is 0.04% and the death rate of all causes (accidents, homicides, suicides, diseases, cancer, infection, etc.) is about 0.8% (Kung et al., 2008). Thus, by this comparison, the total CDC death rate is 667 times more than the late fetal and neonatal death rate per testing session in which pregnant mothers [Hb_{CO}] increase to 5%. This justifies an IRB classification of the risk associated with DL_{CO} testing in pregnant women and the resultant effects on their fetuses as “minimal risk.”

5. Conclusions

Based on the evidence provided in this review, we submit that CO and NO inhalation for testing of pulmonary diffusing capacity is a safe procedure that may be justified by improved medical scare. Given that the elimination half-life of [Hb_{CO}] in maternal blood is about 3.8–4 h (Lawther and Commins, 1970; Selvakumar et al., 1993), or 74 min when given 100% oxygen (Weaver et al., 2000), the following recommendations are provided in Table 4. We suggest different acceptable concentrations for pregnant and non-pregnant women. When CO exposure in a pregnant woman is ≤ 3 min at a concentration of 0.3% (3000 ppm), fetal [Hb_{CO}] will not rise appreciably post-testing. A maximum of 3 testing sessions per trimester is recommended during pregnancy when DL_{CO} is measured (9 sessions in total throughout pregnancy, with a minimum of 48 h between each session). Only if DL_{NO} is measured, then a maximum of 5 testing sessions per trimester is recommended during pregnancy (15 sessions in total, with a minimum of 48 h between each session). If testing pregnant smokers whose maternal [Hb_{CO}] is already at about 5%, then CO exposure that is ≤1 min at a concentration of 0.3% (3000 ppm) can be allowed. Even though each pulmonary diffusion test of 10–15 s in length is separated by 4 min of rest breathing room air, the exposure time within each testing session is suggested to be cumulative over a typical 30–45 min session. Therefore, five 15 s DL_{CO} tests (rebreathing), or four 10 s single-breath DL_{CO} tests equates to 50–75 s of total CO exposure time resulting in an increase of [Hb_{CO}] by 3–4%, since each DL_{CO} test increases [Hb_{CO}] by 0.6–0.8% per test.

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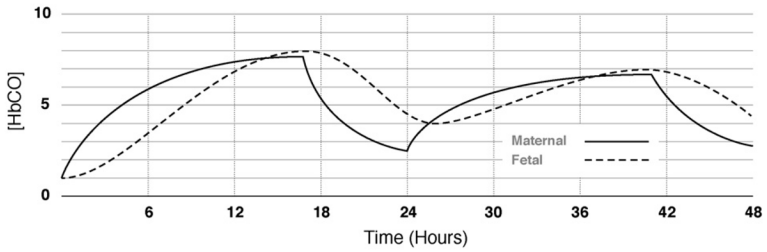

Fig. 1.

The predicted maternal and fetal [Hb_{CO}] when a mother breaths 50 ppm CO for 16 h, followed by an 8 h period in which no CO was inspired. The level of CO exposure is equivalent to smoking about 1–1.5 packs of cigarettes per day, followed by an 8 h sleep period. Figure modified by Longo (1977) based on the mathematical model by Hill et al. (1977).

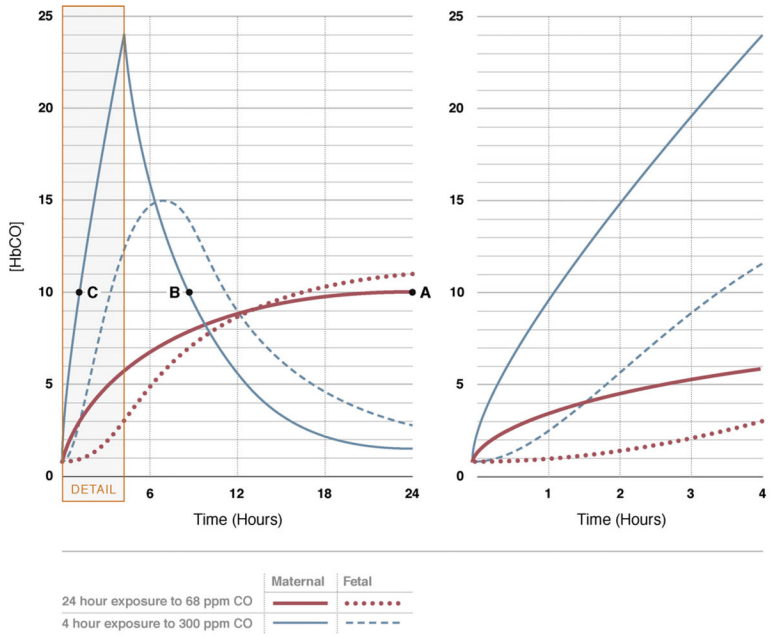

Fig. 2.

Changes in maternal and fetal [Hb_{CO}] during and after a 24 h exposure to 68 ppm CO and a 4 h exposure to 300 ppm CO. If the maternal value of 10% Hb_{CO} were obtained at equilibrium (point A), the fetal [Hb_{CO}] would be equal to about 11%. If the sample was taken at point B during the washout phase, the fetal [Hb_{CO}] would be about 14%. However, if the blood sample was taken during the uptake phase (point C), the fetal [Hb_{CO}] would be 2%. Not only is it impossible to predict fetal [Hb_{CO}] on the basis of a single maternal blood sample, but the length of time necessary to reduce the fetal [Hb_{CO}] depends on whether the concentration has already peaked or is still rising. In the case of lung diffusing testing, which could require up to about 3 min of 3000 ppm CO inhalation, the maternal [Hb_{CO}] may increase to 5%, but the fetal Hb_{CO} would hardly increase. This is typified by the enhanced "Detail" on the right panel in figure. Figures reproduced from Longo (1977).

Table 1

Studies that measured DL_{CO} in pregnant women.

Study	Number and type of pregnant women	Number of diffusing capacity tests done per subject per session	Conclusion of the study
McAuliffe et al. (2003)	112 (sea level) and 192 (4300m) healthy subjects from Peru	2 tests per session. 1 session total (cross-sectional study)	DL_{CO} measured in women living at altitude versus without altitude in the first, second, and third trimester compared to non-pregnant controls. DL_{CO} was higher at altitude and DL_{CO} decreased by about 15% by the third trimester.
McAuliffe et al. (2002)	68 women with twin pregnancies and 140 women with singleton pregnancies (all healthy)	2 tests per session. 1 session total (cross-sectional study)	To compare the differences in lung function between women with twin or singleton pregnancies, various lung function tests were performed. DL_{CO} did not change between the first and the third trimester in women with either twin or singleton pregnancies. DL_{CO} was 10% lower compared to non-pregnant women.
Milne et al. (1977)	21 healthy subjects	2 tests per session; 9 sessions total	DL_{CO} was measured at 8 different time points during pregnancy and 3–5 months post-partum. DL_{CO} decreased by 16% by the third trimester. [Hb] was lowest at 20–23 weeks gestation.
Lehmann (1975)	23 healthy subjects; 8 of those reporting spontaneous reported dyspnea with pregnancy	About 2 tests per session. 5 sessions total	DL_{CO} was measured at 12, 24, 32, and 36 weeks of gestation, and 12 weeks post-partum. Women with dyspnea in early pregnancy (12th week gestation) had a 10% decrease in DL_{CO}. Non-dyspneic women did not show a decrease in DL_{CO} by the 12th week.
Norregaard et al. (1989)	39 healthy subjects; (10 in each trimester and 9 post-partum)	2 series of repeated measurements (seated and supine). So 4 tests in total per session. 5 sessions in total	Lung function and postural changes with pregnancy (first, second, and third trimester), and about 2–4 weeks post-partum. DL_{CO} decreased by about 15% by the third trimester, regardless if the measurement was done in the sitting or supine position. No change in DL_{CO} between sitting and supine.
Gazioglu et al. (1970)	24 subjects; 8 healthy, 8 valvular heart disease, 8 chronic pulmonary disease	At least 2 times per session. 4 sessions total	DL_{CO}, DM, Vc were measured at 10, 24, and 36 weeks gestation, and 10 weeks post-partum. In normal subjects, DL_{CO} and Dm equally decrease by 14% by 36 weeks gestation. No change in Vc. In those with emphysema, DL_{CO} and Vc increased by 36 wks gestation with no change in DM. Those with pulmonary sarcoidosis had no change in DL_{CO}, DM, or Vc.
Garcia-Rio et al. (1996)	23 subjects; 11 healthy with dyspnea, 12 healthy asymptomatic	At least 2 times per session. 4 sessions total	DL_{CO} was measured 12, 24, 36 weeks gestation, and 16 weeks post-partum. DL_{CO} was not altered during pregnancy in either the non-dyspneic or dyspneic group. There was no difference in DL_{CO} in either group. The increase in dyspnea in pregnant women could be due to an excessive increase in sensitivity to CO_2 or hypoxia.
Boggess et al. (1995)	9 subjects; interstitial and restrictive lung disease	About 2 times per session. 1 session total	3 women had DL_{CO}s ≤ 40% predicted; 6 women ≥44% predicted in the first trimester. All women had vital capacity's ≤84% predicted. There was an association between DL_{CO} and vital capacity ($r^2 = 0.63$). Mean birth weight was (50th percentile) was not different between those with the most severe restrictive lung disease or the least severe restrictive lung disease. Restrictive lung disease can be tolerated in pregnancy. Exercise intolerance was common and patients may require early supplemental oxygen, DL_{CO} < 50% better predicted the need for oxygen supplementation than did vital capacity < 1.5 L.

All diffusion testing was accomplished with the single-breath DL_{CO} technique. No study reported adverse outcomes on mothers or their babies before or after birth.

Table 2

Studies and case reports of inhaled NO use in pregnant women.

Study	Number and type of pregnant women	Concentration and length of time NO was inhaled	Outcome
Robinson et al. (1999) (case report)	1 subject; 28 weeks gestation to delivery at 32 weeks and post-partum, with h/o HIV, pulmonary hypertension, peripartum cardiomyopathy	5–20 ppm, 4 weeks continuous inhaled NO with episodic monitoring of methemoglobin levels.	Inhaled NO reduced pulmonary artery pressure and right ventricular pressure, prolonged continuous inhaled NO therapy may be an effective therapy in the management of pulmonary hypertension during pregnancy.
Lust et al. (1999) (case report)	1 subject; delivery and post-partum, Eisenmenger's syndrome	Per nasal cannula during delivery, after delivery 10 ppm, ICU NO was delivered via transtracheal catheter. 10 h.	Continuous inhaled NO reduced initial pulmonary arterial pressure and improved oxygenation. Inhaled NO may be used to improve oxygenation and antithrombotic effects of NO may limit the increase in pulmonary arterial pressure expected with increased cardiac output throughout labor among pts with pulmonary vascular disease.
Goodwin et al. (1999) (case report)	1 subject; 36 weeks gestation with Eisenmenger's syndrome	20 ppm during labor. 80 ppm decreased to 60 ppm by 3rd day post-partum. Continuous during second stage of labor (45 min) and post-partum 3rd day, discontinued after 48 h.	Inhaled NO can be used to correct the hypoxemia of Eisenmenger's syndrome. Administration of NO led to improved oxygenation and lowered pulmonary arterial pressures. Baby survived, woman died.
Decoene et al. (2001) (case report)	1 subject, unexpected pulmonary hypertension that had an emergency C-section	5 ppm 24 h continuous during labor, delivery and post-partum.	Administration of inhaled NO enabled optimal control of pulmonary hypertension. Use of inhaled NO can improve the management of urgent C-section in women with unexpected pulmonary hypertension.
Lam et al. (2001) (case report)	1 subject, primigravida with primary pulmonary hypertension	20 ppm, decreased to 10 ppm 8.5 h after delivery, NO delivered through an endotracheal tube (93 h total).	NO can be used to successfully treat primary pulmonary hypertension in pregnancy.
Bonnin et al. (2005)	15 subjects with severe pulmonary hypertension, 3 were administered NO	50 ppm.	In the 3 out of 15 subjects who were administered NO, 2 babies survived after delivery. The baby that died was delivered at 21 weeks gestation. The babies that survived at 32–34 weeks gestation. Maternal mortality was found to be 36% with pulmonary arterial hypertension. Pregnancy should be discouraged in patients with severe pulmonary hypertension.
McMillan et al. (2002)	3 with pulmonary hypertension secondary to systemic lupus erythematosis (SLE) and anti-phospholipid syndrome	40 ppm during C-section (1st case). Second case, the amount of NO was not specified. NO inhalation did not occur in third case.	1st case patient died post-C-section. 2nd case patient died of severe heart failure after C-section. 3rd case more mild Pulmonary hypertension that was diagnosed earlier in pregnancy and had multidisciplinary management of pregnancy which is necessary for pregnant women with pulmonary hypertension.

Table 3

Original studies of inhaled NO use in pre-term infants.

Study	Number and gestational age of infants	Concentration and length of time NO was inhaled	Outcome
Chock et al. (2009)	Infants with a gestational age of 27 weeks with a history of premature rupture of membranes 6 infants received NO, 6 infants received placebo	5–10 ppm NO, duration of 30 min to 14 days.	Arterial P_{O_2} increased by 40 mmHg in the NO group versus a decrease of 11 mmHg in the placebo group. The mortality rate and incidence of bronchopulmonary dysplasia were not different between the two groups.
Hintz et al. (2007)	Infants < 34 weeks of age with respiratory failure. 198 subjects received NO and 200 received placebo	5–10 ppm NO for 76 + 73 h to 14 days.	Inhaled NO did not reduce mortality or improve neurodevelopment outcomes by 18–22 months of age. However, inhaled NO did not worsen neurodevelopmental outcomes either.
Delsing et al. (2007)	4 sets of twins with twin-to-twin transfusion syndrome who had severe persistent pulmonary hypertension of the newborn	20 ppm for 2 days given to 4 sets of twins (8 neonates).	All twin-to-twin transfusion syndrome infants with severe persistent pulmonary hypertension reacted promptly to inhaled NO.
Mestan et al. (2005)	70 infants at 27 weeks gestational age were given inhaled NO. 80 infants were given placebo	10 ppm day 1. 5 ppm for the next 6 days.	This was a follow-up study to examine neurodevelopmental outcomes of children at 2 years of age who were given inhaled NO at birth. Patients treated with inhaled NO had approximately half the risk of abnormal neurodevelopment compared to placebo. There was a 47% decrease in the risk of cognitive impairment compared to placebo group.
Konduri et al. (2004)	Neonates born at ≥34 weeks gestation with hypoxic respiratory failure. 150 pre-term infants given NO and 149 control subjects	5 ppm of NO which was increased to 20 ppm for 57 ±48 h.	Inhaled NO improves oxygenation but does not reduce the incidence of the use of extracorporeal membrane oxygenation or mortality when initiated at an oxygen index of 15–25 compared with > 25 in term and near-term neonates with respiratory failure.
Konduri et al. (2007)	299 neonates born at ≥34 weeks gestation were randomized to receive NO or placebo. A total 150 neonates were given NO. 266 survived to age 18–24 months	5 ppm of NO which was increased to 20 ppm for 57 ±48 h.	This was the follow-up study to the one published in 2004 (Konduri et al., 2004). Early inhaled NO therapy for hypoxic respiratory failure in term and near term infants is not associated with an increase in neurodevelopmental impairment or hearing loss at 18–14 months postnatal.
Schreiber et al. (2003)	207 newborns, aged 34 weeks or less who were mechanically ventilated were randomized into NO or placebo group. A total of 105 neonates received NO	Initial dose 10 ppm for 1 day, then 5 ppm on days 2–6 (7 days total).	Significant reduction in death or bronchopulmonary dysplasia at 36 weeks of age with inhaled NO compared to controls. The use of nitric oxide in premature infants with respiratory distress syndrome decreases the incidence of chronic disease and death.
Kinsella and Abman (2007)	80 pre-term neonates, with gestational age 34 weeks or less, 7 days age or less, and severe hypoxemia who were on mechanical ventilation	5 ppm or placebo, 7 days then weaned. (40 neonates actually received NO).	Even though oxygenation improved 1 h after inhaled NO, there was only a trend toward decrease in bronchopulmonary dysplasia and no differences in rates of severe intracranial hemorrhage.
Kinsella et al. (2006)	793 newborns ≤34 weeks gestation were randomly assigned to receive NO or placebo. A total of 395 neonates received NO	Randomly assigned to receive 5 ppm NO or placebo for 21 days.	No significant difference in the incidence of death or bronchopulmonary dysplasia between patients receiving NO or not. For infants with a birth weight of 1000–1250 g, inhaled NO reduced the incidence of bronchopulmonary dyplasia and overall brain injury. Inhaled NO did not increase incidence of pulmonary hemorrhage or other adverse events.
Clark et al. (2000)	248 neonates with pulmonary hypertension enrolled ≥34 weeks gestation. Babies were 4 days old. 126 randomly assigned to NO group, 122 assigned to control group.	20 ppm for 24 h, then 5 ppm for 96 h.	30-day mortality rate was the same in both groups (8%). Chronic lung disease developed less often in neonates treated with NO. Inhaled NO reduced the extent to which extracorporeal membrane oxygenation is needed in neonates with hypoxemic respiratory failure and pulmonary hypertension.
Van Meurs et al. (1997)	11 neonates, aged ≤34 weeks gestation with respiratory failure 4 h or more after birth.	Four concentrations of inhaled NO were used: 1, 5, 10, 20 ppm, and placebo for 15 min.	No significant elevations of methemoglobin were found with NO inhalation (0.6% before inhalation to 0.8% after 15 min of inhalation). NO inhalation improved the arterial P_{O_2} to alveolar P_{O_2} ratio in those with respiratory failure.

Table 4

Recommendations for CO and NO inhalation at rest and during exercise in pregnant women and non-pregnant individuals per testing session.

Condition	Recommended CO inhalation exposure time and dosage resulting in a recommended acceptable [Hb_{CO}] concentration	Recommended NO inhalation exposure time and dosage
1. Men and non-pregnant women who are asymptomatic		
Rest (AHA Class A1–3)	[Hb_{CO}] = 10% when 0.3% CO is inhaled for ≤6 min after which breathing room air ensues[a]	80 ppm over 6 min
Exercise (AHA Class A1–3)	[Hb_{CO}] = 5% when 0.3% CO is inhaled for ≤3 min after which breathing room air ensues	80 ppm over 3 min
2. Men and non-pregnant women with cardiovascular disease		
Rest (AHA Class B–D)	[Hb_{CO}] = 5% when 0.3% CO is inhaled for ≤3 min after which breathing room air ensues	80 ppm over 3 min
Exercise (AHA class B and C)	[Hb_{CO}] = 5% when 0.3% CO is inhaled for ≤3 min after which breathing room air ensues	80 ppm over 3 min
3. Asymptomatic pregnant women[b]		
Rest (AHA Class A1–3)	[Hb_{CO}] = 5% when 0.3% CO is inhaled for ≤3 min after which breathing room air ensues[c]	80 ppm over 3 min
Exercise (AHA Class A1–3)	[Hb_{CO}] = 5% when 0.3% CO is inhaled for ≤3 min after which breathing room air ensues[c]	80 ppm over 3 min
4. Pregnant women with cardiovascular disease[b]		
Rest (AHA class B–D)*	[Hb_{CO}] = 4% when 0.3% CO is inhaled for ≤3 min after which breathing room air ensues[c]	80 ppm over 3 min
Exercise (AHA Class B and C)	[Hb_{CO}] = 4% when 0.3% CO is inhaled for ≤3 min after which breathing room air ensues[c]	80 ppm over 3 min

The American Heart Association (AHA) Risk Stratification Criteria (Fletcher et al., 2001; Fuster et al., 1996) Class A. Apparently Healthy

1. Children, adolescents, men < 45 years of age, and women < 55 years of age who have no symptoms or known presence of heart disease or major cardiovascular disease risk (CVD) factors.
2. Men ≥45 years of age or women ≥55 years who have no symptoms or known presence of heart disease with less than two major CVD risk factors.
3. Men ≥45 years of age or women ≥55 years who have no symptoms or known presence of heart disease with more than two major CVD risk factors.

Class B. Presence of known, stable cardiovascular disease with low risk for complications. Includes individuals with any of the following diagnoses:

1. Coronary artery disease who condition is stable and who have the clinical characteristics described below.
2. Valvular heart disease, excluding severe valvular stenosis or regurgitation.
3. Congenital heart disease.
4. Cardiomyopathy, ejection fraction ≤30%.
5. Exercise test abnormalities that do not meet the criteria for Class C.

Clinical characteristics

a. New York Heart Association Class 1 or 2.
b. Exercise capacity ≤6 METS.
c. No evidence of congestive heart failure.

d. No evidence of myocardial ischemia or angina at rest or on the exercise test at or below 6 METS.

e. Appropriate rise in systolic blood pressure during exercise.

f. Absence of sustained or non-sustained ventricular tachycardia at rest or with exercise.

Class C. Those at moderate to high risk for cardiac complications during exercise and/or unable to self-regulate activity or understand recommended activity level. Includes individuals with any of the following diagnoses:

1. Cardiovascular disease with the clinical characteristics outlined below.
2. Valvular heart disease, excluding severe valvular stenosis or regurgitation.
3. Congenital heart disease.
4. Cardiomyopathy, ejection fraction ≤30%.
5. Complex ventricular arrhythmias not well-controlled.

Clinical characteristics

a. New York Heart Association Class 3 or 4.

b. Exercise capacity < 6 METS.

c. Angina of ischemia ST depression at workload < 6 METS.

d. Fall in systolic blood pressure below resting during exercise.

e. Non-sustained ventricular tachycardia at rest or with exercise.

f. Previous episode of primary cardiac arrest.

g. A medical problem that the physician may be life threatening.

Class D. Unstable cardiovascular disease with activity restriction. Includes individuals with:

1. Unstable angina.
2. Severe and symptomatic valvular stenosis or regurgitation.
3. Congenital heart disease.
4. Heart failure that is not compensated.
5. Uncontrolled arrhythmias.
6. Other medical condition that could be aggravated by exercise. No physical exercise is recommended.

CVD risk factors are:

1. *Age*: Men ≥45 years of age; women ≥55 years of age.
2. *Family history*: Myocardial infarction, coronary revascularization, or sudden death before age 55 years in father or other first degree male relative, or before 65 years of age in mother or other female first degree relative.
3. *Cigarette smoking*: Current smoker or those who have quit within the past 6 months.
4. *Sedentary lifestyle*: Not participating in a least 30 min of moderate intensity (40–60% of oxygen uptake reserve) on at least 3 days per week for at least 3 months.
5. *Obesity*: Body mass index ≥30 kg/m^2 or waist girth > 102 cm (40 in.) for men and >88 cm (35 in.) for women.
6. *Hypertension*: Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, confirmed by measurements on at least two separate occasions, or on anti-hypertensive medication.
7. *Dylipidemia*: Low density lipoprotein cholesterol ≥130 mg/dl (3.37 mmol/l) or high density lipoprotein cholesterol < 40 mg/dl (1.04 mmol/l) or on lipid lowering medication. If total serum cholesterol is all that is available use ≥200 mg/dl (5.18 mmol/l).
8. *Prediabetes*: Impaired fasting glucose ≥100 mg/dl (5.50 mmol/l) but <126 mg/dl (6.93 mmol/l) or impaired glucose tolerance from a 2 h oral glucose tolerance test ≥140 mg/dl (7.70 mmol/l) confirmed by measurements on at least two separate occasions.

CVD risk factors are obtained from the American College of Sports Medicine Handbook (ACSM, 2009).

[a] If neurophysiological skills are being tested in a research study (i.e. visuomotor coordination, visuospacial functioning, short and long term semantic memory) (Amitai et al., 1998), Hb_{CO} levels are recommended not to exceed 5%.

[c] Inhalation of 100% oxygen to reduce the half-life of CO to 74 min (Weaver et al., 2000) is not recommended because of the potential toxic effects of oxygen on the mother and fetus. Tests of diffusing capacity (with 0.3% CO inhalation) increase Hb_{CO} by about 0.6–0.8% per 10 s breath-hold maneuver (Forster et al., 1954; Frey et al., 1987) or 15 s rebreathing maneuver. Therefore, one can use this as a template for estimating the [Hb_{CO}] when it is not measured (i.e. one diffusion test increases Hb_{CO} by 0.6–0.8%). Four rebreathing diffusion tests of 15 s each or 4 single-breath diffusion tests of 10 s each should be separated by 4 min between each test. The total CO exposure time in this example is therefore 40–60 s and is estimated to increase Hb_{CO} by 2.4–3.2% (0.6% × 4 tests, or 0.8% ×4 tests).

[b] A maximum of 3 testing sessions per trimester is recommended during pregnancy in which maternal Hb_{CO} can increase up to 5% per session when 0.3% CO exposure is ≤ 3 min. Therefore, a maximum of 9 sessions in total throughout pregnancy are recommended, and sessions should be separated by 48 h. If only DL_{NO} is measured during pregnancy, then a maximum of 5 testing sessions per trimester is thought permissible when ≤80 ppm NO is inhaled for ≤3 min per session (15 sessions in total throughout pregnancy). If testing pregnant smokers, in which maternal levels are already 5%, and a measurement of diffusing capacity is needed, then CO exposure that is ≤1 min at a concentration of 0.3% (3000 ppm) can be allowed.

The repeatability for DL_{NO} (within session variability) is 17 ml/min/mmHg in non-pregnant women and men (Zavorsky and Murias, 2006). The reproducibility of DL_{NO} (week to week variability) from these same subjects is 20 ml/min/mmHg (Murias and Zavorsky, 2007). Thus, we not expect the variability of DL_{NO} to be different during pregnancy since DL_{NO} is minimally affected by hemoglobin changes (van der Lee et al., 2005). Furthermore, as changes in hemoglobin concentration only vary by 1 g/dl throughout pregnancy or over a 28-day menstrual cycle (McAuliffe et al., 2002; Vellar, 1974), the small 10–15% reduction in DL_{CO} in the third trimester of pregnancy has little to do with maternal hemoglobin variability. The repeatability and reproducibility of DL_{CO} is 3 and 5 ml/min/mmHg, respectively in a non-pregnant state (Murias and Zavorsky, 2007; Zavorsky and Murias, 2006) and is not expected to vary during pregnancy.